STUDIES IN THE SYNTHESIS OF MECHANISM-BASED STEROID ENZYME INHIBITORS AND RELATED COMPOUNDS

BY

HELENA MAJGIER-BARANOWSKA

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES OF THE UNIVERSITY OF MANITOBA IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF

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This work, I dedicate to my loving parents, to my mother, Aniela, and my father, Jan who passed away in July, 08, 1994.

ABSTRACT

The main goal of the thesis was to synthesize compounds which would selectively inhibit a function of steroid enzymes. Alteration of the hormonal level in the body by enzyme inhibition is a part of medicinal therapy in a number of disease states including hormone dependent prostate and breast carcinomas. In this thesis, three enzymes have been targeted: 3α,20β- [EC 1.1.1.53] and 17β,20α-hydroxy dehydrogenases ("cortisone reductase") [EC 1.1.1.62] and estrogen synthetase (aromatase) [P450 XIXA1]. The presence of 20α-hydroxysteroid dehydrogenase in human mammary tumours may indicate an inhibitory role for that enzyme in breast carcinomas. Aromatase, on the other hand, controls the endogenous production of estrogens. Estrogen dependent breast cancer is known to respond to manipulation at the level of estrogen. Thus, inhibition of aromatase prevents estrogen production, and leads to regression in tumour growth. The goal of this thesis was to develop drugs for the treatment of estrogen receptor positive breast cancer.

The first part of the research was aimed at the synthesis of substituted cyclosteroid derivatives designed as substrates for the above enzymes which are able to act as mechanism-based inhibitors. The second part of the thesis dealt with the synthesis of 4-hydroxyestrogen derivatives, which are the natural metabolites of estrogens. For the synthesized compounds, ^{1}H and ^{13}C NMR spectra as well as electron ionization mass spectra were determined. The structures of synthesized compounds were analysed by COSY, HSQC, HMBC, NOE experiments, and were confirmed by X-ray crystallographic analysis.

Two spirocyclopropanol steroid isomers, 20α- and 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one, have been synthesized as potential mechanism-based

inhibitors of 17β,20α- and 3α,20β-hydroxysteroid dehydrogenase, respectively. The key synthetic steps involved formation of a 17,20-silyl enol ether intermediate followed by the Simmons-Smith reaction. A newly introduced couple, Zn-Au, follows the order of reactivity Zn-Au > Zn-Ag > Zn-Cu. Enzyme evaluation studies, in the presence of NAD^+, showed that neither 20α- nor 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one was effective as an inhibitor of steroid 17β,20α- and 3α,20β-oxidoreductase, respectively.

Inhibitors of aromatase have been synthesized *via* reductive cyclization of androst-4-ene-3,17-dion-19-al and 5α-androst-1-ene-3,17-dion-19-al with Zn in 50% aqueous acetic acid or Li in liquid ammonia followed by acetylation and oxidation. 19(R/S)-Acetoxy-5β,19-cyclo-androst-1-ene-3,17-diones have been synthesized *via* palladium oxidation of trimethylsilyl enol ethers. Attempts to synthesize unsaturated analogues of 19(R/S)-acetoxy-1β,19-cyclo-5α-androstane-3,17-dione, by the same procedure, were not successful. Enzyme evaluation studies showed that 19(S)- and 19(R)-acetoxy-5β,19-cycloandrost-1-ene-3,17-dione showed 9% and 44% of inhibitory activity, respectively. The synthesized 19(R)-hydroxy- and 19(R)-acetoxy-1β,19-cyclo-5α-androstane-3,17-dione showed 40-50% of inhibition when tested on human placental aromatase microsomes.

The ratio of products with the hydroxy group above ring A upon reductive cyclization was investigated. It was discovered that reductive cyclization of androst-4-ene-3,17-dion-19-al both in Zn/50% aqueous acetic acid and Li/NH_3 gave the same product, namely the thermodynamically <u>less</u> stable 19(R)-hydroxy-5β,19-cycloandrost-3,17-dione. However, reductive cyclization of androst-1-ene-3,17-dion-19-al with Zn/50% acetic acid gave thermodynamically <u>more</u> stable product, the 19(R)-hydroxy-1β,19-cyclo-5α-androstane-3,17-dione, but with Li/NH_3 the thermodynamically less stable, 19(S)-hydroxy-1β,19-cyclo-5α-androst-3,17-dione (70%), was produced. This thermodynamically less stable product upon treatment with base or acid did not epimerize to the more stable isomer unlike the 19(R)-hydroxy-5β,19-cycloandrostane-3,17-dione isomer. Mechanisms for the reductive cyclization of androst-4-ene-3,17-dion-19-al and 5α-androst-1-ene-3,17-dion-19-al as well as for

epimerization of 19(R)-hydroxy-5β,19-cycloandrostane-3,17-dione have been proposed. Investigations of the reductive cyclization were also extended to androst-4-ene-3,17-dione derivatives with different functional groups at C-19, namely the 19-protected oxime and 19-methyl ester. Reductive cyclization, of conjugated double bond versus protected oxime, of tert-butyldimethylsilyl-19-oxime-androst-4-ene-3,17-dione with Li-NH_3 resulted in the elimination of the C-10 angular substituent to yield estr-5(10)-ene-3,17-dione and 17β-hydroxy-estr-5(10)-en-3-one. Reductive cyclization of androst-4-ene-3,17-dione 19-methylcarboxylate, by treatment with either zinc in 50% aqueous acetic acid or lithium in liquid ammonia, gave steroid dimers, bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate).

In the pursuit of a more efficient method for the synthesis of 4-hydroxyestrogens from estr-4-ene-3,17-dione *via* 4ξ,5ξ-epoxysteroid derivatives, two different strategies were applied: (1) acidic aromatization of 4ξ,5ξ-epoxyestr-1-ene-3,17-dione, and (2) pyrolysis of 4-chloro-4ξ,5ξ-epoxyestra-3,17-dione.

By using the first synthetic method, 4-hydroxyestrogens were obtained as highly pure compounds in a good yield (50-79%). The conversion of estr-4-ene-3,17-dione into 4α,5α-epoxyestra-3,17-dione was achieved by using an efficient, one-step *versus* the reported six-step, procedure by treatment of estr-4-ene-3,17-dione with 1,1,1-trifluoromethyl(methyl)dioxirane. 1,1,1-Trifluoromethyl(methyl)dioxirane was also found to be the convenient reagent to introduce an oxygen function into the weakly nucleophilic 3,4-double bond of 4-chloro-estr-4-ene-3,17-dione.

By using the second synthetic method, 4-hydroxyestrone was synthesized together with other compounds. The number and type of 4ξ,5ξ-epoxyestra-3,17-dione depended on the conditions employed. One of the new characterized compounds was determined to be estra-5,7,9-triene-4,17-dione with aromatic ring B and a carbonyl function shifted from C-3 to C-4. Mechanisms of its origin have been proposed.

The research demonstrated that by synthesizing the model steroid compounds, the inhibition mechanism of the cancer-promoting, steroid-specific enzymes can be studied. Rationalization of structure-activity relationship was based on the results of aromatase inhibition.

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GLOSSARY

Ac	acetyl, CH_3CO^+
AIBN	azobisisobutyronitrile
Bn	benzyl
COMT	catechol-O-methyltransferase
Diglyme	diethylene glycol, dimethyl ether
DMF	N,N-dimethylformamide
BSA	benzeneselenic anhydride
BSTFA	bis(trimethylsilyl)trifluoroacetamide
BTMSA	bis(trimethylsilyl)acetylene
CSA	camphorsulfonic acid
DDQ	dicyanodichloroquinone
EIMS	electron ionization mass spectrometry
EPR	electron paramagnetic resonance
EXF	extended X-ray absorption fine structure spectroscopy
FAB	fast atom bombardment
FABMS	fast atom bombardment mass spectrometry
FCC	flash column chromatography
HSD	hydroxysteroid dehydrogenase
$M^{+\cdot}$	molecular ion
MCD	molecular circular dichroism
MS	mass spectrometry
mp	melting point
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect

Oxone^{R}	monopersulfate compound, $2KHSO_5.KHSO_4.K_2SO_4$ (MW 614.78)
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
ppm	parts per million
iPr_3Si	tri-*iso*propylsilyl
Py	pyridine
rt	room temperature
TBDMSi	tert-butyldimethylsilyl
TC	total crude
TESi	triethylsilyl
THF	tetrahydrofuran
TMSi	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
TsOH	p-toluensulfonic acid
Ph_2Se_2	diphenyl diselenide

CHAPTER 1

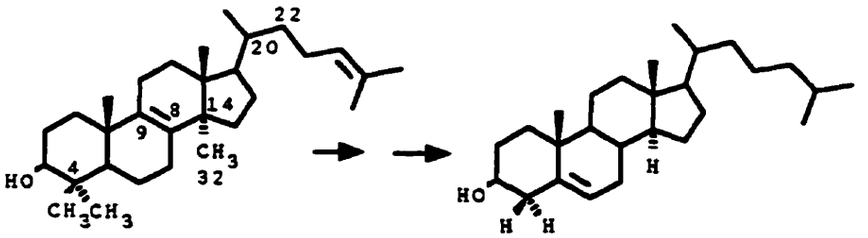
INTRODUCTION

1.1.0 STEROID HORMONE BIOSYNTHESIS

Studies of steroid hormones have been a major interest of research in the disciplines of biology and chemistry over the past 60 years (Kalvoda, 1992). Steroids are responsible for a wide array of messenger signals in physiological functions, e.g. they contribute to fertility, fetal, muscular, sexual organ development, and sexual differentiation. Furthermore, they also contribute to the control of intravascular fluid volume, salt balance, glucose and fat metabolism, as well as to blood pressure regulation, and inflammatory response (Lieberman *at al.*, 1990, 1984).

Steroids are produced in a variety of mammalian tissues. In the gonads, their biosynthesis occurs with the production of sex steroids, and in the adrenal glands it give rise to glucocorticoids and mineralocorticoids. Some steroids are also produced in peripheral tissue such as adipose tissue, skin, muscles, and tumor cells. The activities of steroids are associated with subtle and complex regulation which occurs at the level of steroidal biosynthesis and degradation (Stryer, 1981).

The major sterols are cholesterol and ergosterol. The biosynthetic pathway for these steroids is common up to lanosterol but then diverges. Cholesterol and ergosterol synthesis, in the body, starts from acetic acid *via* acetyl coenzyme A and then to mevalonic acid, squalene oxide and to lanosterol. Lanosterol, through a series of oxidative removals of methyl groups, side chain double bond reduction, and double bond isomerization (C-8-C-9 → C-5-C-6), is further enzymatically converted to the essential steroid cholesterol (Scheme 1.1), (Danielson and Sjovall, 1985). All of the enzymes in the lanosterol to cholesterol conversion have been partially purified and



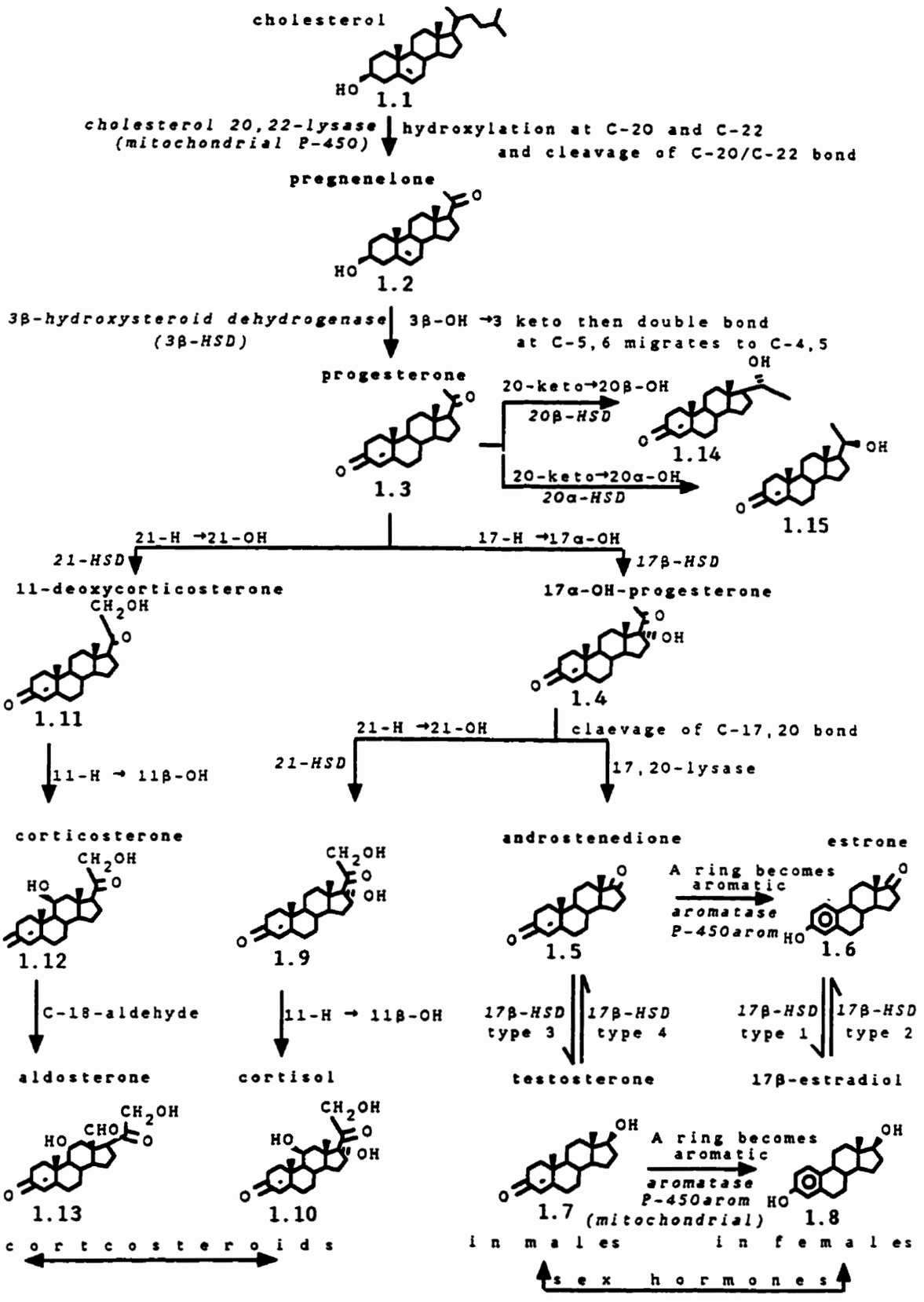
Lanosterol Cholesterol

Scheme 1.1 C-Demethylation during cholesterol biosynthesis.

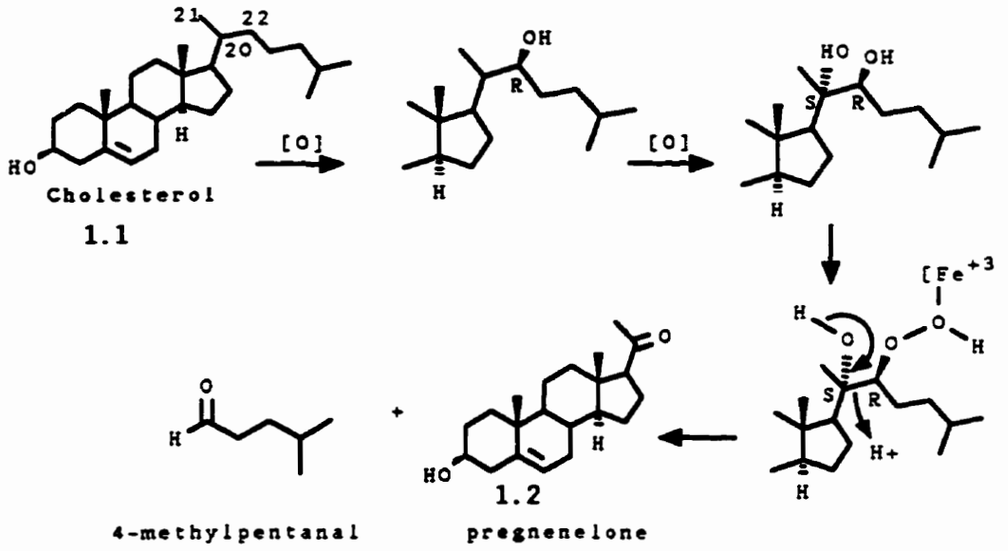
characterized (Fischer *et al.* 1989, Trzaskos *et al.*, 1986).

Cholesterol is one of the most abundant steroids in mammalian cells and is thought to play a critical role in maintaining the integrity of cellular membranes (Chapman *et al.*, 1985). Moreover, cholesterol is the precursor of all endogenous steroidal hormones as shown in Scheme 1.2. The cytochrome P-450 enzymes are involved in the conversion of cholesterol to these hormones. Cytochrome P-450 enzymes are found in adrenal, ovary, testis, placental tissue and pathological tissue (Waterman *et al.*, 1986).

One of a family of enzymes, isozyme, cytochrome P-450$_{scc}$, converts cholesterol to pregnenelone and 4-methylpentanal (Scheme 1.3). The P-450$_{sec}$ catalyzes three sequential oxidative steps, each of which consumes one molecule of oxygen and one molecule of NADPH. The three steps are 22(R)-hydroxylation, 20(S)-hydroxylation and fission of the C-20-C-22 bond. The first two hydroxylations proceed with retention of configuration. The carbon-carbon bond cleavage may occur *via* intercepting the activated oxygen [Fe^{3+}-O] complex, generated in the third step, by one of the hydroxyl groups, 22(R)-OH or 20(S)-OH. Proton removal from the hydroxyl adjacent to the resulting hydroper-

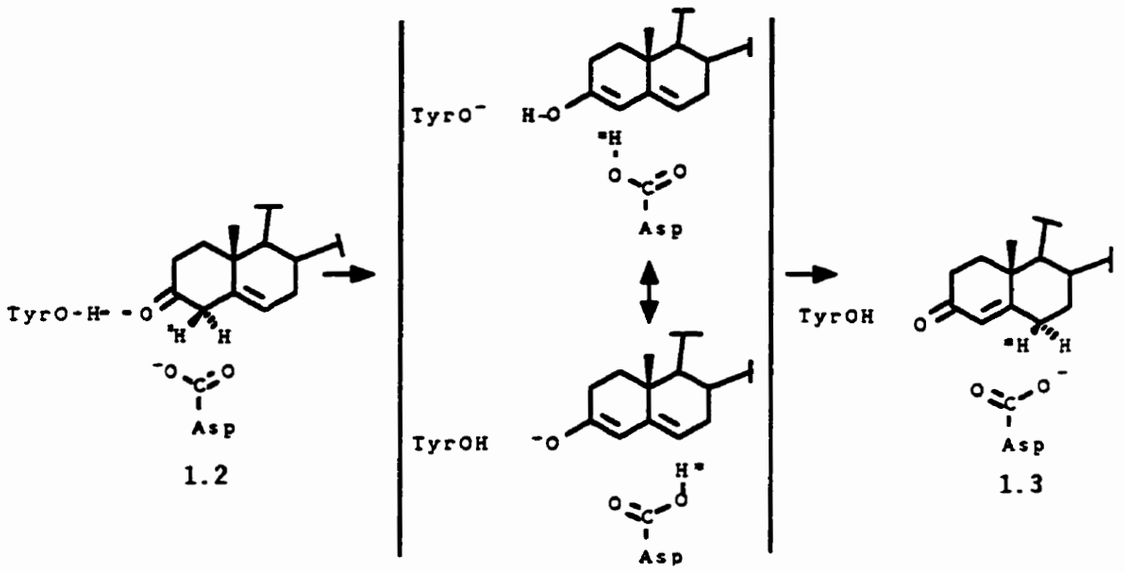


Scheme 1.2 Steroid hormone biosynthesis.



Scheme 1.3 Mechanism of the cholesterol chain cleavage by cytochrome P-450$_{sec}$.

oxide then initiates the C-C bond fission to give the final products. The conversion of pregnenolone **1.2** to progesterone **1.3** (Scheme 1.2) in mammalian systems is believed to involve only one enzyme, which possesses both dehydrogenase (3β-hydroxysteroid dehydrogenase) and double bond isomerization function (Δ^5-3-ketosteroid isomerase), (Brandt and Levy, 1989). This enzyme catalyses the conversion of a variety of Δ^5-3-ketosteroids to the corresponding conjugated Δ^4-3-ketosteroids. Presumably, the isomerization reaction proceeds through an enzyme-bound dienol as shown in Scheme 1.4. Mutagenic analysis suggests that the intermediate is formed by abstraction of the 4β-hydrogen of the Δ^5-ketosteroid by Asp-38, with Tyr-14 acting to polarize the carbonyl group, by either hydrogen bonding or proton transfer. Subsequent protonation of the dienol at C-6β by the conjugate acid Asp-38 gives the conjugated Δ^4-3-ketosteroid.



Scheme 1.4 Isomerization of pregn-5-ene-3,20-dione to progesterone.

Progesterone **1.3** is an important steroidal hormone in pregnant women. It also participates in fetal development and serves as a precursor to the corticosteroids and sex steroids, androgens and estrogens.

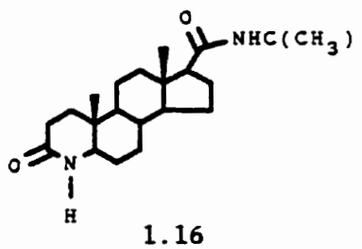
The corticosteroids, including the glucocorticoid, cortisol **1.10**, and the mineralocorticosteroid, aldosterone **1.13**, are produced in the adrenal cortex, which are located in the adrenal glands. These hormones contain carbon skeletons identical to progesterone **1.3** but differ in the number of hydroxy or carbonyl substituents. The enzymes responsible for corticosteroids production are all cytochrome P-450 isozymes, located in the adrenal cortex. A microsomal 21-hydroxysteroid hydroxylase is responsible for the formation of 11-deoxycorticosterone **1.11** (Bumpus and Dus, 1982). Studies by Ogishima *et al.* (Ogishima *et al.*, 1989) suggest that there is a single (mitochondrial) enzyme, which is responsible for aldosterone **1.13**

production from 11-deoxycorticosterone **1.11**. This enzyme, found in the zona glomerulosa located in the outer adrenal cortex of the adrenal glands, can catalyze 11β-hydroxylation, 18-hydroxylation of 18-methyl steroids, and 18-hydroxylation of 18-hydroxymethyl steroids. A second enzyme found in the zona reticularis-fasciculata was found to catalyze 11β-hydroxylation as well as hydroxylation of the 18-methyl group, but not aldosterone **1.13** production (Ogishima *et al.*, 1989). Suhara *et al.* have shown that an 11β-hydroxylase enzyme may be responsible for 19-hydroxylation and the production of 19-norsteroids (Suhara *et al.*, 1988)

Glucocorticoids (i.e. cortisone and hydrocortisone from the adrenal cortex) are responsible for the transformation of proteins into glucose and liver glycogen. On the other hand, mineralocorticoids (i.e. desoxycorticosterone **1.11** and aldosterone **1.13**) are of vital importance in the maintainance of electrolyte and water balance. They are released in response to the concentrations of sodium, potassium, and chloride ions and water in the blood. Aldosterone maintains the concentration of sodium, potassium, and chloride ions in the blood within the narrow limits essential for life.

In the adrenal and testis glands a microsomal cytochrome P-450 enzyme is responsible for 17-hydroxylation of progesterone **1.3** (17α-steroid hydroxylase) and for the 17,20 C-C bond cleavage (17,20-lysase) of 17α-hydroxyprogesterone **1.4** to form a weak androgen, androst-4-ene-3,17-dione **1.5**. Reduction of the 17-ketone **1.5** by 17β-hydroxysteroid dehydrogenase 3 (17β-HSD type 3) affords the more powerful androgen, testosterone **1.7** (Geissler *et al.*, 1994; Anderson and Moghrabi, 1997). Furthermore, many of the androgenic effects of testosterone are thought to be mediated by the 4,5-reduced steroid

5α-dihydrotestosterone. The enzyme responsible for the reduction of testosterone, 5α-reductase, resides in some cell types which are responsive to androgen action. Presumably, this enzyme can then potentiate the effect of testosterone by giving rise to higher levels of intracellular 5α-dihydrotestosterone, which binds to the androgen receptor protein. Therefore, blockage of this enzyme is a method of control of benign prostatic hyperplasia (Russell and Wilson, 1994). The 4-aza-steroid **1.16**, from Merck, Sharpe, and Dohme, is on the market under the brand name PROSCAR (Scheme 1.5).

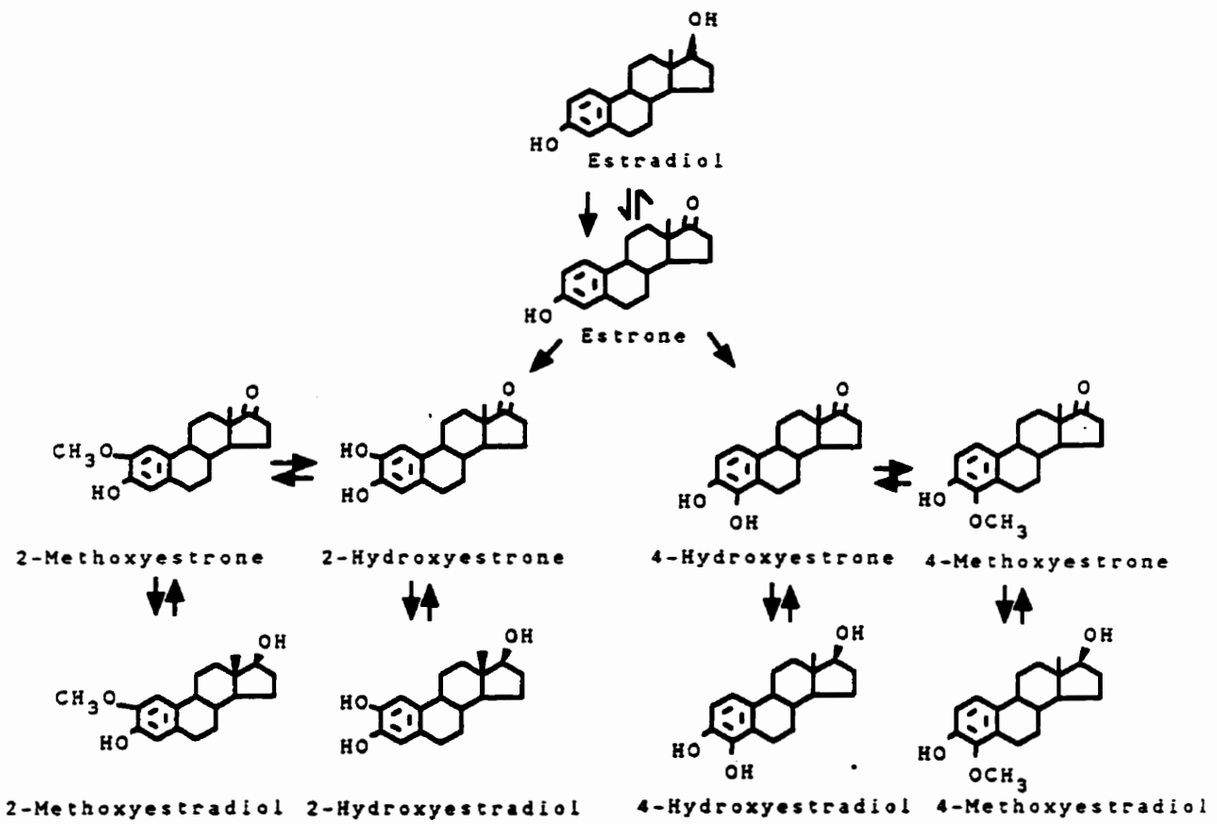


1.16

Scheme 1.5 4-Aza-steroid, inhibitor of 5α-reductase (Proscar).

The last major steroid hormone products in the biosynthetic pathway from progesterone are estradiol and estrone, which are derived from testosterone and androstenedione, respectively. The enzyme responsible for this androgen to estrogen transformation is also a microsomal cytochrome P-450, known as estrogen synthetase or aromatase. Both androstenedione and testosterone are direct substrates for aromatase and give rise to estrone and estradiol, respectively. Of these estrogens, estradiol is the more potent estrogen. Estrogens are further enzymatically oxidized, in the body, to catechols, e.g. 2- and 4-hydroxyestrones. They are synthesized in various human organs, e.g.

the liver, the pituitary, the hypothalamus and even in breast cancer tissue. In Scheme 1.6 are shown the metabolic pathways for the formation of the various catechol estrogens and their methyl ethers. Estradiol is largely converted to estrone by 17β-HSD type 2 (Anderson and Moghrabi, 1997; Labrie *et al.*, 1997). Estrone is further metabolized to 2- and 4-hydroxyestrones, which are rapidly methylated by the catechol O-methyl transferase enzyme (COMT) to 2- and 4-methoxyestrones. The transformation of 2- or 4-methoxyestrogens back to 2- or 4-hydroxyestrogens also occurs. Ball *et al.* (Ball *et al.*, 1972) demonstrated that catechols have higher affinity for the catechol O-methyl transferase enzyme than for



Scheme 1.6 Metabolic pathway for catechol estrogens and their methyl ethers (Martucci, 1983).

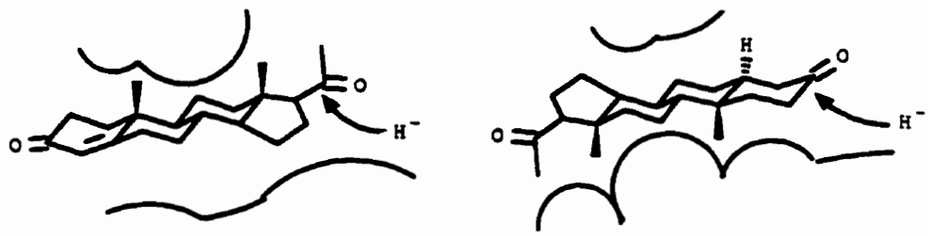
catecholamines. Thus, they have been recognized as potent inhibitors of the methylation of catecholamines. In addition, Schutze *et al.* (Schutze *et al.*, 1994, 1993) showed that 2- and 4-hydroxyestrogens interact with the estrogen receptor and could be transcriptionally active. The effects of 4-hydroxyestrogens are more pronounced than 2-hydroxyestrogens.

The relative equilibrium between estradiol and estrone does not depend only on the levels of testosterone and androstenedione, as estrone and estradiol may be also interconverted by type 1 17β-HSD and type 2 17β-HSD type 2, respectively (Labrie *et al.*, 1997). These enzymes are present in placenta and other tissues that synthesize estrogens. X-Ray crystallographic studies of type 1 17β-HSD at the 2.20A resolution have revealed a fold characteristic of the short-chain dehydrogenase (Gosh *et al.*, 1995).

A recent site-directed mutagenesis study of type 1 17β-HSD showed that His221 as well as Tyr155 is essential for the enzymatic activity (Puranen *et al.*, 1994).

Another oxidoreductase, 20α-hydroxysteroid dehydrogenase, depending on the pH and coenzyme concentration (NAD^+ and NADH), is able to interconvert progesterone to its 20(S) (20α)-hydroxy derivative (Pons *et al.*, 1977). 17β-Hydroxy- and 20α-hydroxysteroid dehydrogenases have been associated with a number of biochemical processes, e.g. regulation of pregnancy (Kuhn and Briley, 1970; Matsuda *et al.*, 1990), testicular function (Fan *et al.*, 1974) and as an enzymatic marker for pre-T and T lymphocytes (Weinstein, 1982). Further, it has been shown from *in vitro* studies that human breast tissues contain high levels of 17β-hydroxysteroid dehydrogenase (Blomquist *et al.*, 1987; Braselton *et al.*, 1974).

Another oxidoreductase, 3α,20β-hydroxysteroid dehydrogenase [EC 1.1.1.53] of *Streptomyces hydrogenans*, also called "cortisone reductase", (Hubener, 1959) is *able* to catalyze the interconversion of 3-oxo-5α-steroids to the 3α-alcohols and 20-oxosteroids **3** to 20(*R*) 20β-alcohols **14** (Scheme 1.2). Schematic representations of the positions of the 20-oxo and the 3-oxo-steroids in the active site of the enzyme are given in Scheme 1.7.



progesterone 5α-pregnane-3,20-dione

Scheme 1.7 Binding of 20-oxo- and 3-oxosteroid to 3α,20β-hydroxysteroid dehydrogenase.

Evidence that the 3α-and 20β-HSD activities are due to one enzyme comes from kinetic competition experiments using a mixture of substrates (Pocklington and Jeffery, 1968, 1969; Gibb and Jeffery, 1971), from the comigration of 3α- and 20β-HDS activities on polyacrylamide disc gels, and from identical rates of their inactivation in acid at pH 4.5 (Blomquist, 1973), as well as from comparison studies of 3α- and 20β-HSD activities with halo steroidal affinity labels (Edwards and Orr, 1978). X-ray crystallographic structure of the enzyme-cofactor (NADH) binary complex has been carried out at 2.6A resolution (Ghosh *et al.*, 1994; Ghosh *et al.*, Orr, 1991). The structure determination of 3α,20β-HSD demonstrated the presence of

a single cofactor site and a single substrate site per unit confirming the two steroid orientations-one cofactor site model (Ghosh *et al.*, Orr, 1991).

The amino acid partial (Orr *et al.*, 1990) and complete (Marekov *et al.*, 1990) sequence data of 3α,20β-HSD have been published. They showed that 3α,20β-HSD belongs to the class of nonmetallo-short-chain alcohol dehydrogenase. Spectral and kinetic analysis (Betz and Warren, 1968) revealed that the reaction mechanism of 3α,20β-HSD is essentially an ordered bi-bi mechanism, with the cofactor binding first and being released last. The 3α,20β-HSD is active as a tetramer with four identical subunits (Blomquist, 1973, 1972; Hubner, 1963, Hubner *et al.*, 1959) and is commercially available. During the reduction process, the enzyme transfers hydride from NADH to various 3-oxo (Hyakawa, 1981) or 20-oxo (Sweet, 1980) steroids to give 3α- or 20(R) 20(β)-hydroxysteroid derivatives, respectively. The enzyme has wide substrate specificity for pregnanes, doing redox chemistry at the C-20 position of progesterones, pregnenelones, 21-deoxycortisones, and A ring saturated pregnanes as well as the originally described cortisones (Kawamura *et al.*, 1980; Hyakawa *et al.*, 1981).

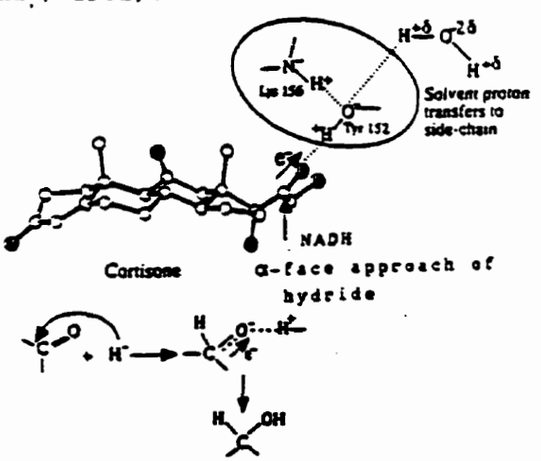


Figure 1.1 Proposed mechanism for stereospecific hydride transfer and proton relay during 20-keto to 20β-hydroxyl conversion (Duax and Ghosh, 1997).

The observed three-dimensional architecture of the catalytic cleft suggests the conserved Tyr152 and Lys156 residues to be regarded as having a primary role in the hydride and proton transfer reaction in SDRs (Duax and Ghosh, 1997). Figure 1.1 illustrates a possible mechanism by which the conserved residues and solvent molecules in the catalytic cavity can catalyze the reaction during 20-keto to 20β-hydroxyl conversion (Duax and Ghosh, 1997).

Glycyrrhizic acid and its metabolite carbenoxolone have been found to be very potent competetive inhibitors of 3α,20β-HSD (Ghosh *et al.*, 1992). X-Ray crystallographic analysis of the complex of carbenoxolone and 3α,20β-HSD at 2.2A resolution revealed the hemissuccinate side chain of carbenoxolone forms a hydrogen bond with the hydroxyl group of the conserved Tyr152 and occupies the position of the nicotinamide ring of the cofactor (Ghosh *et al.*, 1994).

The biosynthetic interrelationship among all of the steroid hormones has important implications for hormonal diseases. A number of clinical situations exist in which steroid hormones, either in normal amounts, or when produced in excess, have a role in the pathogenesis of the disease. For example, a 17β-hydroxysteroid dehydrogenase deficiency is an inborn error of metabolism, which causes a known form of male pseudohermaphroditism (Anderson *et al.*, 1996). The severity and unique characteristics of this disorder were indicative of a specific deficiency in testicular testosterone synthesis described originally by Saez *et al.* (Saez *et al.*, 1972, 1971).

Another interesting biological feature related to these steroids is the similarity between the oxidative biosynthetic enzymes involved in their production. Many are cytochrome P-450 enzymes which in each case utilize a cysteine bound heme and molecular oxygen to effect

hydroxylations and other oxidative reactions. One of the cytochrome P-450 enzymes, aromatase, attracted the interest of many scientists of diverse diciplines. Four international conferences solely dedicated to this enzyme have been held, in 1981, 1985, 1992, and 1996. The published proceedings [*Cancer Research (Suppl.)*, 1982; *Steroids*, 1986; *J. Steroids Biochem. Mol.*, 1993] of the First 1981, Second 1985, and Third 1992 International Aromatase Meetings provide a comprehensive account of the broad range of research interest that have been pursued in the aromatase field.

1.2.0 ESTROGEN SYNTHETASE (AROMATASE)

1.2.1 Aromatase and Breast Carcinoma

Estrogens are known to promote the growth of specific tumours such as breast, prostate, and uterine (Foye, 1995; Brodie, 1985; Johnston, 1984; Bradlow, 1982), which indicates that some tumors retain the mechanism of hormone-dependent transcription of target genes. It ultimately leads to cell proliferation (Dickson and Lippman, 1987; Tsai *et al.*, 1989). Certain forms of cancer of the breast in females have been described. In treating neoplastic disorders the significant part played by estrogen blockade has stirred interest among those related to the solving of the problem of cancer in women.

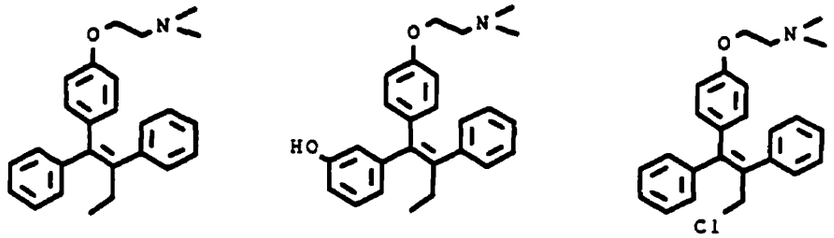
Breast cancer is one of the two leading causes of death by cancer in women. Approximately 183,000 cases of breast cancer in women are diagnosed each year in the United States. In almost 2/3 of the breast cancer patients, their tumors contain estrogen receptors. About 50% of metastatic breast cancer is estrogen receptor positive. Between 50-70% of those patients showing estrogen receptor positive breast cancer respond to hormone therapy. Patients with breast cancer that contain both estrogen and progesterone receptors have indicated an even higher

response rate of approximately 75-80%. Approximately, 1/3 respond to endocrine therapy. Improvement in the correlation of hormone therapy responsiveness among breast cancer patients has been brought about by the introduction of steroid receptor assays.

Pathologic conditions also treated by anti-estrogen therapy include: endometrial carcinoma, endometriosis, fibrocystic disease, male pubertal gynecomastia, prostate cancer, and idiopathic oligospermia.

The inhibition of estrogen action by therapeutic intervention is centered on two approaches: (1) the development of estrogen receptor antagonists, i.e. antiestrogens; or (2) lowering the estrogen levels *in vivo*.

Recent studies (Webster *et al.*, 1988) have suggested that the antiestrogen receptor complexes are not able to stimulate transcription. Models *in vivo* have demonstrated that antiestrogens affect tumor cells directly as well as result in reductions in circulating estrogen and prolactin (Jordan *et al.*, 1980). In estrogen



E/Z isomers
Tamoxifen

Droloxifene

Toremifene

Scheme 1.8 Chemical structure of tamoxifen, droloxifene, and toremifene: the anti-breast cancer drugs.

receptor antagonist development, the best established compound is the stilbene derivative, tamoxifen (ICI 46,474), Bardin and Catterall, 1981) as well as its two analogues droloxifene (Jordan *et al.*, 1977) and toremifene (Kalio *et al.*, 1986), (Scheme 1.8).

Tamoxifen is a weak agonist as well as antagonist. So, the benefit of this antagonist may not be long lasting. Eventually breast tumours may resist the drug (Early Breast Cancer T.C.G., 1992). This may lead to the disease recurrence in the patients. The agonist (estrogenic) activity of the drug tamoxifen is believed to result in side effects which include tumour flares and vaginal cornification.

The second approach, lowering the estrogens levels *in vivo*, may be achieved by endocrine ablation or medicinal therapy. A century ago, pioneering effort in surgical therapy (oophorectomy) led to improved outcomes in breast cancer patients (Beatson, 1896).

A variety of surgical procedures have been employed to reduce estrogen production, i.e. ovariectomy, adrenalectomy, hypophysectomy (Schneider *et al.*, 1994). It is noted that adrenalactomy diminishes the estrogen precursor, androgens, levels. The hypophysectomy procedure is used to prevent the formation of ACTH which is an estrogen production stimulator (Namer, 1989). Morbidity and occasional mortality can occur with the surgical procedure.

Also, estrogen syntheses take place in peripheral tissue such as fat, muscle, liver, brain, and breast tissue as well as in gonads, adrenal, and placenta. Peripheral aromatization becomes the main source of estrogen production in women after menopause. This increases with age. For example, synthesis in the muscle and adipose tissue contributes to the total production of estrogens in 25-30% and 10-15%, respectively. Therefore, traditional surgical procedures are limited

as complete approaches to removing endogenous estrogen in breast cancer treatment, as significant reduction of estrogen may continue in peripheral tissue.

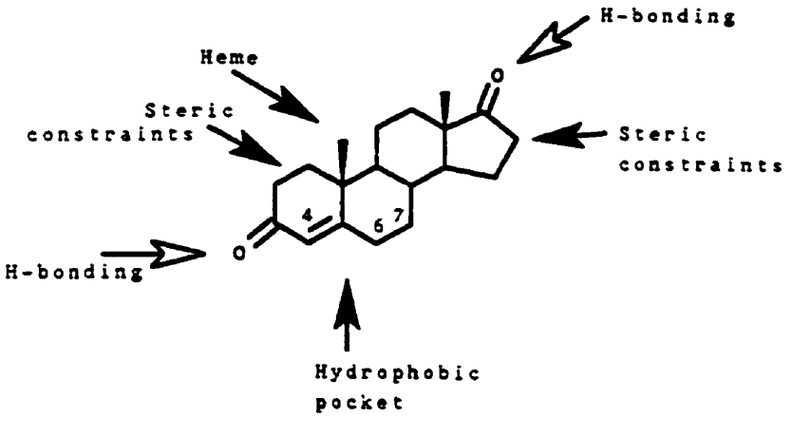
Alternative approaches to reduce estrogen levels, however, are gaining interest. Considerable attention to inhibition of aromatase, which catalyses the final step of estrogen production, (i.e. the conversion of steroidal androgens to estrogens) has been a principle target of inhibition study.

1.2.2 Aromatase-Substrate Binding Forces.

Illustration of the structural details of the active-site model involved in substrate-enzyme or inhibitor-enzyme interactions can provide insight into enzyme action and help to determine the structural complementarity of the substrate (inhibitor) and the enzyme, which is crucial for the design of effective inhibitors. In general, binding forces involved in substrate (inhibitor)-enzyme interactions include, dipole moment, electrostatic bonding, hydrogen bonding, hydrophobic bonding, van der Waals forces, and London dispersion forces. Besides non-covalent bonding, covalent bonding is also found in inhibitor-enzyme interactions. Covalent bonding is necessary for irreversible mechanism based (suicide) inhibitors. One of the most striking features of aromatase inhibitors is their structural variety, from substrate-like molecules such as 4-hydroxyandrost-4-ene-3,17-dione **1.63** (Scheme 1.22), (Brodie *et al.*, 1977) to others such as the glutarimide derivative, rogletimide **1.40** (Scheme 1.15), (Laughton *et al.*, 1990), which bear little resemblance to a steroid.

Scheme 1.9 shows elements of the active-site geometry of aromatase deduced from experimental data (Laughton *et al.*, 1993). Based on these

data, a lack of tolerance of increased steric bulk at positions C-1 and C-2 is found. In the region C-4, C-6, and C-7 a hydrophobic pocket is observed. The substrate binding pocket appears to give a tight fit in the region of the D-ring, based on the poor binding steroid in derivatives with the D ring opened (Laughton and Neidle, 1990; Abdul-Hajj, 1986; Brueggemeier *et al.*, 1978). However, the same study also indicated that interactions of the enzyme active-site with the C-17 substituent appeared to have limited importance.



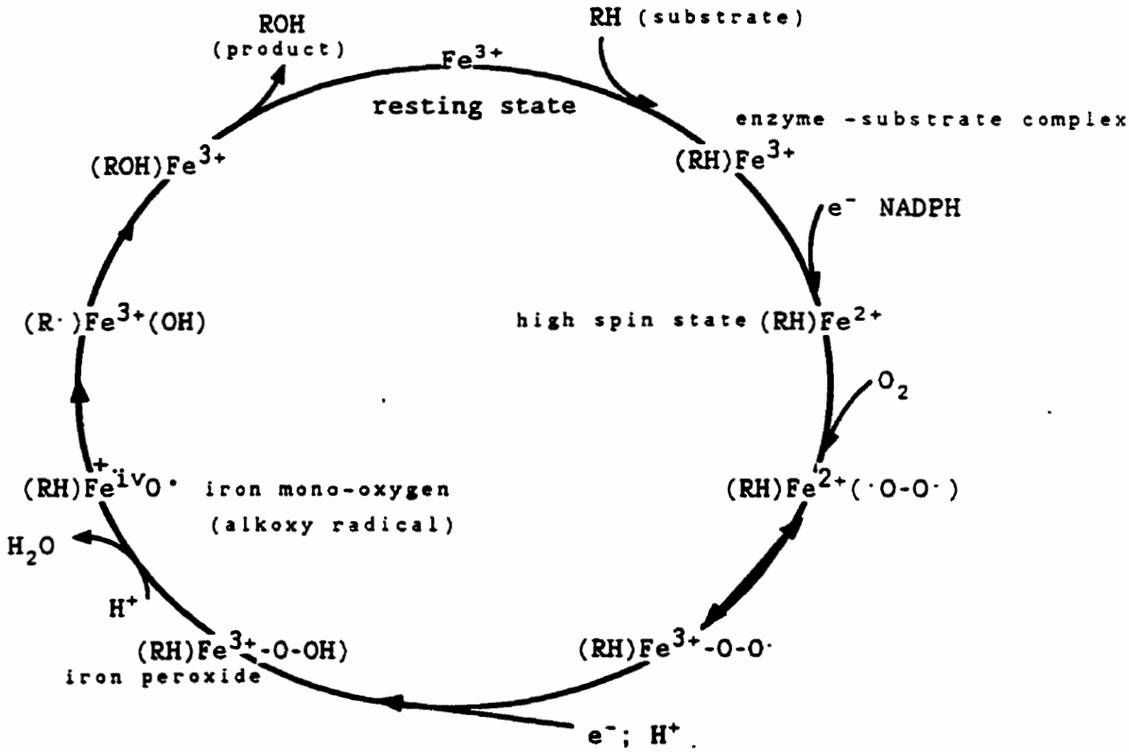
Scheme 1.9 The active-site geometry of aromatase, deduced from experimental data (Laughton *et al.*, 1993).

The orientation of the heme group relative to the steroid has been inferred from the sites of oxidation (Cole and Robinson, 1989), from the contribution of the C-19-methyl group to the inhibition of cyanide binding to the heme (Kellis and Vickery, 1987), and from the structures of inhibitors that interact directly with the heme group (Kellis *et al.*, 1986; Wright *et al.*, 1985)

On the basis of the these observations, it has been predicted that the heme is positioned so that the distance of the heme-iron atom from atoms C-1, C-2, and C-10 of the steroid will be about 7A, and the angle between heme and the steroid A-ring mean planes will be about 45° (Kellis and Vickery, 1987).

1.2.3 Aromatase Mechanism

Aromatase belongs to the cytochrome P-450 enzyme family. It functions with flavoprotein NADPH-dependent reductase. Much has been learned, about the structure and function of cytochrome P-450 enzyme



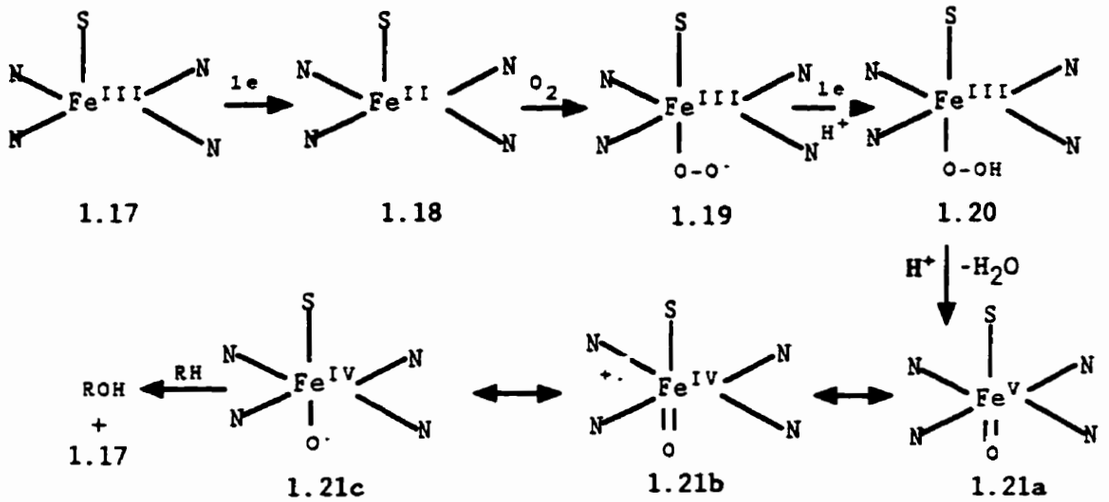
Scheme 1.10 Postulated cytochrome P-450 cascade of hydroxylations (White and Coon, 1980).

systems, from mechanistic studies of bacterial camphor hydroxylase, liver microsomal detoxifying enzymes, and metalloporphyrin model systems (Ortiz de Montellano, 1986). The main monooxygenation reactions performed by the cytochrome P-450 active-oxygen species are indicated in Scheme 1.10 (White and Coon, 1980; Mansuy *et al.*, 1989).

Aromatase, in its resting state (in the absence of substrate), exists in equlibrium with two forms of cytochrome P-450: a hexacoordinate low-spin iron(III) complex bearing two axial ligands and pentacoordinate high-spin iron(III) complex with one ligand. The hexacoordinate (octahedral) heme iron (Fe^{3+}), in the low-spin state (one unpaired electron), is surrounded by the following ligands: the four nitrogens from pyrrole rings of the porphyrin, one thiolate from a cysteine residue, and one water molecule. The thiolate and water molecule are in an axial orientation. The pentacoordinate high-spin iron(III) complex exists with cysteine as the only axial ligand. Upon substrate binding, $(RH)Fe^{3+}$, which occurs, in general, on a protein-binding site close to the heme, the equilibrium is shifted toward the pentacoordinated iron (square pyramidal). The sixth ligand, i.e. water molecule, is expelled (presumably for steric reasons or entropy driving forces). Simultaneously, the heme iron becomes high spin (five unpaired electrons) and this change is accompanied by a characteristic UV-absorption spectrum change of the Soret band maximum from about 420 nm to about 390 nm. The high spin iron possesses a larger atomic radius and can no longer fit into the centre of the porphyrin.

In the presence of NADPH and the reductase, the iron (Fe^{3+}), in the high-spin enzyme-substrate complex is reduced by one electron to give the high-spin pentacoordinate Fe^{2+}-complex. It is thought that the

rate of reduction is dependent upon the high spin-low spin equilibrium discussed above, with the high spin form favouring reduction. The high-spin pentacoordinate Fe^{+2}-complex is able to bind various ligands such as CO, isocyanates, nitrogenous bases, phosphines, and dioxygen. Binding of dioxygen leads to relatively stable hexacoordinate low-spin complex, $(RH)Fe^{2+}(O_2)$ (Scheme 1.10). This O_2-Fe interaction has been



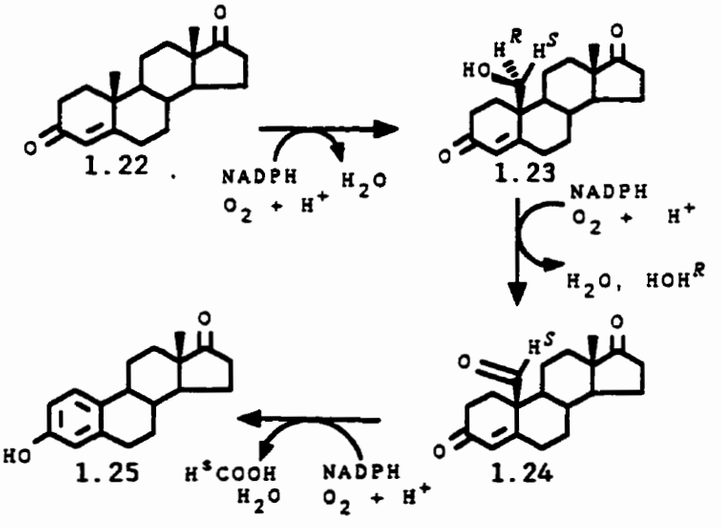
Scheme 1.11 The $Fe^{III}OOH$ species formed by the sequence **1.17-1.21** is converted into the oxo derivative **1.21**.

formulated as involving either unreduced oxygen or superoxide (with Fe^{3+}) as the available evidence is consistent with either formulation. Next, a second electron is transferred to the iron (making one full-equivalent of NADPH per cycle) followed by a proton addition to yield the iron peroxide intermediate, $(RH)Fe^{+3}$-OOH. Thereafter, fragmentation of this unstable intermediate by cleavage of the O-O bond in the peroxide furnishes an iron-oxo species, $(R^{\cdot})Fe^{IV}O^{\cdot}$. This

iron-oxo species is believed to perform the hydroxylation of substrate.

The iron-oxo species (Scheme 1.11, **1.21a-1.21c**) are considered to have the Fe^{4+} porphyrin radical cation oxidation state based on analogy to the peroxidases and catalases where a number of physical techniques (EPR, ENDOR, EXFS, NMR, Mossbauer, magnetic susceptibility, MCD, UV) has been used to determine the oxidation state of the iron (for reviews see Akhtar and Wright, 1991; Paterson and Prough, 1986).

The intramolecular redox reaction **1.21a** → **1.21b** involved in the formation of the radical cation **1.21b** is presumably aided by the greater stability of Fe^{IV} over Fe^{V}. The $(\cdot^{+})Fe^{IV}\text{-}O\cdot$, **1.21c**, behaves like an alkoxy radical and participates in the hydroxylation reaction *via* a free radical mechanism, as shown by the formation of both racemic and rearranged products. The aromatase reaction is shown in Scheme 1.12.



Scheme 1.12 Conversion of androst-4-ene-3,17-dione to estrogen by human placental aromatase: elimination of the 19(pro-*R*)-H as H_2O and the 19(pro-*S*)-H as HCOOH (Akhtar *et al.*, 1982).

Since 1955, when Meyer (Meyer, 1955) first showed that 19-hydroxy-androstenedione **1.23** had been converted to estrone, many investigators have pursued mechanistic studies of the aromatase reaction. A review of all the studies reported to date on the aromatase mechanism will not be attempted. For a comprehensive description of mechanistic studies, the reader is refered to several reviews (Akhtar and Wright, 1991; Ortiz de Montellano, 1986; Geuengerich and MacDonald, 1984; Ruckpaul and Rein, 1984; White and Coon, 1980). However, a short introduction to the aromatase mechanism will be presented in this chapter.

Ryan first reported that the microsomal fraction of human placenta cells can be used to study the aromatase reaction (Ryan, 1958). The conversion of androgen **1.22** to estrogen **1.25** is a process comprised of three steps as shown in Scheme 1.12. The stoichiometry of the process was established by Thompson and Siiteri (Thompson and Siiteri, 1975), who showed that, in the overall aromatization reaction that forms estrogens, three molecules of molecular oxygen and six reducing equivalents from NADPH were consumed. Experiments performed with microsomal (membrane-bounded placental preparations) initially suggested that the three aromatase monoxygenation reactions occurred at a single active site. This suggestion was supported by results obtained with purified aromatase which showed that androst-4-ene-3,17-dione, 19-hydroxyandrost-4-ene-3,17-dione, and androst-4-ene-3,17-dion-19-al were all aromatized by a 55 kDa protein that migrated as single band on an SDS-polyacrylamide gel. Aromatase, $P\text{-}450_{arom}$, catalyzes a sequence of two carbon hydroxylations. The first 19-methyl hydroxylation affords the 19-hydroxy intermediate **1.23** (Meyer, 1955). The reaction occurs with retention of configuration and is accompanied with a normal kinetic isotope effect ($k_H > k_D > K_T$),

(Caspi *et al.*, 1986).

During the second hydroxylation, the 19-hydroxy intermediate **1.23** is oxidized to the 19-aldehyde **1.24**, and the 19-pro-R hydrogen is lost into the water medium (Arigoni *et al.*, 1975; Osawa *et al.*, 1975). No isotope effect was observed. Moreover, Akhtar *et al.* (Akhtar *et al.*, 1986) showed that the aldehyde oxygen atom was derived solely from the first mole of oxygen consumed, based on studies with ^{18}O which revealed that there was no label incorporation into androst-4-ene-3,17-dion-19-al during the second hydroxylation. The above oxidation process has been rationalized either by invoking stereospecific dehydration of the 19-gem-diol intermediate or by invoking direct hydrogen abstraction from a 19-carbinol radical species. The results of studies by Covey and co-workers (Bensen *et al.*, 1986; Covey, 1987) are consistent with the former pathway, while experiments by Wright and Akhtar (Akhtar *et al.*, 1981) are consistent with either process. Duax and Osawa (Duax and Osawa, 1980) suggested that conformational features of 19-hydroxy-androst-4-ene-3,17-dione might contribute to the stereospecificity of its hydroxylation. Their crystallographic studies of 19-hydroxy-androst-4-ene-3,17-dione. established that the 19-hydroxy group is located over the steroid A-ring in the solid state. In the light of the recently proposed model by Oh and Robinson, (Oh and Robinson, 1993) this solid state confirmation supports the accepted enzyme-bound conformation required to effect stereoselective removal of the 19-pro-R hydrogen.

In the third step, the C-10-C-19 bond is oxidatively cleaved to give estrogen, formic acid and water. Oxygen atoms each of the first and third moles of oxygen consumed, as well as the 19-pro-S hydrogen, are incorporated into the formic acid (Scheme 1.12), (Akhtar *et al.*,

1982). In the case of androst-4-ene-3,17-dione and its C-19 oxygenated analogues, the final oxidative reaction involves stereospecific removal of the 1β- and 2β-hydrogens (Brodie *et al.*, 1969; Fishmann and Guzik, 1969). The situation for 17β-hydroxy androgens, testosterone and its 19-aldehyde analogous is different. In these, only the 1β hydrogen atoms are abstracted stereospecifically while the C-2 hydrogen atoms are eliminated non-stereospecifically. The 2β or 2α hydrogen atoms are lost in variable amounts of up to the 6:5; 2β-H:2α-H ratio (Cole and Robinson, 1988).

Any mechanism proposed for the third step must accommodate the loss of the 1β- and 2β-hydrogens and incorporation of two oxygen atoms from molecular oxygen into the formate released. Several mechanisms for this step have been postulated and examined. Some of them were consistent with the isotopic labeling data and they are discussed below.

One of the earliest theories was proposed in the 1960's by Townsley and Brodie (Townsley and Brodie, 1968). It involved 1β-hydroxylation and subsequent fragmentation. Early support for this mechanism came from the fact that 19-norandrostene-3,17-dione was 1β-hydroxylated by placental microsomes. However, this mechanism was not able to explain the incorporation of an isotopic oxygen into the formic acid. Later, it was modified by Akhtar *et al.*, who proposed the formation of a hemiacetal (Akhtar *et al.*, 1981). Although this mechanism accounts for all the isotopic labeling data, it is not considered likely because formation of the four-membered ring hemiacetal would be energetically unfavourable.

One of the mechanisms involved water attack on the 19-aldehyde with subsequent C-10-C-19 fragmentation and elimination of water to afford

formic acid plus estrogen (Townsley and Brodie, 1968) However, the discovery that an atom from the third equivalent of O_2 was incorporated into formic acid weakened this proposal.

In the early 1970's, Hosda and Fishman (Hosda and Fishman, 1974) proposed a theory based on the formation and non-enzymatic aromatization of 2β-hydroxyandrost-4-ene-3,17-dion-19-al. Indeed, 2β-hydroxyandrost-4-ene-3,17-dion-19-al was found to undergo rapid transformation to estrone in pH > 6 aqueous buffer, in contrast to 2α-hydroxyandrostene-3,17-dion-19-al which was shown to be stable. That the 2β-hydroxy compound did not form estrone, in non-aqueous solvent, led to the initial suggestion that the 19-aldehyde function was attacked by water and the resultant hydrate subsequently fragmented to estrone and formic acid. But, when Akhtar *et al.* (Akhtar *et al.*, 1982) showed that formic acid eliminated in the aromatization step contains the first and third oxygen atoms consumed in the catalytic sequence, Hahn and Fishman (Hahn and Fishman, 1984) modified their postulated mechanism. Akhtar's experiment led them to the postulate that intramolecular addition of the 2β-hydroxyl to the 19-aldehyde moiety to give hemiacetal precedes rupture of the carbon-carbon bond.

Other experimental results, which were consistent with the 2β-hydroxylation mechanism included the following: (1) 1β hydrogen losses during non-enzymatic aromatization of 2β-hydroxy-androst-4-ene-3,17-dion-19-al, as it was observed upon aromatase reaction with its substrate; (2) isolation of tetrol derivative of 2β-hydroxyandrost-4-ene-3,17-dion-19-al (formed by *in situ* NaB^3H_3CN reduction) in 0.13% yield from androst-4-ene-3,17-dione in microsomal incubations; (3) inhibition of aromatase activity, in such an incubation, that 2β-hydroxyandrost-4-ene-3,17-dion-19-al was trapped

with monoclonal antibodies. Thus, the 2β-hydroxylation theory was valid for over ten years (1972-1984).

In 1984, Caspi and his co-workers (Caspi *et al.*,1984) synthesized 2β-^{18}O-hydroxyandrost-4-ene-3,17-dion-19-al, and subjected it to aromatization in the presence and absence of microsomal aromatase. In neither case was the formate found to contain ^{18}O. These results indicated that, although 2β-hydroxyandrost-4-ene-3,17-dion-19-al is formed by microsomal aromatase, it is not an intermediate of the dominant aromatase reaction pathway indicated by ^{18}O labeling experiments. These findings have been challenged, and results from similar experiments showing ^{18}O incorporation into formate from collapse of an isotopically labeled 2β-hydroxy group of 2β-hydroxyandrost-4-ene-3,17-dion-19-al have been presented (Fischman and Hahn, 1987). Recently, in a revised experiment, Caspi *et al.* showed that aromatization of androgens, indeed involves the intermediacy of 2β-hydroxyandrost-4-ene-3,17-dion-19-al (Caspi *et al.*, 1993). Thus, incubation of 2β-[^{18}O]-hydroxyandrost-4-ene-3,17-dion-19-al with placental aromatase gave HC^{18}OOH. Although the results of the experiment were rather scattered (10-91% incorporation), they showed that the 2β-^{18}OH was incorporated in the eliminated formic acid. This incorporation of isotopic oxygen in the formic acid is consistent with the view that one of the alternative routes of estrogen conversion may involve a 2β,19-dioxygenated androgen.

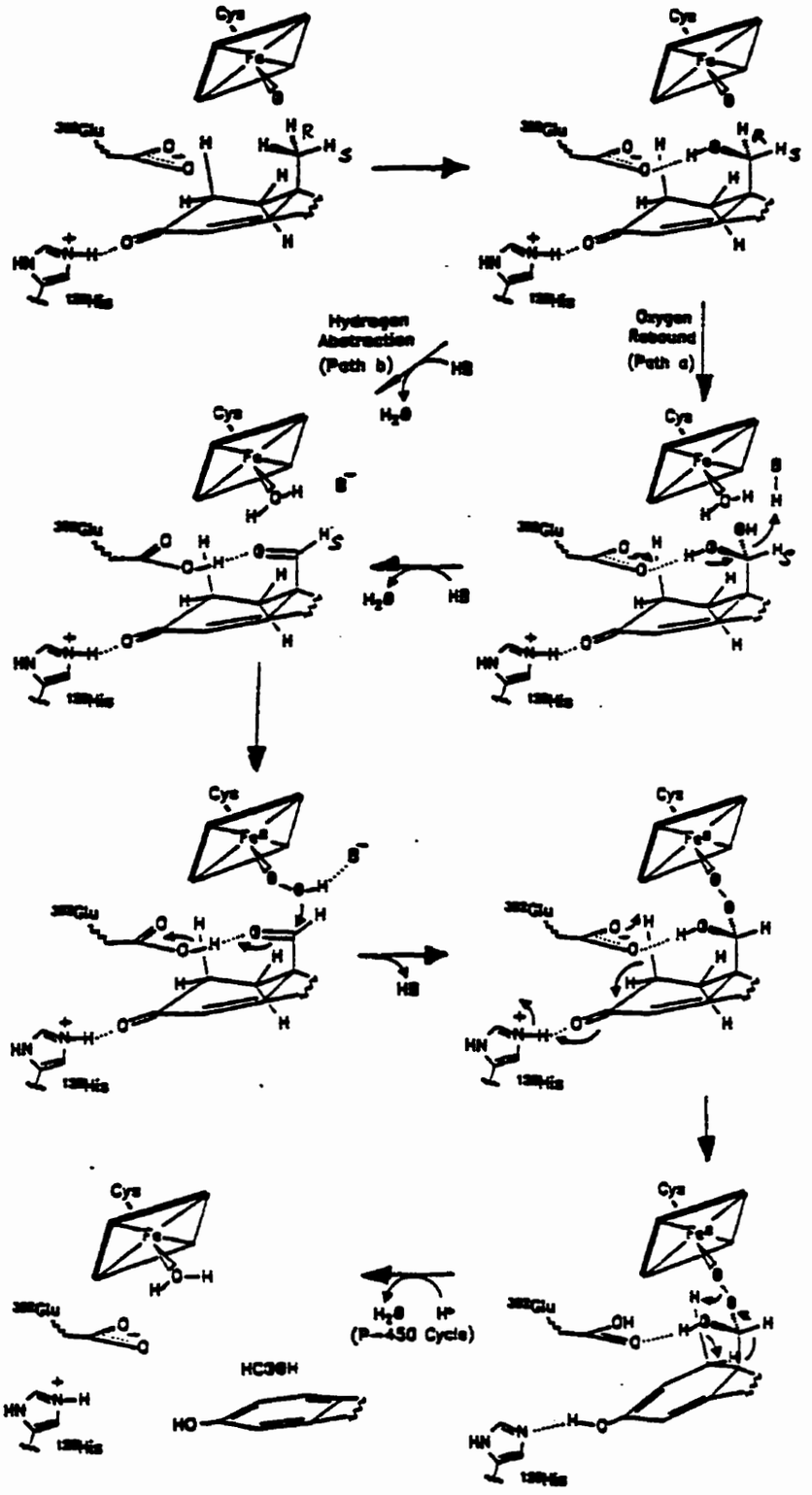
Alternative mechanisms of aromatization have been excluded. These involved 4,5 epoxidation (Morand *et al.*, 1975), 1β-hydroxylation (Townsley and Brodie, 1968), Baeyer-Villiger oxidation at C-19 (Akhtar *et al.*,1982), 10β-hydroxyestr-4-ene-3,17-dione production or Schiff base formation through the 3-keto function that involves water attack

on the 19-aldehyde group.

A theory originally proposed by Akhtar *et al.* invoking nucleophilic attack of an iron peroxy intermediate on the 19-aldehyde intermediate to give a peroxide species is the only one that has remained consistent with experimental data and P-450 mechanistic theory (Akhtar *et al.*, 1976). Thus, the attack of an enzyme bound ferric peroxide ($Fe^{+3}OOH$) on the 19-aldehyde group produces the geminal hydroxyferric peroxide. It was also suggested that the unstable intermediate is able to collapse to estrogen and formic acid by abstraction of 1β-H (Stevanson *et al.*, 1985), hydride shift (1β-H) (Akhtar *et al.*, 1982), or free radical elimination (1β-H·), (Stevanson *et al.*, 1988).

Support for a peroxide mechanism which may promote an acyl-carbon cleavage is also provided by other findings. First, in model studies, Cole and Robinson have shown that the decomposition of a synthetic 19-hydroxy-19-peroxide leads to the formation of an aromatic compound (Cole and Robinson, 1988). Second, the work of Roberts *et al.* and Vaz *et al.* examined the metabolism of a large number of aliphatic aldehydes with eight different drug metabolizing P-450 enzymes (Roberts *et al.*, 1981; Vaz *et al.*, 1991).. It was found that four of these were capable of catalyzing the acyl-carbon fission, in the presence of H_2O_2, as exemplified by the conversion of cyclohexylaldehyde into cyclohexene. Third, Ranjith *et al.*, during their studies on aromatase, have isolated minor products whose origin could be explained from the decomposition of a C-19 peroxy species (Ranjith *et al.*, 1993).

Recently Oh and Robinson (Oh and Robinson, 1993) presented a detailed proposal for aromatase action based on Akhtar's peroxide mechanism (Stevanson *et al.*, 1988), (Scheme 1.13).



Scheme 1.13 Proposed mechanism of aromatization of androstanedione to estrone by Oh and Robinson (Oh and Robinson, 1993).

1.3.0 AROMATASE INHIBITION

Aromatase, P-450$_{arom}$, requires three general catalytic cycles to complete the full conversion of androgens to estrogens. Each of these cycles is particularly vulnerable to inhibition: (1) the binding of the substrates, (2) the binding of molecular oxygen subsequent to the first electron transfer and (3) each of the catalytic steps in which the substrate is actually oxidized.

Compounds that inhibit cytochrome P-450$_{arom}$ can be divided into three mechanistically differentiable categories: (1) compounds that bind reversibly (Type I), (2) compounds that form quasi-irreversible complexes with the heme iron atom (Type II), and (3) compounds that bind irreversibly to the portion or heme group or that accelerate degradation of the prosthetic heme group without demonstrably binding to it.

Inhibitors that interfere in the catalytic cycle prior to the actual oxidative event are reversible, competitive or noncompetitive inhibitors. Compounds that act during or subsequent to the oxygen transfer step are generally irreversible or quasi-irreversible inhibitors. The latter are referred to as mechanism-based, suicide or k_{cat}, inhibitors.

Inhibitors that compete reversibly with substrates for occupancy of the active site include substances that bind to its hydrophobic domain, coordinate to the prosthetic heme iron atom, participate in specific hydrogen bonding or participate in ionic interactions with specific active-site residues. Mechanism-based inhibitors are potentially more enzyme-specific than reversible inhibitors, because the inhibitor must first bind to the enzyme, i.e. (1) it must be recognized and accepted by enzyme, (2) it must then be catalytically activated, and finally,

(3) a reactive species is produced which irreversibly alters the enzyme, and thereby removes it permanently from the catalytic pool.

Three classes of the catalysis-dependent irreversible inhibitors of $P\text{-}450_{arom}$ can be considered:

(1) compounds that bind covalently to the protein;

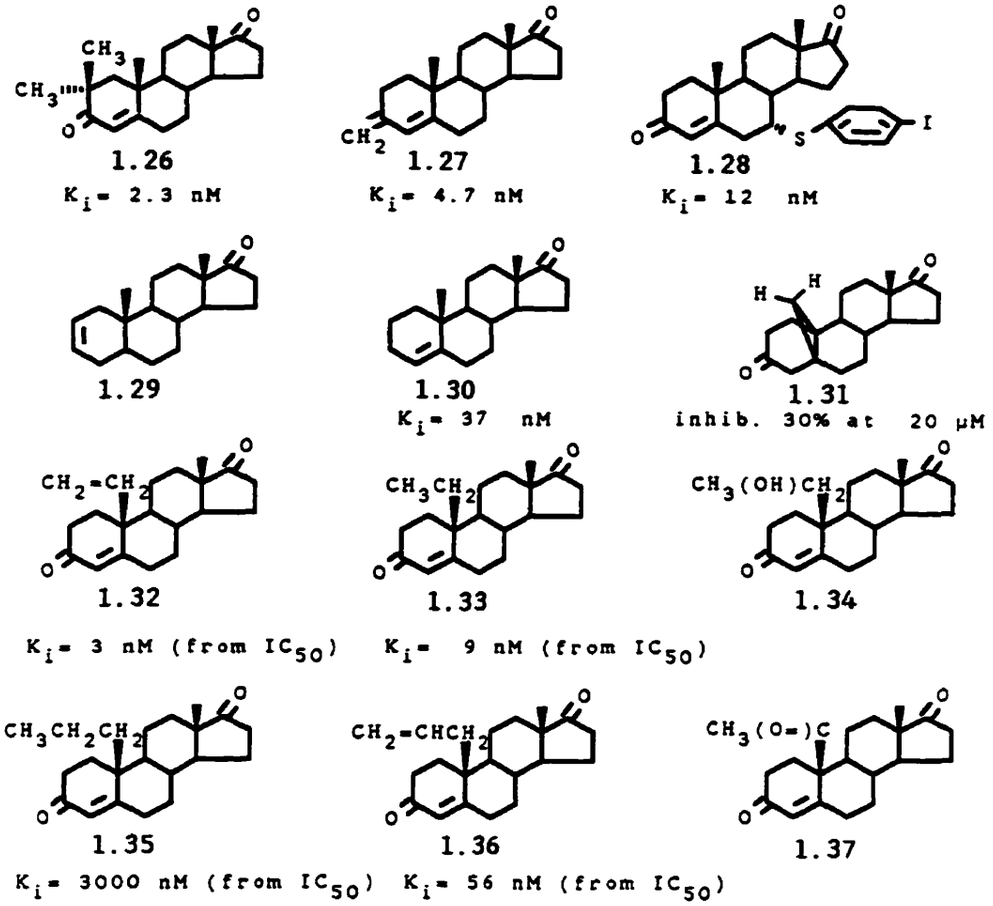
(2) compounds that quasi-irreversibly coordinate to the prosthetic heme iron, and

(3) compounds that alkylate or degrade the prosthetic heme group.

Many non-steroids and steroids have been designed, synthesized, and tested as aromatase inhibitors. However, this discussion will be focused on compounds where the mechanism of interaction has, at least partially, been explored, those that show exceptional inhibitory properties and those that show clinical promise.

1.3.1 Type I, Competitive inhibitors.

Useful inhibitors would be compounds that bind reversibly to the active site, heme, of aromatase as steroid substrate analogues, and which do not dissociate very rapidly. In this way, selective inhibition of aromatase may be achieved by compounds which interfere with androgen aromatization by binding to the enzyme. Such compounds produce Type I high-spin spectra, e.g. changes in the UV-absorption spectrum Soret band of the enzyme-bound heme. As mentioned in the aromatase mechanism chapter, this phenomenon is characteristic for cytochrome P-450 enzymes. A Type I complex induces a shift in the Soret band maximum from about 420 nm to about 390 nm. For this class of inhibitor, the lower the apparent K_i or IC_{50}, the better the inhibitor. This relationship is not necessarily easily extended to drug utility where such considerations as metabolism, bioavailability, toxicity, distribution, and synthetic expense become critical.



Scheme 1.14 Type I, steroidal competitive inhibitors of aromatase.

Many compounds were synthesized and evaluated as Type I competitive inhibitors and some examples are presented in Scheme 1.14, which include 2,2-dimethylandrostene-3,17-dione **1.26** (Furth *et al.*, 1990), 3-methylene-androst-4-en-17-one **1.27** (Miyairi and Fishman, 1986), 7α-thio-(p-iodophenyl)-androstene-3,17-dione **1.28** (Brueggemeier *et al.*, 1987), 5,10β-cycloandrostane-3,17-dione **1.31**, 10β-vinylestr-4-ene-3,17-dione **1.32**, and 10β-ethylestr-4-ene-3,17-dione **1.33** (Marcotte and Robinson, 1982a,b).

As we can see, the K_i values (Scheme 1.14) are very close to the K_m of androstenedione (30 nM). Furthermore, it is interesting that all of the most potent inhibitors have similar K_i values, despite their different structures. Thus, the mode of binding of these competitive inhibitors may be somewhat different from that of the normal substrates, and therefore they are not processed readily.

The varied structures of the above inhibitors make it difficult to deduce key active site features. However, it seems that hydrophobic functionalities lead to increased affinities. For example, by simply increasing the 10β-ethyl chain length by one CH_2 group (as seen in **1.35**) there is an increase in the binding constant by factor of 300 (3.4 kcal/mol). Inserting a CH_2-group into the C-10/C-19 bond of the 10β-vinyl compound **1.32** (as in **1.35**) increases the K_i by a factor of 20 (1.8 kcal/mol), (Marcotte and Robinson, 1982).

Furthermore, the A ring conformation seems to play a critical role, as 5α-androst-2-en-17-one **1.29** is a weak binder (Schearzel *et al.*, 1973), whereas androst-4-ene-17-one **1.30** is a potent inhibitor (K_i= 37 nM), (Numazawa *et al.*, 1989). Compound **1.33** was evaluated initially as a competitive inhibitor of aromatase and later investigated as a substrate for the enzyme. The diasteromeric compounds **1.34**, which are potential monooxygenation products of compound **1.33** (19-CH_2CH_3) were examined as aromatase substrates. It was found that both compounds gave Type I binding difference spectra and are both converted to compound **1.37** (Beusen *et al.*, 1987). The latter result strengthens the idea that a hydrogen bond donor on the α-carbon atom of the side chain at C-10 is important for the subsequent monooxidation of substrate analogues by aromatase.

Compound **1.37** binds well to aromatase and gives a Type I binding

difference spectrum. Nevertheless, it is not oxidized by aromatase. It has been suggested that, because compound **1.37** is a methyl ketone, its rate of hydration to the corresponding *gem*-diol would be considerably slower than that of 19-oxoandrostenedione. Thus the compound may bind to the enzyme but be unable to form the *gem*-diol required for its further monooxidation. This could explain why even the enzyme-generated compound **1.37** is not further modified by aromatase.

The lack of enzyme inactivation by compound **1.36** can be explained by the tight binding of **1.36** to aromatase. Moreover, its C-10 side chain must bind in a such manner to preclude its oxidation (Marcotte and Robinson, 1982).

The structural modification of androst-4-ene-3,17-dione at C-3 gave the 3-methylene compound **1.27**. The compound was able to bind tightly to the enzyme, but it was prevented from oxidation at C-19 (Miyairi and Fishman, 1986). Another modified compound, a C-19 unsubstituted, 5,10β-cycloandrostane-3,17-dione **1.31**, showed only 30% inhibition at 20 μM concentration (Marcotte and Robinson, 1982b).

The 7α-substituted compounds are representative of potent competitive inhibitors of aromatase. For example, compound **1.28** has been shown to inhibit aromatase cultured MCF-7 human mammary cancer cells without exhibiting any estrogenic effects and also caused regression of dimethylbenzanthracene-induced estrogen dependent mammary tumors in rats (Brueggemeier *et al.*, 1987).

1.3.2 Type II, Bathochromic Shift Competitive Inhibitors.

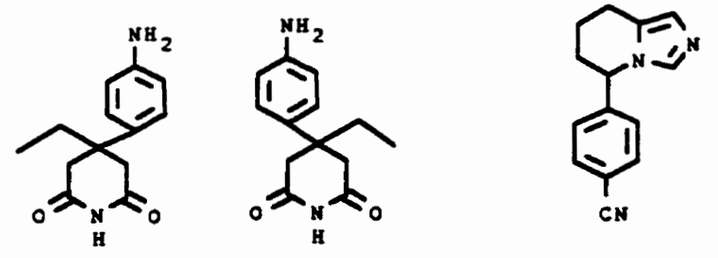
Type II competitive inhibitors are represented by compounds that contain suitably positioned heteroatoms, which are capable of

coordination to the heme iron of cytochrome P-450. This type of binding is reflected in Soret Band shift (bathochromic with respect to Type I binders). The precise Soret band displacement is often diagnostic of the heteroatom type (N, S, O, S^{-2}). The term, Type II, for convenience,is used to refer to all of the Fe-coordinating inhibitors, regardless of the nature of the heteroatom. However, not all heterocycles behave as Type II competitive inhibitors. The structural characteristics of an organic compound as well as the position of the heteroatom will determine the specificity of the interaction. Type II inhibitors are expected to behave as reversible inhibitors, although their K_m can be very low particularly for potent binders. Again this type of inhibitor is divided into two groups, the non-steroidal and steroidal inhibitors.

Aminoglutethimide (AG) **1.38** (Scheme 1.15) was the first non-steroidal aromatase inhibitor to be developed and marketed for the treatment of breast cancer (Santen *et al.*, 1981). Initially, this compound was introduced as an anticonvulsant but its use was restricted, when it was realized that the compound causes adrenal insufficiency. AG was found to be a competitive inhibitor of P-450 enzymes and interferes with desmolase, 11-hydroxylase, 18-hydroxylase, 21-hydroxylase, and aromatase (Chackraborty *et al.*, 1972). AG is a moderately potent aromatase inhibitor (K_i = 0.70 μM, IC_{50} = 44 μM) and exists as a mixture of D- and L-enantiomers. Uzgris *et al.* determined that the D-enantiomer was 30-fold more potent than the L-enantiomer for aromatase inhibition (Uzgris *et al.*, 1977). The aminophenyl nitrogen appears to play a key role for binding although it is not certain which nitrogen atom is actually coordinated to the iron. AG is now used to reduce estrogen formed in peripheral tissue by aromatization of adrenal

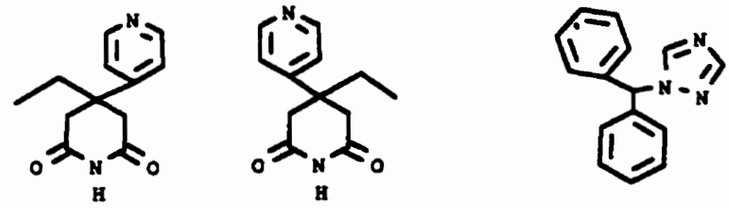
androgens in post-menopausal breast cancer patients. Hydrocortisone is given concomitantly for glucocorticoid replacement. AG produces objective disease remission to the same extend as surgical adrenalectomy, thus establishing it as a useful agent in breast cancer treatment (Santen *et al.*, 1981; Brodie and Santen, 1986).

Modifications of aminoglutethimide were also investigated to improve selectivity for aromatase inhibition while reducing the inhibition of other enzymes. Several other non-steroidal reversible aromatase inhibitors, more selective than aminoglutethimide, have been developed and now are in various stages of clinical evaluation, including fadrazole (CGS 16949A) **1.39**, rogletimide **1.40**, and triazole CGS 20267 **1.41** (Scheme 1.15), (Lang *et al.*, 1993).



L(-) **1.38** D(+)
Aminoglutethimide
Ki= 700 nM

1.39
Fadrazole
(CGS 16949A)
Ki= 1.5 nM

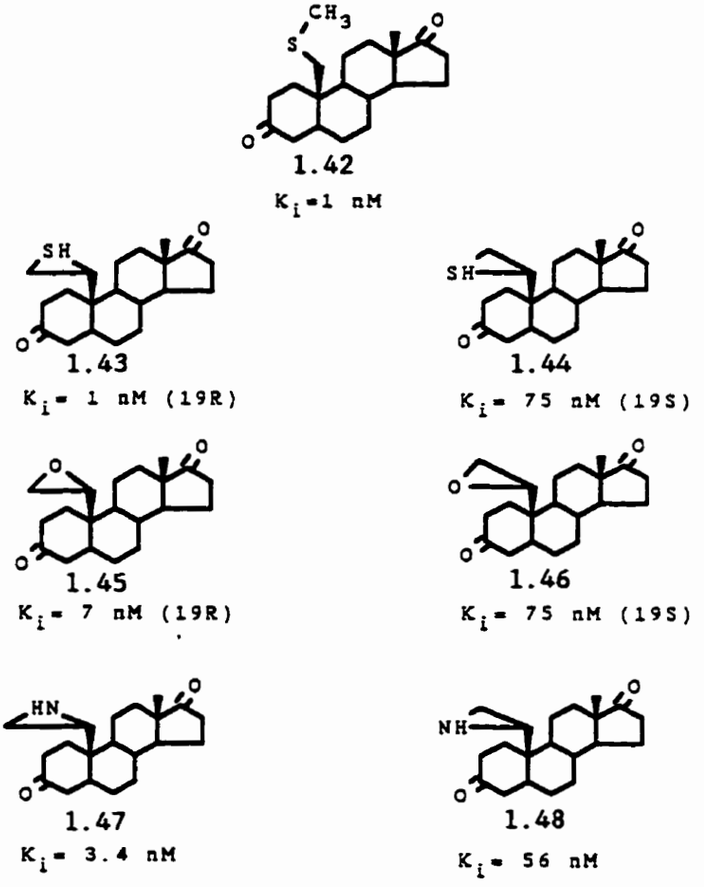


L(-) **1.40** D(+)
Rogletimide

1.41
CGS 20267

Scheme 1.15 Type II, non-steroidal competitive inhibitors of aromatase.

Compound **1.39** (CGS 16949A, K_i = 0.0015 μM) is a selective aromatase inhibitor with excellent potential as a new pharmaceutical agent. A comparison of its K_i value with that of D,L-aminoglutethimide shows that compound **1.39** is about 500 times more potent as an aromatase inhibitor. The compound is devoid of estrogenic, androgenic and anti-androgenic activities (Steele *et al.*, 1987).



Scheme 1.16 Type II, steroidal competitive inhibitors of aromatase.

Good competitive inhibitors (Type II) appear to contain two domains: an Fe-coordinating domain and a hydrophobic domain. The

structural requirements of the hydrophobic domain appear to be critical but the chemical details of the specificity are unknown. However, steroids containing heteroatoms appropriately placed near the heme iron might be expected to behave as Type II competitive inhibitors. Such a hypothesis has been successfully applied to inhibitors designed for the cholesterol side-chain cleavage cytochrome P-450 by Sheets and Vickery (Sheets and Vickery, 1982, 1983a,b). Several compounds of this type having high affinity to aromatase have been synthesized, Scheme 1.16, **1.42-1.48**. This class of compounds includes the 10β-CH_2SCH_3 compound (**1.42**) (Wright *et al.*, 1989) 10β-thiiranes (**43** and **1.44**), 10β-oxiranes (**1.45** and **1.46**) (Kellis *et al.*, 1987) and 10β-aziridines (**1.47** and **1.48**) (Nijar *et al.*, 1996, 1993) derivatives of androst-4-ene-3,17-dione. All of these compounds are competitive inhibitors of aromatase and show UV-absorption spectra that suggest Fe-heteroatom coordination.

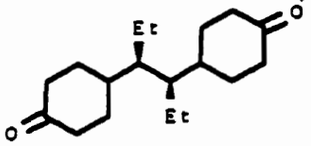
Compound **1.42** is a potent competitive inhibitor of aromatase. Spectroscopic studies have shown that the sulphur atom of this thioether coordinates with the iron atom of the heme group (Wright *et al.*, 1989, 1985). Presumably, the S-methyl group of the thiol sterically forces interaction of the sulphur atom with the heme iron. The Soret absorption band was shifted to 474 nm (indicative of a thiolate anion).

Thiiranyl (**1.43**, **1.44**), oxiranyl (**1.45**, **1.46**), and aziridinyl (**1.47**, **1.48**) compounds behave similarly. The interactions of the oxiranes and thiiranes with the heme of aromatase were characterized spectroscopically. The absolute spectra (e.g. not difference spectra) obtained with purified aromatase showed Soret peaks at 411 nm and 425 nm for the *R* diastereomers of the oxiranes and the thiiranes,

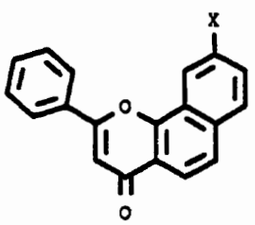
respectively. The tighter binding of the *R* diasteromers of the oxiranes and thiiranes, together with the finding from X-ray diffraction studies that the oxygen atom of the *R* oxirane is over ring A, has been interpreted to show that the heme is located close to C-1, C-2, and C-19. This suggestion is consistent with the finding from hydroxylations, which occurred at the C-1 and C-2 positions in metabolic switching studies, reported for 19,19,19-tritiated androstenedione (Osawa *et al.*, 1987a,b). These diastereomeric aziridines, **1.47** and **1.48**, represent the first 19-N coordinating aromatase inhibitors. Of the two aziridines, the 19(R)-10β-aziridine **1.47** is more potent (Nijar *et al.*, 1996).

1.3.3 Type Undetermined, Competetive Inhibitors.

For the inhibitors, presented in Scheme 17, difference spectra were reported. However, it is unclear whether binding in these compounds produces the Type I difference spectra found with androst-4-ene-3,17-dione. Thus, they will be discussed separately.



1.49



1.50 X = H 7,8-benzoflavone
1.51 X = OH 9-hydroxy-7,8-benzoflavone
K_i = 9 nM

Scheme 1.17 Non-steroidal competetive inhibitors (type undetermined).

Compounds **1.49**, **1.50**, and **1.51** are representative of those non-steroidal compounds that inhibit aromatase even though they do not contain a nitrogen atom to coordinate to the heme iron of the enzyme. The resemblance of the dicyclohexyl compound **1.49** to androst-4-ene-3,17-dione presumably accounts for its ability to bind to the enzyme (Sirett *et al.*, 1984).

The flavones **1.50** and **1.51**, which belong to a class of plant natural products, and some of their *in vivo* metabolites have also been tested as aromatase inhibitors. The [1,2-2'] naphthopyrone **1.50**, also called a 7,8 benzoflavone, is a potent competetive inhibitor of aromatase (K_i = 20 nM). Moreover, a structure-activity study of flavone derivatives showed that 9-hydroxy-7,8-benzoflavone **1.51** was an even more potent competetive inhibitor (K_i = 5 nM). A comparison of molecular models of the hydroxylated α-naphthoflavone derivative and testosterone had led to the proposal that the 7,8-benzochromone ring system of compound **51** is bound in the aromatase active site similar to the steroid ring system of testosterone (Kellis *et al.*, 1986)

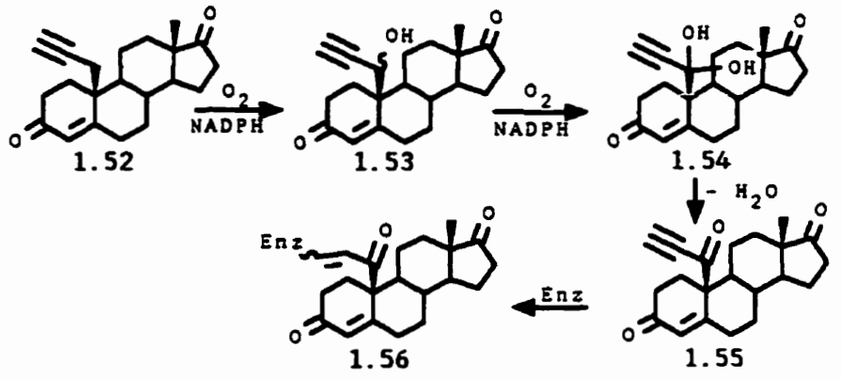
1.3.4 Mechanism-Based (Suicide) Inhibitors.

Because substrates for aromatase have very low K_m values (*ca* 30 nM), even competitive inhibitors with very low K_i values must be used at high dose levels. Thus, in the search for increased efficacy as well as specificity, the attention of scientists was directed to the development of suicide or mechanism-based inhibitors. Such compounds contain a latent electrophilic group, which is intended to be activated by the target enzyme and can provide high specificity. The irreversible nature of the enzyme inactivation process resulting from covalent modification at the active-site provides several advantages. Firstly, mechanism-based irreversible inactivation of aromatase is much

more attractive from a clinical point of view than competetive inhibition because of much lower intracellular drug concentractions. Secondly, as aromatase has a low turnover rate its destruction means that the inhibitor's effect can persist after its removal from the body. Finally, such compounds can serve as valuable mechanistic probes and as active-site mapping agents. The lack of availability of experiments with purified and radiolabelled inhibitors with high specific activities has made it difficult to establish covalent modification of the enzyme. Currently, more is known about the aromatization of androst-4-ene-3,17-dione than is known about its inhibition.

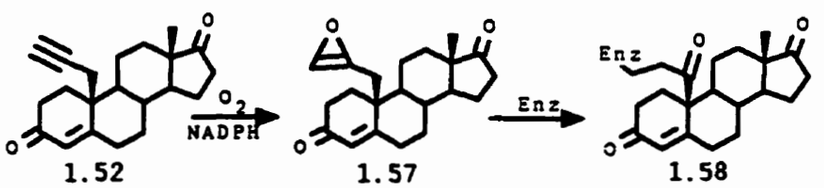
In 1979, the first rationale for the preparation of potential mechanism-based inhibitors of aromatase was reported (Covey *et al.*, 1979). The (R/S)-diastereomeric acetylenic alcohols **1.53** shown in Scheme 1.18, were prepared as analogues of 19-hydroxyandrost-4-ene-3,17-dione, the first intermediate in the aromatase reaction (Covey *et al.*, 1981; Metcalf *et al.*, 1981; Marcotte and Robinson, 1981). It was suggested that monooxygenation of either of these acetylenic alcohols on the propargylic carbon would lead to the *gem*-diol **1.54**, which would dehydrate to the conjugated acetylenic ketone **1.55**. This product, in turn, would lead to covalent modification and inactivation of aromatase *via* a Michael addition reaction with an active site nucleophile. Kinetic evaluation (Covey *et al.*, 1981) showed that only the *S* acetylenic alcohol (K_i= 27 μM, $t_{1/2}[I]$ = 4 min) caused loss of aromatase activity. The acetylenic ketone **1.55** (K_i = 12 μM, $t_{1/2}[I]$ = 21.7 min) was also a potent inactivator of aromatase. Although the acetylenic ketone **1.55** has proved to be a time-dependent inactivator of aromatase, its rate of inactivation (K_{inac} = 5.35 x 10^{-4} s^{-1}) was

slower than that shown by the parent acetylenic compound **1.52** (k_{inac} = 1.11 x 10^{-3} s^{-1}). The proposed mechanism of the acetylenic-aromatase interaction is shown in Scheme 1.19 (Covey *et al.*, 1981). This mechanism has been disproved, when Metcalf *et al.* found that there was no detectable isotope effect associated with the inactivation process for the 19-deuterium propargyl compound (Metcalf *et al.*, 1981).



Scheme 1.18 Proposed aromatase inactivation mechanism by the acetylenic ketone **1.55** (Covey *et al.*, 1981).

An alternative mechanism shown in Scheme 1.19 was proposed by Metcalf *et al.* (Metcalf *et al.*, 1981). According to this mechanism, the close proximity of the acetylenic group to the activated oxygen complex (iron-oxo species) would result in oxygen insertion into the carbon-carbon triple bond.



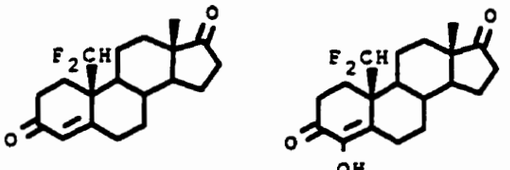
Scheme 1.19 Aromatase inactivation mechanism by the acetylenic compound **1.52** (Metcalf *et al.*, 1981).

The resultant highly reactive oxirene **1.57** would then isomerise to an α-ketocarbene that would bind covalently to the prosthetic heme group. The same mechanism has been proposed by Ortiz de Montellano *et al.* for inactivation of liver microsomal cytochrome P-450 enzymes by terminal acetylenes (Ortiz de Montellano *et al.*, 1986). Although the mechanism of aromatase inactivation by compound **1.52** remains to be established, the compound has considerable potential as a therapeutic agent. Compound **1.52** does not inhibit steroid 5α-reductase, 3α-hydroxysteroid dehydrogenase, 11β-hydroxylase, 18-hydroxylase, or cholesterol-side-chain-cleavage enzyme, and the compound has little or no affinity for androgen, estrogen, and progestin receptors (Johnston *et al.*, 1984a; Brandt, *et al.* 1987). Compound **1.52** inhibits both placental aromatase and aromatase found in human breast tumors and adipose tissue (Perel *et al.*, 1981). It also inactivates rat ovarian aromatase (Brandt *et al.*, 1987; Johnston *et al.*, 1984) and baboon placental aromatase but does not inactivate Rhesus monkey placental aromatase (Johnston, 1987). Compound **1.52** has been shown to block aromatase in the following cultured tumor cell lines: MCF-7 human breast cancer cells (MacIndoe *et al.*, 1986), M5480A murine Leyding tumor cells (Ziminski *et al.*, 1985) and JAr choriocarcinoma trophoblast cells (Johnston *et al.*, 1984b).

Compound **1.52** showed better aromatase inhibitory properties (IC_{50} = 3 mg/kg) than aminoglutethimide (IC_{50} = 250 mg/kg), 6 h after oral administration of drug (Johnston, 1987). The 10β-propargyl compound **1.52** is currently undergoing Phase II clinical trials in the United States (O'Neil Johnson and Cramer, 1992).

Another structural analog of androstenedione, 19,19-difluoro-androsten-4-ene-3,17-dione **1.59** (Scheme 1.20), was designed as a

mechanism-based inactivator of aromatase. Indeed, the difluoro compound was found to be a competitive inhibitor of aromatase (Marcotte and Robinson, 1982a). It also caused time-dependent irreversible inactivation of human placental aromatase in the presence of NADPH and O_2(K_i = 1μM, K_{inac} = 0.023 min^{-1}), (Marcotte and Robinson, 1982c).

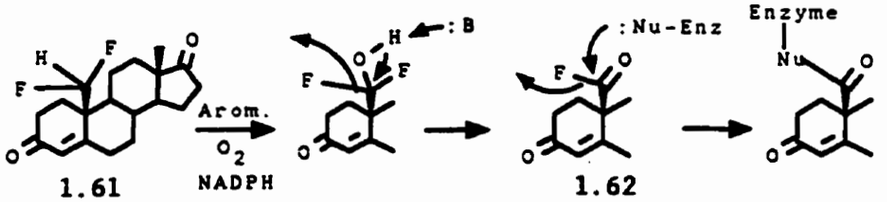


1.59 1.60

$K_{i(comp.)}$ = 1μM

Scheme 1.20 19,19-Difluoro compounds, **1.59** and **1.60**, as mechanism-based inhibitors of aromatase (Marcotte and Robinson, 1982c).

The proposed inactivation mechanism of aromatase by **1.59** is shown in Scheme 1.21 (Cole and Robinson, 1990). Enzymatic hydroxylation at the 19-carbon would give a geminal fluorohydrin, which would spontaneously lose HF yielding an electrophilic acyl fluoride **1.62**, Scheme 1.21. The latter would then react covalently with an active-site nucleophile and thus inactivate the enzyme.



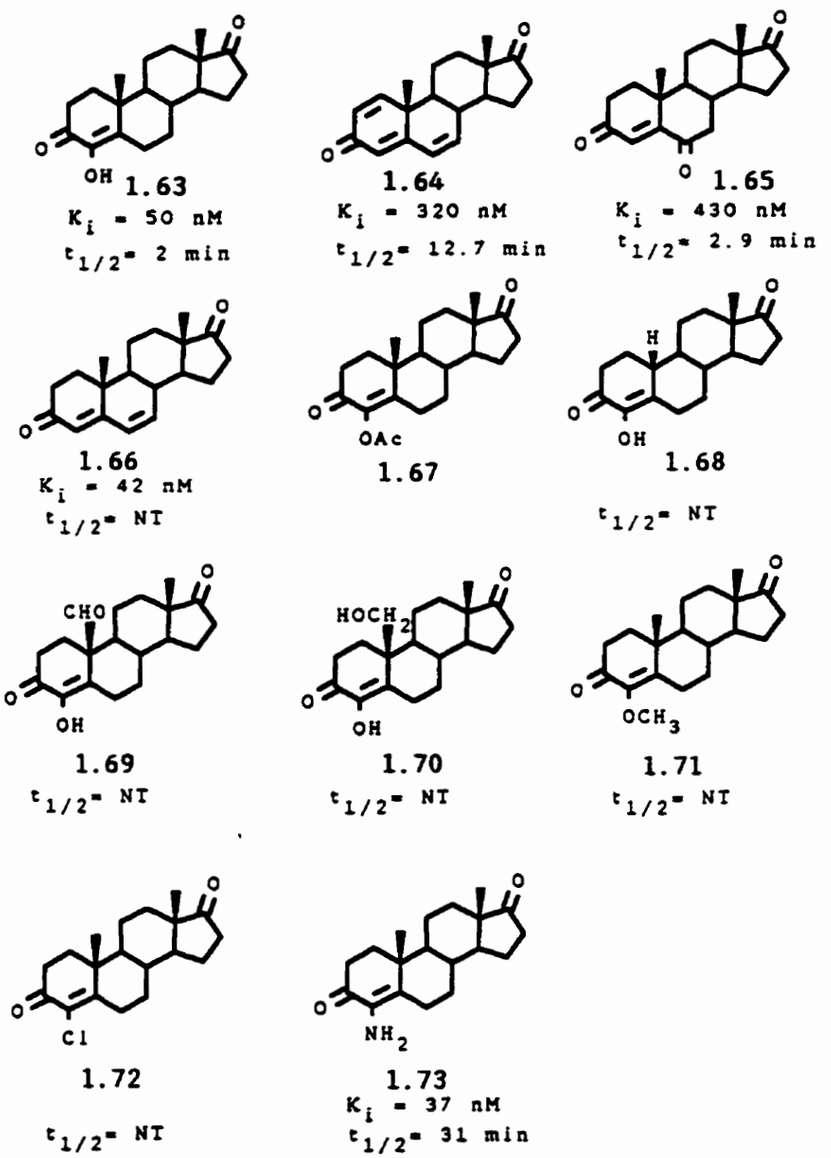
1.61 1.62

Scheme 1.21 Proposed mechanism of aromatase inactivation by 19,19-difluoroandrost-4-ene-3,17-dione (Cole and Robinson, 1990).

Direct evidence for attack by aromatase at the 19-carbon of 19,19-difluoroandrostenedione **1.61** came from studies using tritium labelling at C-19 (Furth and Robinson, 1989). When [19-^{3}H]-19,19-difluoroandrostenedione was incubated with human placental aromatase, a time-dependent release of tritium into the solution occurred. While the tritium-release process was dependent on protein concentration and NADPH, it was suppressed in the presence of 19(R)-epoxide **1.45**, a powerful inhibitor of the enzyme. On the other hand, deuterium labelling data showed that [19-^{2}H]-19,19-difluoroandrostenedione inactivated the enzyme at the same rate as the nondeuterated parent showing no isotope effect (Furth and Robinson, 1989). It indicated that the 19-C-H bond cleavage is not the rate-limiting step in the inactivation process by the 19,19-difluoro compound. The 4-hydroxy analogue **1.60** synthesized was reported to be an inhibitor of human placental aromatase (IC_{50} = 3.3 μM) (Mann and Pietrzak, 1987).

Initially, compounds **1.63-1.65** were described as potent competitive inhibitors of aromatase (Brodie *et al.*, 1981). It was later shown that these compounds cause a time-dependent loss of aromatase activity only in the presence of O_2 and NADPH (Covey and Hood, 1982). A large amount of research (Covey and Hood, 1986), established the endocrinological and pharmacological profiles of compounds **1.63-1.65** (Scheme 1.22) as aromatase inhibitors. However, the mechanism(s) responsible for aromatase inactivation by **1.63-1.65** are still unknown. Compound 1.63 (Formestane), has been shown to have activity against human ovarian aromatase in grannulosa cell cultures (Koss *et al.*, 1986). In 1992, Ciba-Geigy introduced Formestane, 4-OHA **1.63**, into the market as intramuscular formulation under the name Lentaron (Combs, 1995). In clinical trials in Britain, 4-OHA has shown promise in promoting tumor

regression in patients with advanced breast cancer (Stein *et al.*, 1990). The 4-O-acetyl derivative **1.67** of 4-OHA **1.63** was also demonstrated to be a time-dependent inactivator of aromatase (Brodie *et al.*, 1979).



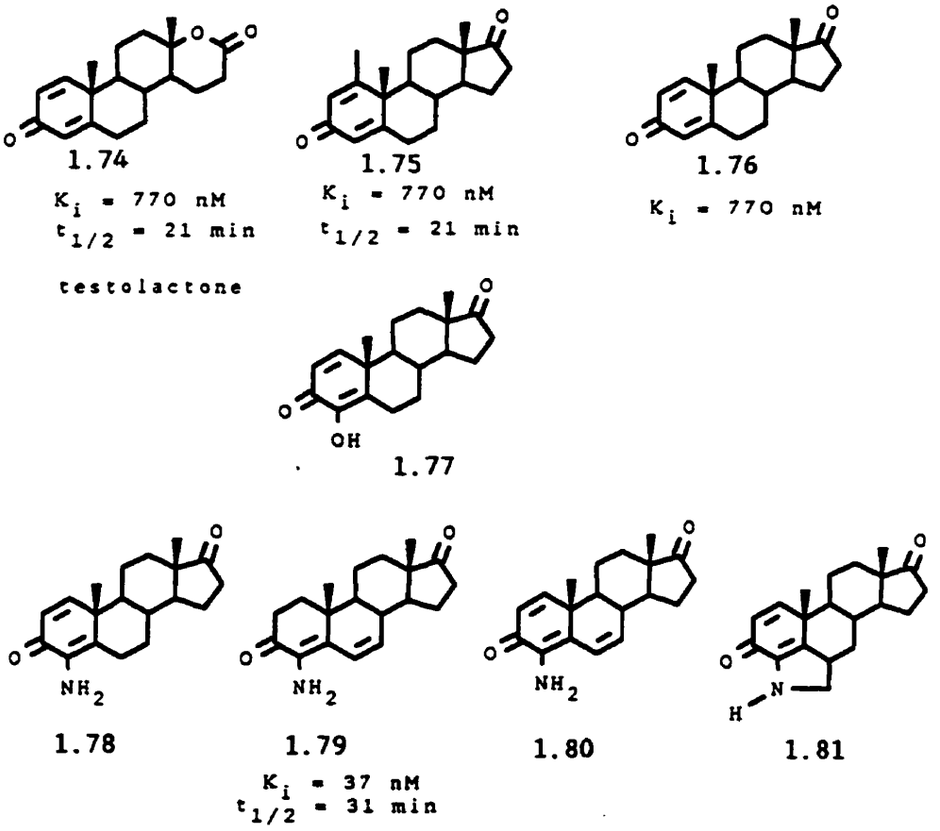
Scheme 1.22 Chemical structures of selected mechanism-based inhibitors of aromatase.

It is likely that this compound is hydrolyzed to 4-OHA before inactivation occurs. Presumably, the estrase activity in the human placental microsomes is responsible for the conversion of 4-OAc **1.67** to 4-OHA **1.63** (Covey and Hood, 1982). In the same paper, Covey and Hood, showed that 19-nor-4-hydroxyandrost-4-ene-3,17-dione **1.68** was capable of binding to the enzyme, but it did not inactivate aromatase. Moreover, they concluded that the 19-oxygenated intermediate could be the inactivating species. Later, Newitt and Robinson (Newitt and Robinson, 1986) showed that 4-hydroxy-19-norandrost-4-ene-3,17-dione **1.69** was a poor inactivator relative to 4-OHA **1.63**. No studies have been reported with the 19-hydroxy analogue **70**. It is also interesting to note that the 4-OMe **1.71** and 4-Cl **1.72** are not time-dependent inactivators, underlining the importance of the free OH group (Marsh *et al.*, 1985).

The triene **1.64** differs from androstenedione by the presence of two additional double bonds. When compounds **1.64** and **1.66** were evaluated, it was shown that the C-1-C-2 double bond, not the C-6-C-7 double bond, was the structural feature responsible for aromatase inactivation (Covey and Hood, 1982). For example, compound **1.74** (Scheme 1.23), which also has the C-1-C-2 double bond and a modified D ring, testolactone has been clinically used since 1962. Knowledge of its efficacy for the treatment of breast cancer (Segaloff *et al.*, 1962) preceded the discovery that the compound lowers circulating estrogen levels in humans (Sitteri and Thomson, 1975). As soon it was realized that $\Delta^{1,4}$-3-ketosteroid could inactivate aromatase, compound **1.74** was evaluated and found to be a weak-binding, slow inactivator of the enzyme (K_i = 770 nM, $t_{1/2}$= 21 min), (Johnston and Metcalf, 1984). It was also found that the similarly modified D-ring compound without the

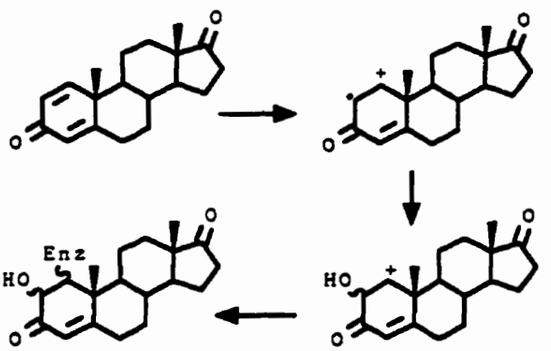
C-1-C-2 double bond was not a mechanism-based inactivator of aromatase (Johnston and Metcalf, 1984).

More highly substituted 1,4-diene, 4,6-diene, and 1,4,6-triene compounds have been synthesized and evaluated as aromatase inhibitors: substitutions include 1-methyl **1.75** (Henderson *et al.*, 1986), 4-hydroxy **1.77** (Marsh *et al.*, 1985) and 4-amino **1.78-1.81** (Scheme 1.23), (Di Salle *et al.*, 1990, 1993). All of these were found to be potent time-dependent inhibitors of aromatase.



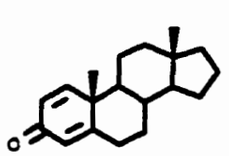
Scheme 1.23 1,4-Diene-, 4,6-diene, and 1,4,6-triene of androstane-3,17-dione as mechanism-based aromatase inhibitors.

Only one mechanism has been proposed to explain why androstenedione analogues containing a C-1-C-2 double bond could be aromatase inactivators (Covey and Hood, 1982) The inactivation mechanism, shown in Scheme 1.24, considers the removal of an electron from the C-1-C-2 double bond of compound **1.76**, by, presumably, the peroxy species, to produce a radical cation. Oxygen insertion then converts the radical cation to a carbocation, and this electrophilic species covalently modifies the enzyme, inactivating it.



Scheme 1.24 Proposed mechanism of aromatase inactivation by Covey and Hood (Covey and Hood, 1982).

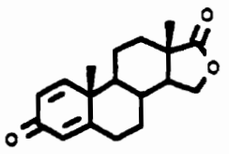
Sherwin *et al.* (Sherwin *et al*, 1989) have synthesized analogues of androsta-1,4-dien-3-ones with modified D rings. They found that the 17-desoxy compound **1.82** (K_i = 1.1 μM, $t_{1/2}$ = 15.2 min) showed less than a 4-fold decrease in incubation rate or binding affinity relative to compound **1.76**. Similarly, the corresponding D-ring butyrolactone **1.83** (K_i = 390, $t_{1/2}$ = 6.2 min) was still a potent suicide inactivator. In contrast, analogous inhibitors with open D ring were poor inhibitors.



1.82

K_i = 1.1 μM

$t_{1/2}$ = 15.2 min



1.83

K_i = 390 nM

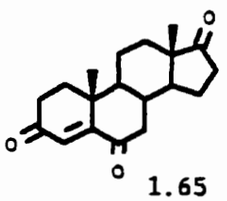
$t_{1/2}$ = 6.2 min

Scheme 1.25 Time-dependent inhibitors of aromatase with modified ring D (Sherwin *et al.*, 1989).

The next group of mechanism-based inhibitors to be considered are those structurally related to the 6-ketosteroid **1.65** (Scheme 1.26) (Schwarzel *et al.*, 1973). The mechanism of aromatase inactivation of compound **1.65** is unknown. The potential involvement of sequential monooxidation reactions at C-19 in the activation pathway has been evaluated (Covey and Hood, 1982). The 19-hydroxy compound **1.84** (Scheme 1.26) is a mechanism-based inactivator, and its high affinity for, and rapid inactivation of, aromatase suggest that this compound could be part of the reaction pathway leading to aromatase inactivation. The tetraoxo-compound **1.85** is, likewise, a mechanism-based inhibitor. However, its poor affinity and slower rate of inactivation suggest that this compound may not be an intermediate in the major pathway for inactivation by compound **1.65**. Compounds **1.87a** and **1.87b** also inactivated enzyme in a time-dependent manner in the presence of NADPH (Covey and Hood, 1981).

Numazawa *et al.* (Numazawa *et al.*, 1993) found that the 19,19-difluoro **1.89** and 19-acetoxy,19-acetylenic **1.90** derivatives of androst-4-ene-3,6,17-trione are time-dependent inactivators of aromatase. Rate of inactivation of compounds **1.89** and **1.90** decreased,

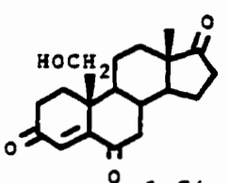
when the substrate androstanedione was included in the incubation mixture. In the nucleophilic protection experiments, L-cysteine failed to protect aromatase from inactivation of the inhibitors. Thus, the authors concluded that, presumably, covalent-bond formation between the enzyme and the reactive intermediate occurred rapidly at the active site.



1.65

K_i = 0.43 μM

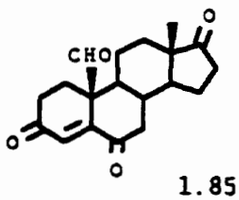
$t_{1/2}$ = 2.9 min



1.84

K_i = 2.75 μM

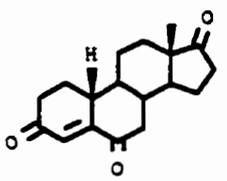
$t_{1/2}$ = 2.9 min



1.85

K_i = 39.4 μM

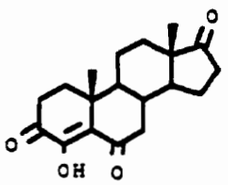
$t_{1/2}$ = 6.4 min



1.86

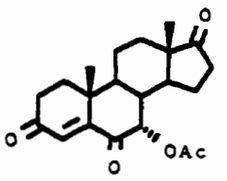
K_i = ND[a]

$t_{1/2}$ = 9 min (10 μM)[b]



1.87a

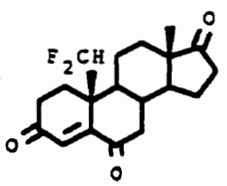
K_i = 61 nM



1.87b

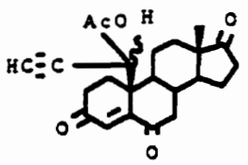
K_i = 310 nM

$k_{inact.}$ = 0.090 min^{-1}



1.89

k_i = 0.0.25 min^{-1}



1.90

K_i = 0.077 min^{-1} 19(S

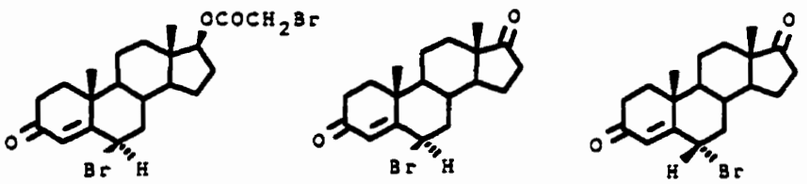
K_i = 0.065 min^{-1} 19(R

a ND (not determined)

b not the extrapolated $t_{1/2} = \infty$,
but the $t_{1/2}$ at the concentration indicated in parenthese

Scheme 1.26 Kinetic constants of inactivation of human placental microsomal aromatase by androst-4-ene-3,6,17-trione and structurally related compounds.

The synthesis and evaluation of the brominated androstenedione derivatives **1.91-1.93** have also been described, Scheme 1.27. Osawa and Coon (Osawa and Coon, 1987) have reported that two 6β-bromoandrostenedione epimers, **1.91** and **1.92**, are potent irreversible inhibitors for human placental aromatase.



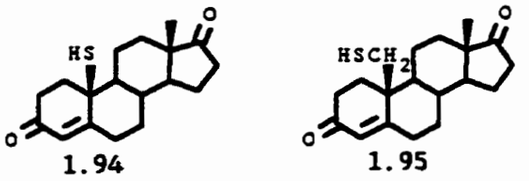
1.91
K_i = 25 μM
k_{inac} = 0.0274 min^{-1}

1.92
K_i = 0.8 μM
k_{inac} = 0.025 min^{-1}

1.93
rever. compt.

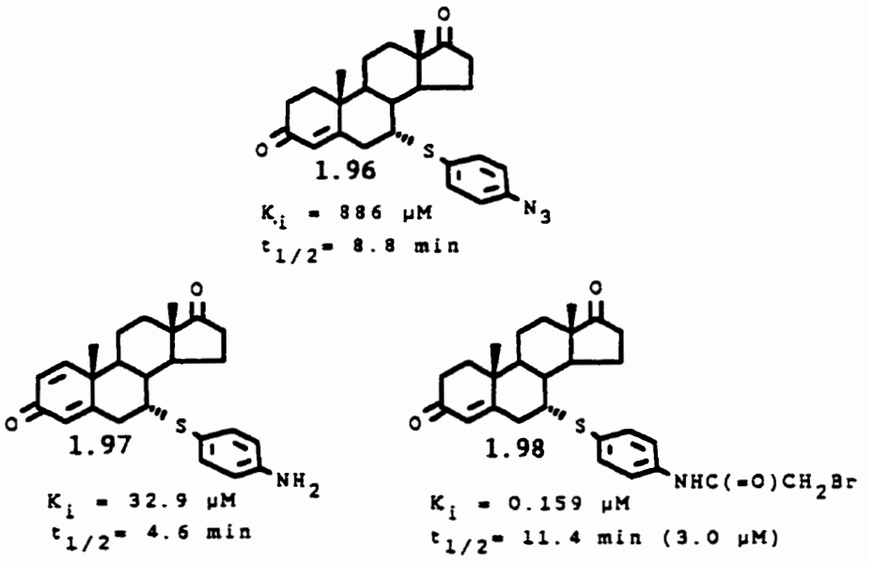
Scheme 1.27 6ξ-Bromo derivatives of androst-4-ene-3,17-dione as aromatase inhibitors (Osawa and Coon, 1987).

Bednarski and Nelson (Bednarski and Nelson, 1989) have found that some thio-substituted compounds obeyed many of the classical criteria for suicide inhibitors (Scheme 1.28). For example, both the 10β-mercapto **1.94** (K_i = 106 nM; k_{iact} = 0.0032 s^{-1}) and 19-mercapto **1.95** (K_i = 34 nM; k_{iact} = 0.0012 s^{-1}) compounds caused time-dependent inactivation of aromatase in the presence of NADPH and O_2. The inactivation process was shown to be irreversible (by extended dialysis of the enzyme to remove unbound inhibitor). The mechanism of inactivation remains unclear, although it was suggested that oxidation to a potent electrophile, i.e. sulfenic acid might be involved which could react with a nucleophile in the enzymatic active site.



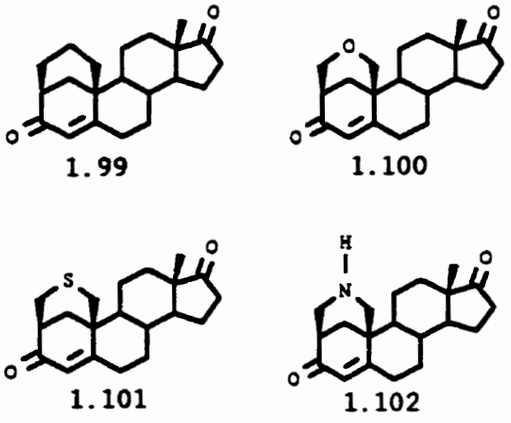
Scheme 1.28 Thio-substituted mechanism based inhibitors of aromatase (Bednarski and Nelson, 1989).

Compounds **1.96 and 1.97** are photoaffinity and compound **1.98** affinity labels (Scheme 1.29) of aromatase (Snider and Brueggemeier, 1987, 1985). The C-1-C-2 double bond present in compound **1.98** makes it a very potent mechanism-based inhibitor of aromatase.



Scheme 1.29 Photoaffinity **1.96** and **1.97** and affinity **1.98** labels of aromatase (Snider and Brueggemeier, 1987, 1985).

All four bridged compounds, **1.99-1.102** (Scheme 1.30), have been shown to be potent time-dependent inhibitors of human placental aromatase. Kinetic analyses were not presented (Burkhart *et al.*, 1992; Peet *et al.*, 1992).



Scheme 1.30 2,19-Bridged androstenedione derivatives as mechanism-based inhibitors of aromatase (Burkhart *et al.*, 1992).

1.4.0 Research Objective and Achievements

Alteration of hormonal levels by enzyme inhibition can be of benefit in a number of disease states, e.g. the hormone dependent prostatic and breast carcinomas (Foye, 1995). Enzyme inhibition is also valuable as a probe to investigate the nature of the enzyme's active-site, the mechanism of enzyme activity, and the role of certain enzymes and "minor" metabolites.

The main goal of this research was to synthesise compounds that would selectively inhibit endogenous production of specific hormones in the body. Three enzymes have been targeted, aromatase and 20α- and 20β-hydroxysteroid dehydrogenase. Various modes of enzyme inhibition are possible. Three general approaches are competitive inhibition, active-site-directed inhibition, and mechanism-based inhibition. The latter approach is based on the design of an enzyme substrate, on which the enzyme carrying out its normal function, produces a highly reactive electrophilic species, which is immediately attacked by an active-site nucleophile to form a covalent bond. This can be further divided with respect to the stability of the covalent bond into irreversible inhibition and pseudoirreversible inhibition; the latter when the reverse reaction occurs relatively easily.

An essential objective of this thesis was to establish the general hypothesis that a secondary cyclopropyl derivative incorporated into a molecule can serve as a functional group which, through metabolic oxidation to a cyclopropanone, will react with an enzyme active-site nucleophile to form a covalent bond. Covalent bond formation at an enzyme active-site is the most effective means of enzyme inactivation. However, a reactive molecule capable of covalent bonding at an active-site could also, because of its reactivity, bond to other

molecules before effective distribution to the active-site occurs. Thus, it would lack site selectivity and perhaps cause unwanted side effects.

The principle of mechanism-based-enzyme (suicide) inhibition overcomes this limitation by introducing a high degree of selectivity for the target enzyme. A mechanism-based inhibitor is an inherently stable molecule which must:

(i) act as a substrate for a specific enzyme,

(ii) be chemically and pharmacologically inert prior to enzymatic reaction,

(iii) have a structure such that the enzyme can carry out its normal catalytic reaction on it. This reaction must yield a highly reactive electrophilic species which can form a strong covalent bond and effectively block the activity of the enzyme (Abeles, 1980).

It is assumed that the electrophile produced will react with an active-site nucleophile before escape from the site can occur. Of course, if the reactive species diffuses away from the enzyme-active site before covalent bond formation can occur, this specificity is diminished or lost. The process for mechanism-based enzyme inactivation is represented as follows:

$$E + I \rightleftarrows E\text{-}I \rightarrow E\text{-}I^{*} \rightarrow (E\text{-}I)_{inactive}$$

E = enzyme; I = inhibitor;

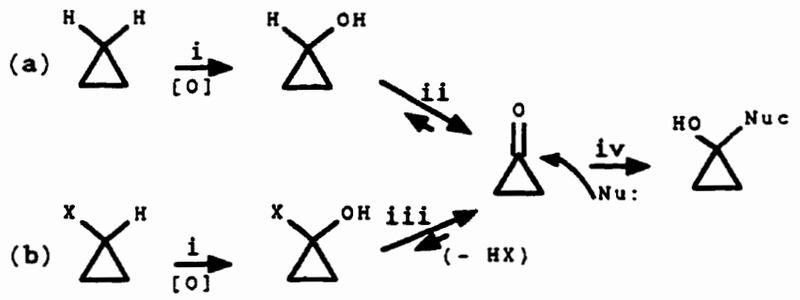
E-I = enzyme inhibitor complex;

$E\text{-}I^{*}$ = active electrophile derivative;

$(E\text{-}I)_{inactive}$ = covalently bonded enzyme-electrophilic derivative.

Based on a definition of suicide inhibitors, we may say that the "inhibitor" (I) is rather a "pro-inhibitor", while the resulting electrophile (E--I*) is the "real inhibitor" or a "metabolically activated inactivator". Thus, a mechanism-based inhibitor is an intrinsically unreactive molecule (I) which acts as an enzyme substrate to form a (E--I) complex. Next, the (E--I) is converted by the enzyme, performing its normal reaction, into a reactive form (E--I*), which then forms a covalent bond $(E\text{-}I)_{inactive}$ with the enzyme at the active-site. The formation of the covalent bond in the $(E\text{-}I)_{inactive}$ complex effectively blocks the active-site in an irreverible manner.

We propose that certain cyclo- and cyclopropanosteroid derivatives could act as mechanism-based inhibitors of steroid enzymes through the reaction sequences shown in Scheme 1.31. In favourable circumstances, hydroxylation of a cyclopropane ring gives a secondary cyclopropanol, which on further oxidation by an appropriate oxidoreductase (dehydrogenase), yields a cyclopropanone. Next, the highly reactive



where X = OH, Cl, F, $OCOCH_3$ NH_2, NO_3

Scheme 1.31 (a) and (b) **i**, hydroxylating enzyme; **ii**, dehydrogenase and NAD^+ or $NADP^+$; **iii**, elimination reaction; **iv**, attack of a nucleophile at an electrophile.

cyclopropanone (Wasserman, 1974) produced could react rapidly with a nucleophile at the enzyme active-site to form a covalent bond (Scheme 31, path a). Both naturally occurring and synthetic cyclopropanoid compounds that are irrevesible inhibitors of particular target enzymes exist (Walsh, 1982; Hoffman and Silvermann, 1980; Peak *et al.*, 1980; Wiesman and Abeles, 1979).

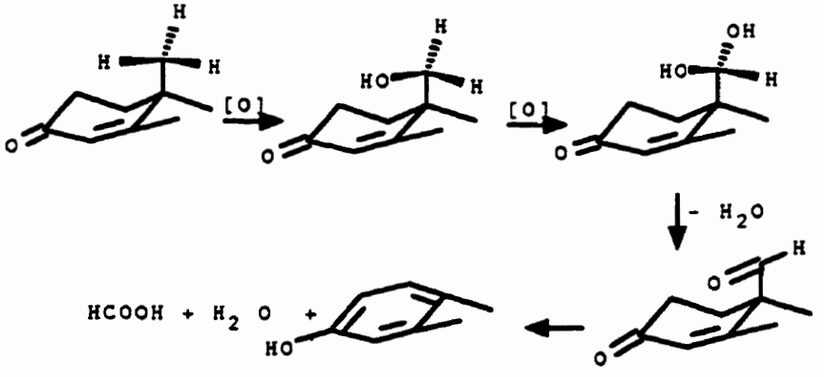
Moreover, this concept of cyclopropanol enzyme inhibition is supported by the reported irreversible inhibition of methanol oxidase by cyclopropanol through covalent bond formation (Sherry and Abele, 1985).

Cyclopropanol derivatives, e.g. cyclopropanol esters, can act as pro-alcohol groups *in vivo*. A modification of this sequence (Scheme 1.31, path b), applicable to hydroxylating enzymes (aromatase = estrogens synthetase) rather than a dehydrogenase, requires cyclopropanone substitution with an electronegative group, e.g. F, Cl, Br, OH, NH_2, or NO_2 to generate a cyclopropanone after enzymatic hydroxylation. In general, unsubstituted cyclopropane ring derivatives are resistant to metabolic hydroxylation (Templeton and Kim, 1976). However, this problem can be overcome by the presence of substituents in the cyclopropane ring which activate the adjacent C-H bond for metabolic oxidation. For example, 5β,19-cycloandrostane-3,17-dione has been shown to be a very weak inhibitor of aromatase (Marcotte and Robinson, 1982b). Although the 5β,19-cycloandrostane-3,17-dione has been shown to be a weak inhibitor of aromatase, both 2β,4β-cyclo-5α-androstane-3ξ,17β-diols (Skirving *et al.*, 1986) acted as irreversible, but unselective, inhibitors of 3β-hydroxysteroid dehydrogenase, supporting the concept that cyclopropanol derivatives can act as mechanism-based enzyme inhibitors (Orr *et al.*, 1988).

AROMATASE (ESTROGEN SYNTHETASE) INHIBITORS.

Aromatase is a key cytochrome P-450 monooxygenase enzyme system involved in the conversion of the male sex hormones (androgens) into female sex hormones (estrogens), e.g. androstene-3,17-dione → estrone, testesterone → estradiol, and 16α-hydroxytestesterone → 16α-hydroxy-estradiol.

Much of the detailed mechanism of the conversion of the steroid ring A androgens into the aromatic ring has been explained. Comprehensive review of aromatase function, mechanism, and biological significance has been published (Tan, 1992; Covey, 1988) as have been the result of the Proceedings of the Third International Aromatase Conference (Proc. the 3th Int. Arom. Conf., 1993). The multistep process is summarized in Scheme 1.32.

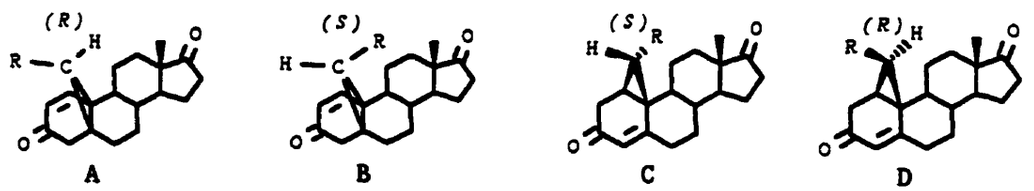


Scheme 1.32 Aromatase C-19 hydroxylations leading to aromatization.

It is generally agreed that the aromatization process in the steroid A-ring begins with two sequential radical oxidations at C-19 for which two equivalents of oxygen and NADPH are required (Bausen *et al.*, 1987; Stevenson *et al.*, 1988). These two oxidative steps are thought to be typical cytochrome P-450 type hydroxylations, occurring with retention of configuration. Recently, a new active-site model has

been proposed (Oh and Robinson, 1993) which accounts for the stereochemical consequences observed at C-19 (Scheme 1.13). For our purposes, a knowledge of the initial C-19 hydroxylation and the nucleophilic interaction of the enzyme active site with an electrophile is necessary. Evaluation of steroids and non-steroid compounds acting as aromatase mechanism-based inhibitors shows that C-19 steroids closely related to the natural substrates, androstenedione and testosterone, are the most effective inhibitors. For example, 4-hydroxyandrost-4-ene-3,17-dione, a time-dependent inhibitor, has been shown to be effective with no serious side effect (Brodie *et al.*, 1986b; Coombes *et al.*, 1984). It is now on the market as a intramuscular formulation, Formestane.

The objective of these studies is to synthesize unsaturated steroid cyclopropane derivatives designed as substrate for the aromatase enzyme to act as mechanism-based inhibitors. The proposed structures consist of isomeric pairs of corresponding unsaturated analogues. Selected C-19 chloro, hydroxy, acetoxy, nitro, and amino, derivatives of the unsaturated cycloandrostanes, **A** to **D**, are outlined in Scheme 1.33.



R= F, Cl, OH, AcO, NH_2, NO_2

Scheme 1.33 Compounds proposed to be synthesized and tested as the mechanism-based aromatase inhibitors.

It can be assumed that the initial hydroxylation will occur to a hydrogen atom located over ring A and that the hydroxyl group formed becomes hydrogen bonded to the active-aminoacid residue (Glu-302), (Oh and Robinson, 1993). The second hydroxylation is not required in this case as one hydroxyl group (or equivalent halogen, amino, or nitro group) is already present. Loss of H_2O, HX, NH_3, or HNO_2 can then occur to generate a carbonyl group (in this case a reactive cyclopropanone). Depending upon active-site requirements, both isomers (R) and (S), may act as aromatase inhibitors, but the isomer with the cyclopropyl hydrogen located over or out-of ring A would probably be most effective. The anticipated difference between the activities of the (R)- and (S)- isomers would add support for the proposed model by Oh and Robinson (Oh and Robinson, 1993).

The compounds we proposed to synthesize and test as aromatase inhibitors have the required hydrogen located over ring A (Scheme 1.33, compounds **B**). Other compounds have the required hydrogen out of ring A (Scheme 33, compounds **D**). The presence of the C-3 and C-17 carbonyl groups, which are able to form hydrogen bonds at the active-site, is important for receptor attachment (Oh and Robinson, 1993). The 17-keto group is more effective than the 17βα-hydroxy group. Introduction of unsaturation in ring A leads to the unsaturated derivatives, which more closely approximate the structure of the natural substrate, androst-4-ene-3,17-dione. MMX geometry optimization unambiguously shows the structural differences and similarities for both the saturated and unsaturated molecules proposed. In the case of the unsaturated compounds, the A/B ring geometry remains similar to the geometry of A/B ring region of androst-4-ene-3,17-dione, as shown by the superimposed structures (Figure 1.2).

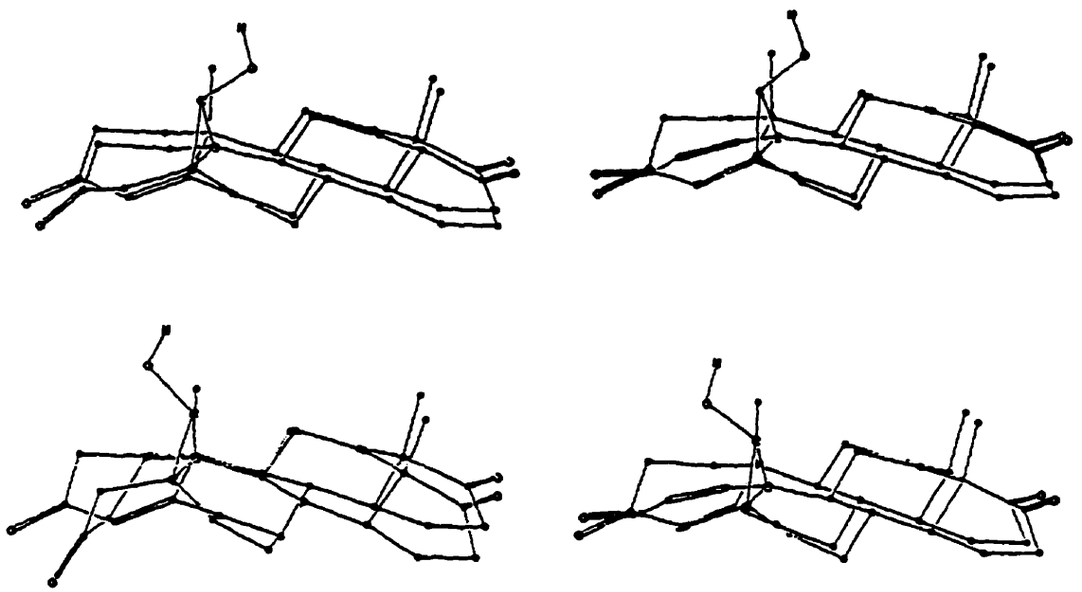
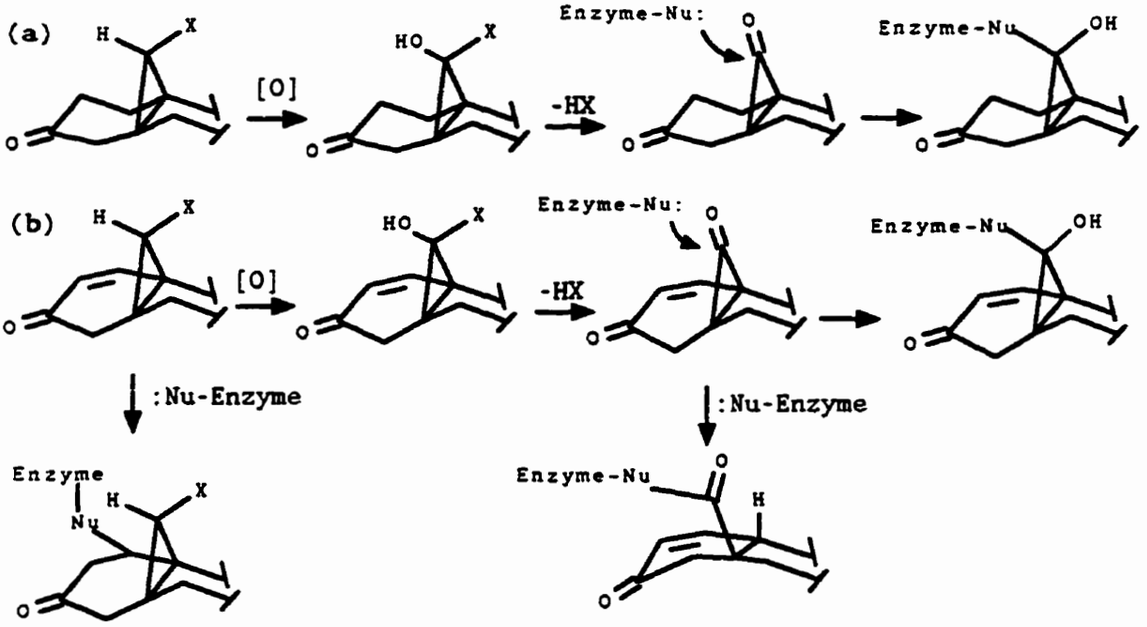


Figure 1.2 A structure of androst-4-ene-3,17-dione superimposed with saturated and unsaturated 19(R)- and 19(S)-hydroxy-5β,19-cycloandrostane-3,17-dione.

Moreover, introduction of the unsaturation at the C(1)-C(2) bond not only makes ring A more planar but also adds resonance energy and thus increases the inhibition properties (Bohl *et al.*, 1989).

Whereas the cyclopropane ring hydrogens themselves are resistant to metabolic hydroxylation (Templeton and Kim, 1976; Burger, 1971), the electronegative substituent can be expected to make the geminal hydrogen more reactive because electron-withdrawing groups on carbon can facilitate carbon radical formation (Hine, 1962).

The proposed mechanisms of aromatase inactivation are given in Schemes 1.34 and 1.35. Saturated derivatives can bond to the enzyme active-site as outlined in Scheme 1.34(a) and Scheme 1.35(a).

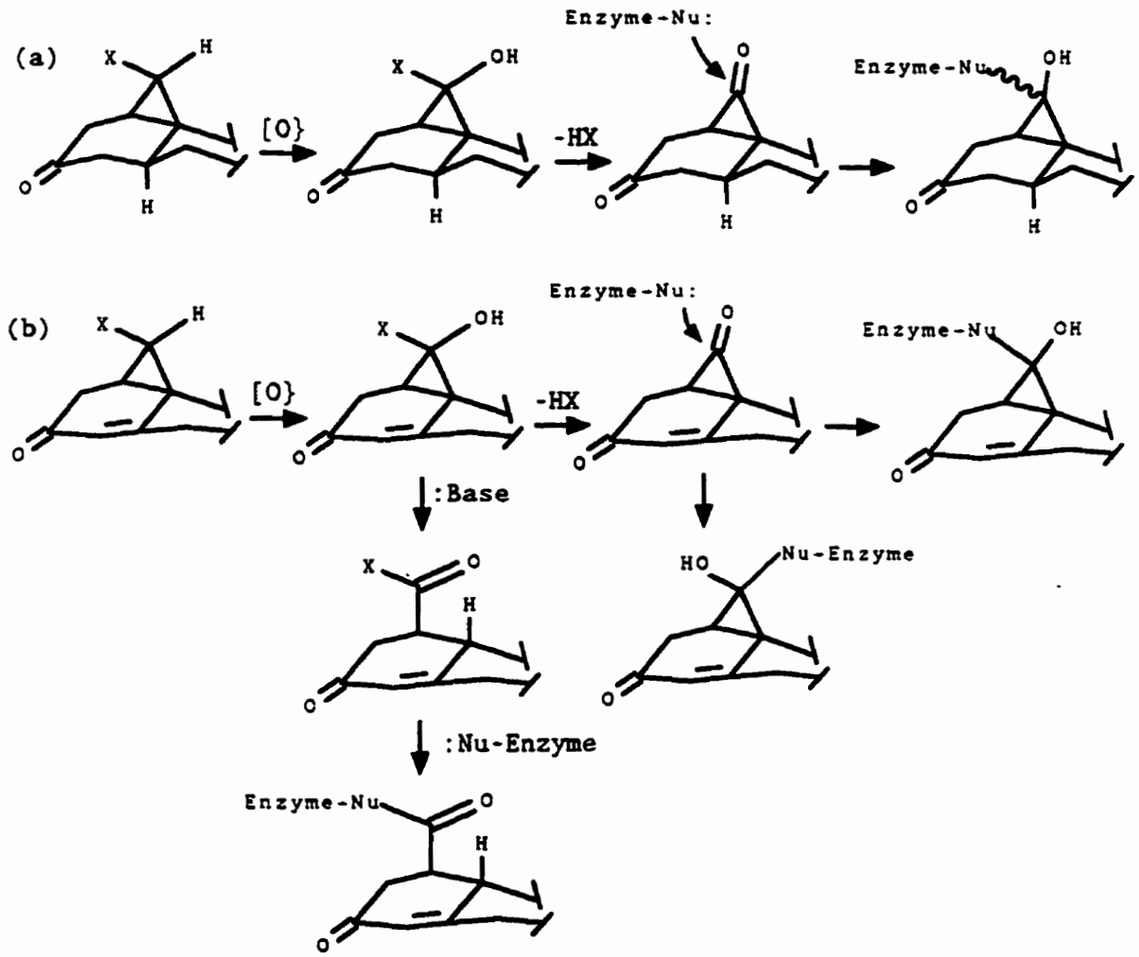


Scheme 1.34 Proposed mechanisms of aromatase inactivation by 19(R)-substituted-5β,19-cycloandrostane-3,17-dione (a) and its unsaturated analogue (b).

The unsaturated derivatives can undergo further reaction, as shown in Scheme 1.34(b) and Scheme 1.34(b), giving a strong covalent link to the enzyme. The C(1)-C(2) unsaturated 19-acetate derivatives, shown in compounds **A** and **B** (Scheme 1.33), have been synthesized *via* TMS-enol derivatives of the saturated analogous followed by treatment with $Pd(OAc)_2$. A synthesis of unsaturated 19(R/S)-hydroxy-5β,19-cyclopropane-3,17-dione and 19(R/S)-hydroxy-1β,19-cyclopropane-3,17-dione isomers has been developed in this laboratory (Templeton *et al.*, 1994a).

The method referred to above for the introduction of the C-1 double bond was not applicable to introduction of the C-4 double bond to yield the target compound 19(R)- and 19(S)-acetoxy-1β,19-cyclopropano-

androst-4-ene-3,17-dione. Examination of structural models shows that in the 1β,19-cycloandrostane derivatives, the cyclopropane ring hydrogens are also in favourable positions to undergo aromatase hydroxylation. Moreover, as shown by superimposed structures (Figure 1.3), a structure of 19(R)-acetoxy-1β,19-cycloandrostane-3,17-dione closely approximates the structure of androst-4-ene-3,17-dione, even without introduced unsaturation in ring A.



Scheme 1.35 Proposed mechanism of aromatase inactivation by 19(R/S)-substituted-1β,19-cycloandrostane-3,17-dione (a) and its unsaturated analogue (b).

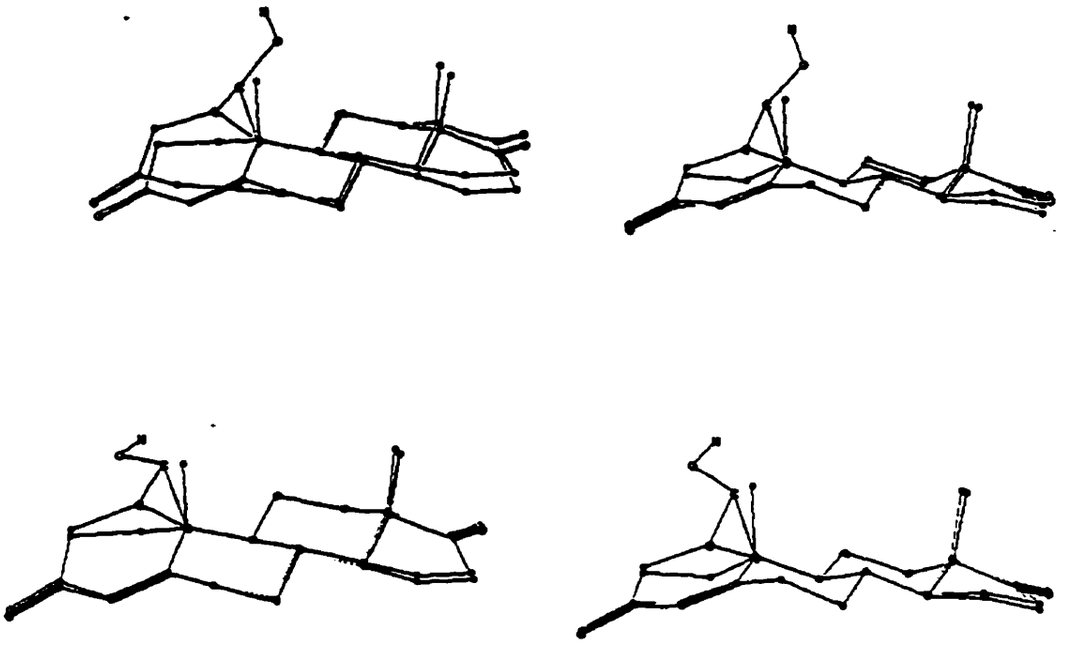
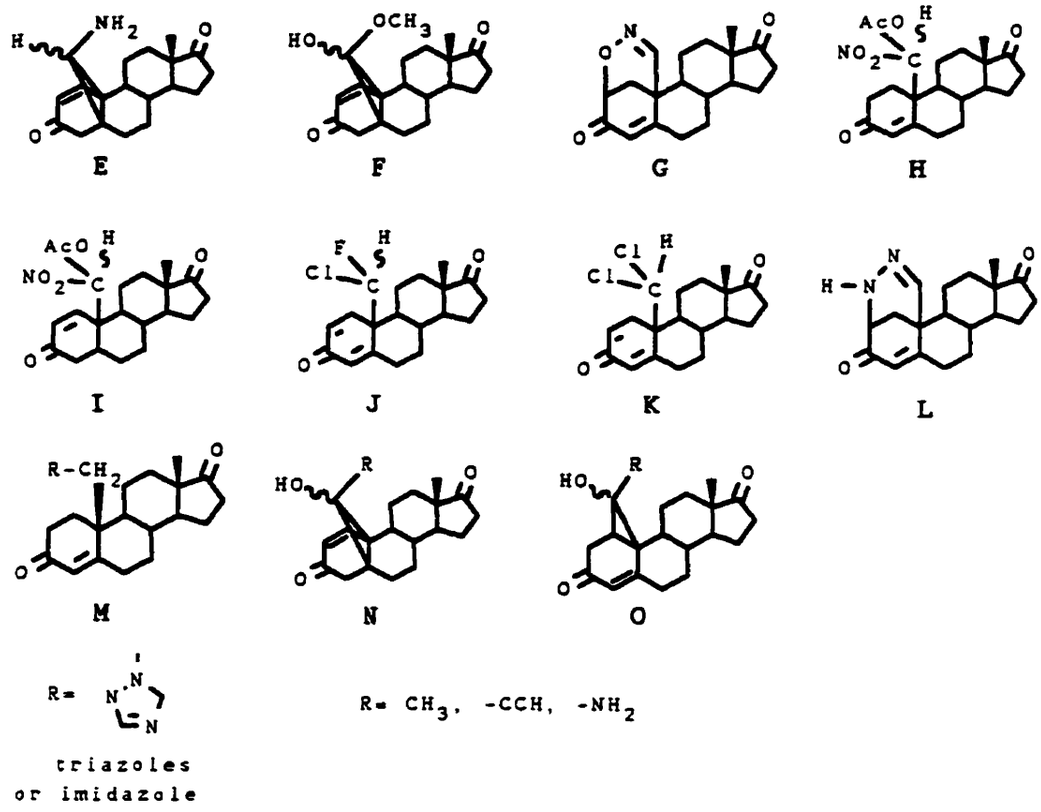


Figure 1.3 A structure of androst-4-ene-3,17-dione superimposed with saturated and unsaturated 19(S)- and 19(R)-hydroxy-1β,19-cycloandrostane-3,17-dione.

Additionally (Scheme 1.36), an attempt was made to synthesise the following compounds: the monosubstituted 19,19-(R/S)-amino-5β,19-cyclopropane derivatives **E**, the 19-acetoxy,19-methoxy-5β,19-cyclopropane **F**, the 2,19-O-N=CH briged derivative **G**, and 19-acetoxy,19-nitrate unsaturated derivatives, **H** and **I**. The synthetic approaches to the above compounds are described in following chapters. Compounds **E**, **F**, **H-K**, **N**, **O** were considered to be time-dependent and compounds **G**, **L**, **M** as the effective competitive inhibitors. Compounds, **J-O** have been proposed, but no attempt was made to synthesize them.



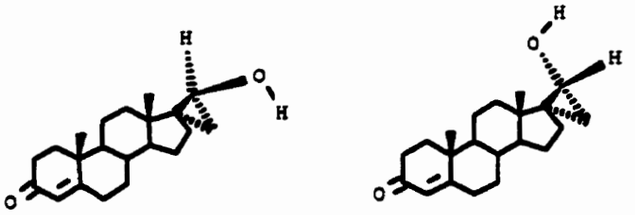
Scheme 1.36 Compounds proposed as aromatase inhibitors: (a) mechanism-based **E**, **F**, **H-K**, **N**, **O**; (b) competitive **G**, **L**, **M**.

17β,20α- and 3α,20β-HYDROXY STEROID DEHYDROGENASE (OXIDOREDUCTASES) INHIBITORS

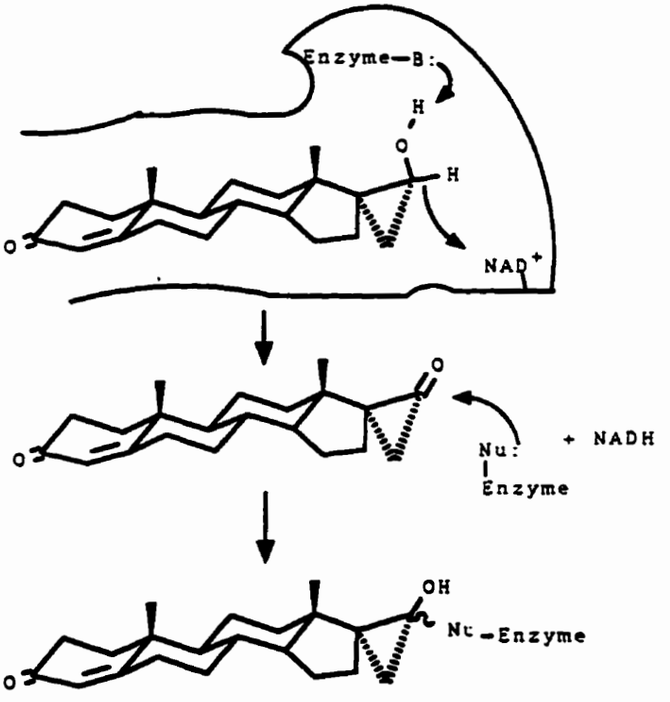
The concept of cyclopropanol derivatives acting as irreversible inhibitors has also been applied to 17β,20α- and 3α,20β-hydroxy dehydrogenase (oxidoreductase).

Synthetic approaches to two C-17 steroid spirocyclopropanols, 20α- and 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one, have been developed (Templeton *et al.*, 1994, Orr *et al.*, 1994), (Scheme 1.37). The syntheses are described in Chapter 4.

The enzymatic conversion of the C-17 steroid spirocyclopropanols was assumed to occur through oxidation of the 21-hydroxyl group to a highly reactive cyclopropanone at a receptor active-site which could lead to covalent bond formation and irreversible enzyme inhibition (Scheme 1.38).



Scheme 1.37 Chemical structures of 20α- and 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one.



Scheme 1.38 Proposed action of 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one at the enzyme active-site.

However, neither 20α- nor 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one has been adequately tested as enzyme inhibitors (Dr. J.C. Orr personal communication).

4-Hydroxyestrone and 4-hydroxyestradiol are the products of further metabolic oxidations of estrogens in the body. However, as the synthetic methods developed are not always adequate, we designed three methods with the anticipation of obtaining higher yields. The syntheses are described in Chapter 3.

In summary, a purpose of this study was to search for new aromatase and 20ξ-hydroxysteroid dehydrogenases inhibitors and to determine the structure-activity relationship of the synthesized compounds at the enzyme active site. The nature of the enzyme active-site (Duax and Gosh, 1997; Thomas and Strickler, 1983; Kawamura *et al.*, 1981) and role of formed metabolites (Houben and Bullock, 1987; Naito *et al.*, 1986; Khun and Briley, 1970) are areas of ongoing research interest.

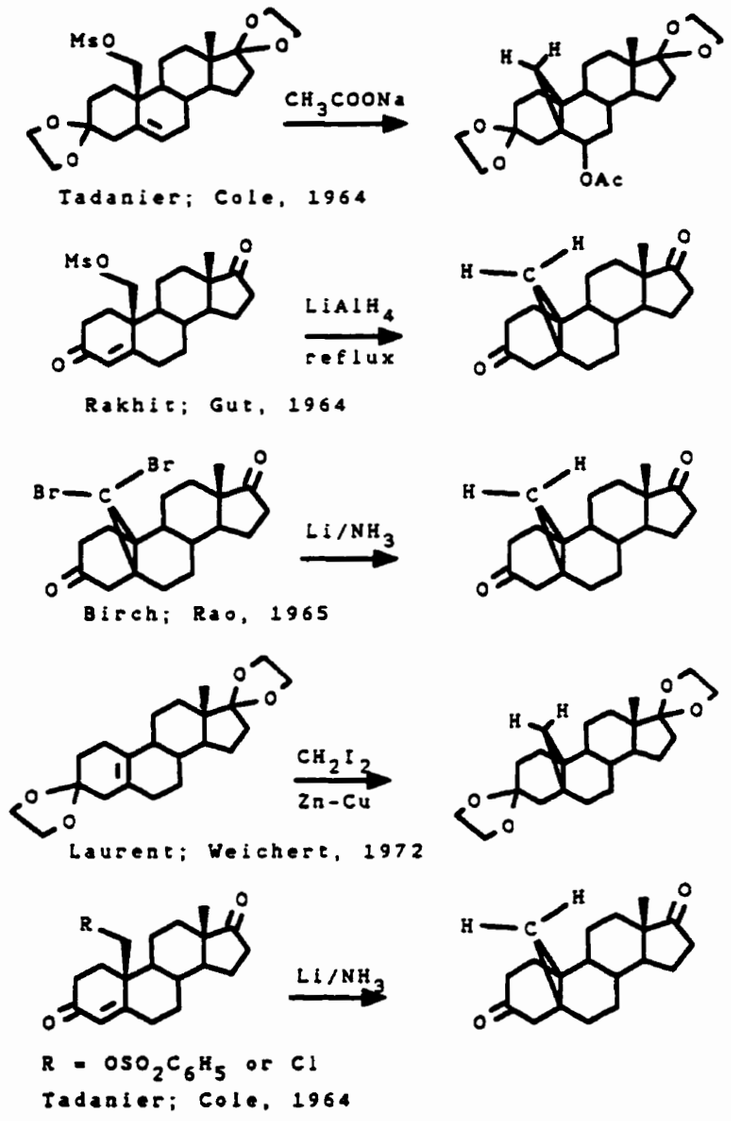
CHAPTER 2

SYNTHESIS OF POTENTIAL MECHANISM-BASED INHIBITORS OF AROMATASE

2.1.0 Synthesis of 19(R/S)-acetoxy-5β,19-cycloandrost-1-ene-3,17-dione.

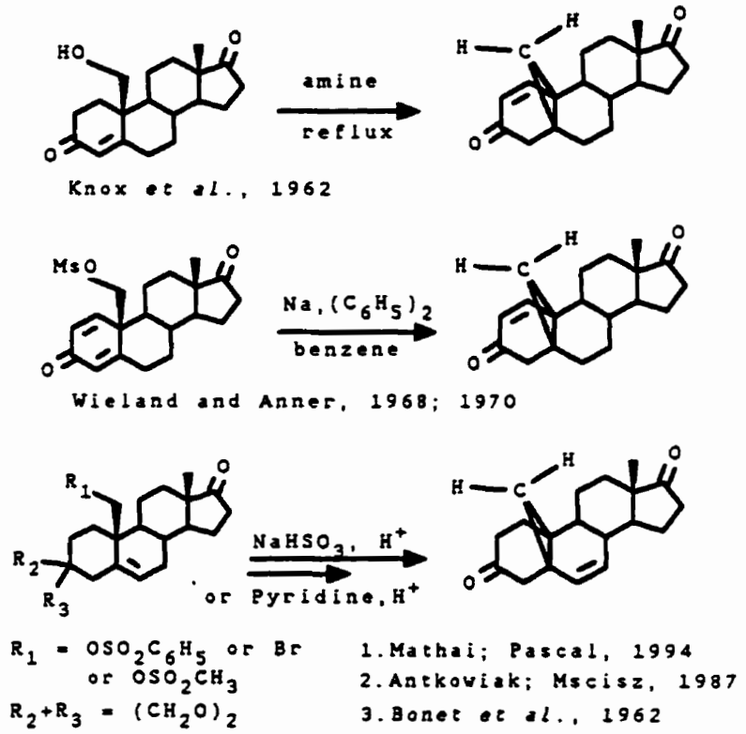
Introduction

The reported synthesis of saturated unsubstituted 5β,19-cyclosteroids (Scheme 2.1) involved: elimination of a 19-mesylate 5-ene with acetate ion (Tadanier and Cole, 1964) or the 4-ene with hydride ion



Scheme 2.1 The reported synthesis of saturated unsubstituted 5β,19-cyclosteroids.

(Rakhit and and Gut, 1964), reduction of the 19,19-dibromo-5β,19-cyclosteroid (Birch and Rao, 1965), addition of the Simmons-Smith reagent to the steroid 5(10)-double bond (Laurent and Weichert, 1972), reductive elimination of a C-19 sulfonate or halogen in the steroid 4-en-3-one with Li or Na in liquid ammonia (Tadanier and Cole, 1964; Knox *et al.*, 1965; Santaniello and Caspi, 1976), or zinc in aqueous acetic acid (Rakhit and Gut, 1964; Dyer and Harrow, 1979; Holland and Taylor, 1978, 1981).

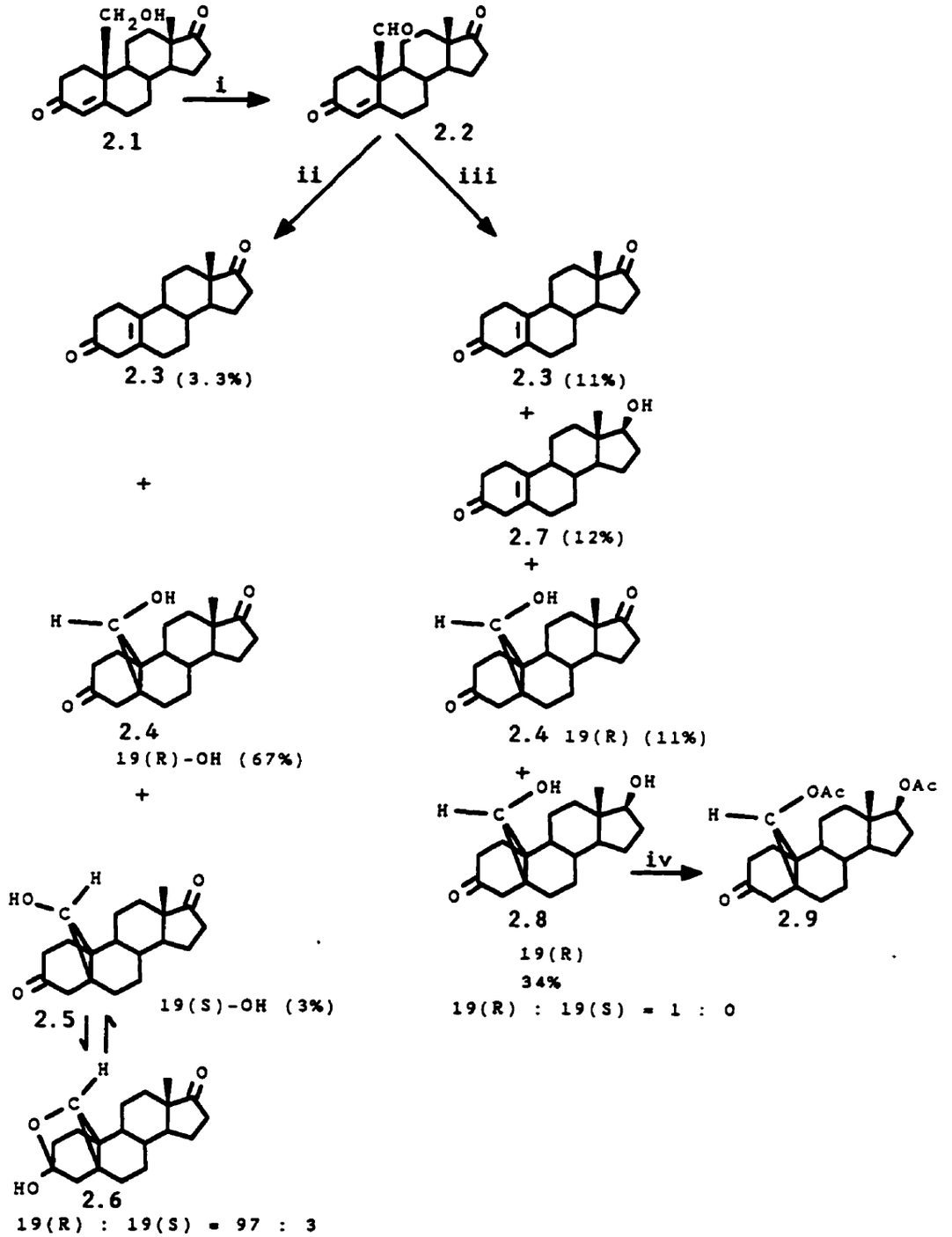


Scheme 2.2 The reported synthesis of unsaturated unsubstituted 5β,19-cyclosteroids.

The synthesis of unsaturated unsubstituted 5β,19-cyclosteroids with a double bond at C-1 involved (Scheme 2.2) reaction of 19-hydroxyandrost-4-ene-3,17-dione with diethyl-(2-chloro-1,1,2-trifluoroethyl)-amine

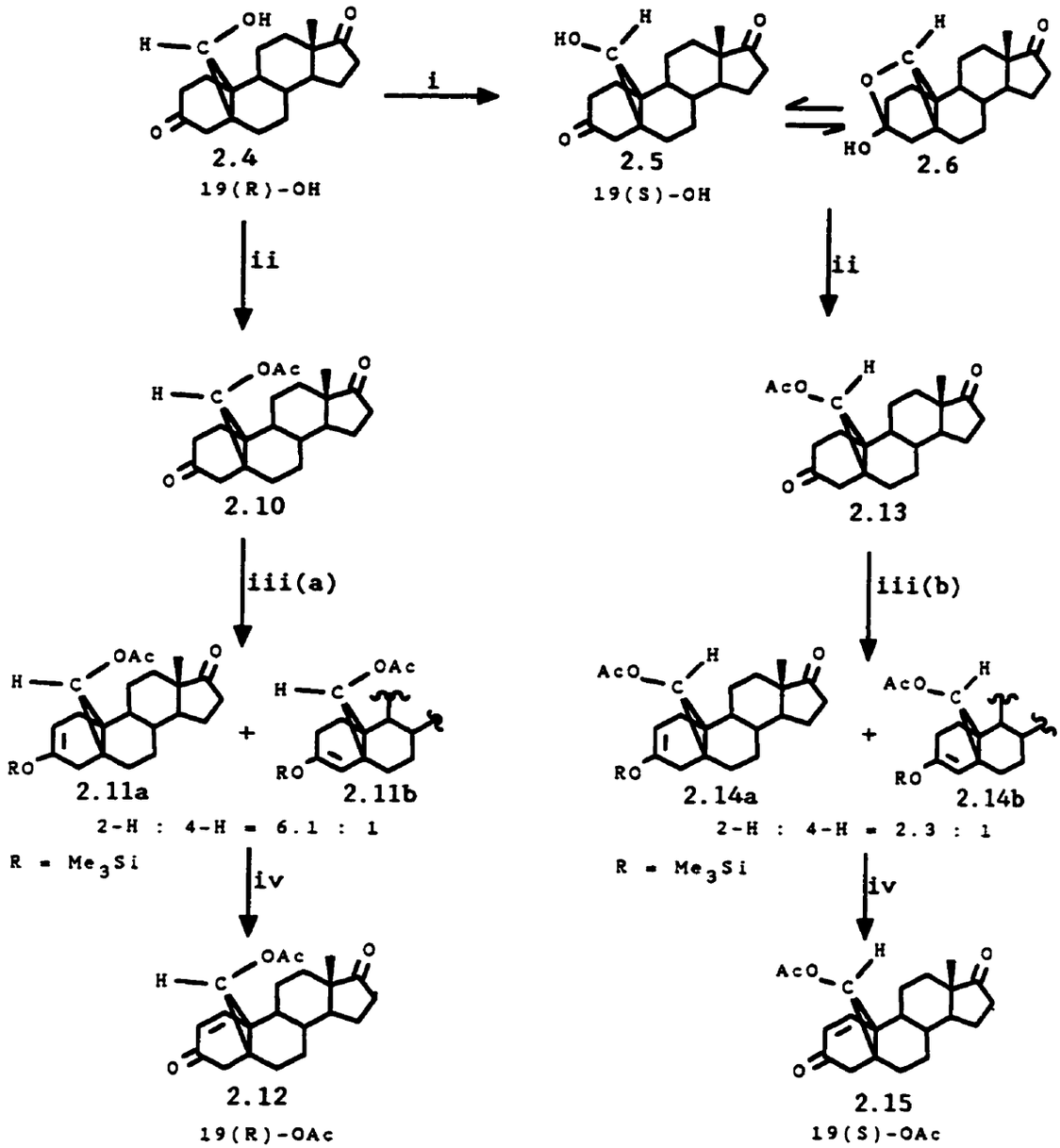
followed by unique rearrangement of the steroid 19-diethyl-[2-chloro-1,1,2-difluoroethyl]-amine derivative (Knox *et al.*, 1962, 1963) or reaction of the 19-mesylate-1,4-dien-3-one with Na-biphenyl in THF (Wieland and Anner, 1968; 1970). On the other hand (Scheme 2.2), solvolysis of a 19-tosylate-5-ene with $NaHSO_3$ (Antkowiak and Mscisz, 1987) or 19-mesylate-5-ene with pyridine (Bonet *et al.*, 1962) or 19-bromo-5-ene with $NaHSO_3$ in DMF (Mathai and Pascal, 1994) produced C-6 unsaturated 5β,19-cyclosteroids.

Recently, Templeton *et al.* reported the first synthesis of isomeric 19-monosubstituted derivatives of 5β,19-cyclosteroids, namely 19(R/S)-chloro-5β,19-cycloandrostane-3,17-dione (Templeton *et al.*, 1994a, 1996b) and 19(R/S)-hydroxy-5β,19-cycloandrostan-3,17-dione **2.4** and **2.5/2.6** (Templeton *et al.*, 1994b, 1996a) (Scheme 2.3). While collaborators were working, in this laboratory, on the synthesis of 19(R/S)-chloro-5β,19-cycloandrostane two synthetic strategies were suggested to produce 19(R/S)-chloro-5β,19-cycloandrostane-3,17-dione. One of the strategies considered the synthesis of 19(R/S)-chloro-5β,19-cycloandrostanes *via* 19(R/S)-hydroxy-5β,19-cycloandrostan-3,17-dione intermediate followed by substitution of the 19(R/S)-hydroxy group with a chlorine atom *via* the S_N1 or S_N2 reaction (Majgier-Baranowska, personal communication to Weigang Lin, 1992) and the second one (2) *via* a reductive cyclization of the *gem* 19,19-halogens (Majgier-Baranowska, personal communucation to Weigang Lin, 1993). Later, an attempt to finalize one of the concepts, i.e. to exchange the 19-hydroxy group with a nucleophilic chlorine atom by treatment of 19(R)-hydroxy-5β,19-cyclosteroid with concentrated HCl in CH_2Cl_2 was unsuccessful and led to the ring opening yielding 5β-androstane-3,17-dion-19-al (Lin, 1994). To adopt the second strat-



Reagents: i, PCC, CH_2Cl_2; ii, Zn, 50% aqueous CH_3COOH, 20 °C; iii, Li, NH_3, -78 °C, THF; iv, Ac_2O, DMAP, CH_2Cl_2.

Scheme 2.3 Reductive cyclization of androst-4-ene-3,17-dion-19-al 2.2 with Zn in 50% H_2O-CH_3COOH and with Li in NH_3.



Reagents: **i**, 0.5M KOH/MeOH or CH_3COOH/THF or Lewis acid ($SnCl_2.H_2O$, $BF_3.OEt_2$); **ii**, Ac_2O, DMAP, CH_2Cl_2; **iii**, **(a)** TMSiOTf, *i*-Pr_2EtN, CH_2Cl_2, -78 °C, "reverse addition"; **(b)** TMSiOTf, Et_3N, DMF, 0 °C; **iv**, $Pd(OAc)_2$, CH_3CN.

Scheme 2.4 Synthesis of 19(R)- **2.12** and 19(S)-acetoxy-5β,19-cyclo-androst-1-ene-3,17-dione **2.15**.

egy, the 19,19-dihalogen steroid derivatives are required to be synthesized. The synthesis can be achieved by either a reaction of the 19-aldehyde steroid with appropiate reagents or by insertion of dihalogen carbenes ($:CCl_2$ or :CClF) into the C-10 position, or by treatment of the 19-oxime steroid derivative with Cl_2 in the presence of Lewis acids (Tordeux *et al.*, 1992).

The objective of this project was to introduce 1,2-unsaturation into ring A of the saturated 19(R/S)-acetoxycycloandrostane **2.10** and **2.13** (Scheme 2.4). The key synthetic step, in the preparation of the 19(R)- **2.12** and 19-(S)-acetoxy-5β,19-androst-1-ene-3,17-dione **2.15** (Scheme 2.4), required regioselective introduction of the unsaturation into ring A without affecting the strained 5β,19-cyclopropane ring. In search for mild oxidation conditions, attention was paid to oxidation of the appropriate silyl enol derivatives **2.11** and **2.14** (Scheme 2.4).

In general, formation of α,β-unsaturated carbonyl compounds can be achieved by oxidation of saturated ketones. Established methods are based on introduction of heteroatoms (halogen, S, and Se) at the α-position and their *syn* elimination. The following reagents or methods can be employed: Ph_2Se_2, CSA, and 3-iodylbenzoic acid (Barton *et al.*, 1989), Ph_2Se_2, 3-iodylbenzoic acid (Barton *et al.*, 1981), benzeneselenic anhydride (Barton *et al.*, 1982a,b; 1980, 1978), *t*-butylhypochlorite (Beereboom *et al.*, 1953), dicyanodichloroquinone (1961); Shimizu *et al.*, 1966), selenylation (Reich *et al.*, 1993, 1973,; Clive 1978, 1973; Sharpless *et al.*, 1973), sulfurylation (Trost and Salzmann, 1973; Trost *et al.*, 1976), bromination-dehydrobromination (Stotter, 1973),

Similarly, oxidations of enol silyl or acetyl ethers lead to α,β-unsaturated ketones when DDQ-BSTFA (Bhattacharya *et al.*, 1988),

DDQ-collidine (Fleming and Paterson, 1979), DDQ-BTMSA (Ryn *et al.*, 1978), palladium acetate (Ito *et al.*, 1978), trityl tetrafluoroborate and DDQ alone (Jung *et al.*, 1977), as well as selenylating agents such as $C_6H_5Se(O)Cl$ (Reich, 1975), PhSeCl (Reich and Wollowitz, 1993), and $(C_6H_5SeO)_2O$ (Barton *et al.*, 1980) are used.

As reported previously (Lin, 1994), attempts to introduce a double bond into ring A of 19(R)-acetoxy-5β,19-cycloandrostane-3,17-dione by treatment with either DDQ or benzeneselenic anhydride in benzene under reflux resulted in ring opening to afford 5β-androstane-3,17-dion-19-al, 5β-estrane-3,17-dion-5β-al, and an unidentified product. After several unsuccessful attempts, finally, introduction of the 1,2-double bond, into ring A, was achieved by employing modified Ito and Sagausa oxidation of the TMsilyl enol ethers **2.11** and **2.14** (Scheme 2.4). This was done with an equimolar amount of palladium acetate in acetonitrile.

Results and Discussion

Saturated 19(R/S)-hydroxy-5β,19-cycloandrostanes, **2.4** and **2.5/2.6**, (Scheme 2.3) were synthesized, with a slight modification, following methods developed in this laboratory (Templeton *et al.*, 1996). Androst-4-ene-3,17-dion-19-al **2.2** was prepared by PCC oxidation of 19-hydroxyandrost-4-ene-3,17-dione **2.1**. PCC (acidic) instead of PDC (basic) was used to minimize formation of by-products. Treatment of the 19-aldehyde **2.2** with zinc in aqueous acetic acid gave a mixture of estr-5(10)-ene-3,17-dione **2.3** (3.3%) together with the 19(R)-alcohol (62.5%) **2.4** and a mixture 19(S)-alcohol/hemiacetal **2.5/2.6** (3%), (Templeton *et al.*, 1994, 1996a). However, treatment of the aldehyde **2.2** with zinc in glacial acetic acid yielded also the 19(R)-alcohol **2.4** (60 %). The reaction to be completed required a longer time (4 h *vs* 1.5). Surprisingly, no estra-5(10)-ene-3,17-dione **2.3** was formed as

shown by the ^1H NMR spectrum of the crude product. This may indicate that the initial attack of a molecule of water on the 19-aldehyde group is required to produce estr-5(10)-ene-3,17-dione **2.3** (Templeton *et al.*, 1996).

Additionally, to explore the remarkable degree of stereoselectivity, a metal ammonia reduction of the 19-aldehyde **2.2** was designed. In particular, this experiment was performed to determine the ratio of products with the 19-hydroxy group projected above rings A and B.

Treatment of the aldehyde **2.2** with lithium in liquid ammonia gave the reductive cyclization product the 19-(R)-alcohol **2.4** (11%) as obtained with zinc in the aqueous acetic acid treatment together with the corresponding 17β-alcohol **2.8** (34%), and the 5(10)-olefin **2.3** (11%) and its 17β-alcohol **2.7** (12%). The 17β-alcohol **2.8** was further characterized as the diacetate **2.9**.

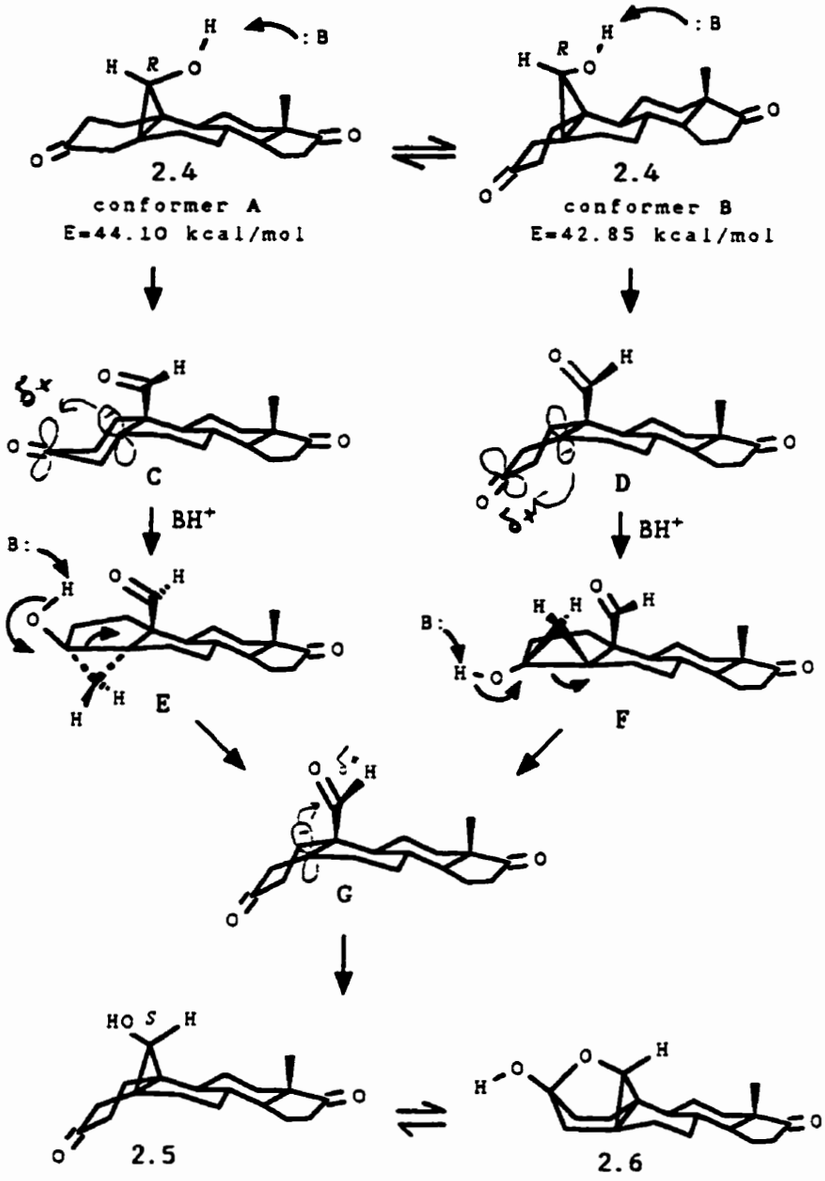
As reported (Templeton, Ling *et al.*, 1994b), treatment of the 19(R)-alcohol **2.4** with dilute solutions of either HCl-THF or methanolic KOH caused its isomerization to the thermodynamically more stable 19(S)-alcohol **2.5**. Similar treatment of the 19(R)-alcohol **2.4** with CH_3COOH-THF or Lewis acids, $ZnCl_2.2H_2O$ in CH_3CN or $BF_3.OEt_2$-Et_2O in THF, at room temperature led to the 19(S)-hydroxy/hemiketal mixture **2.5/2.6**. It was also noted that the 19(R)-alcohol **2.4** was unstable even in CH_2Cl_2 solution, at ambient temperature, and epimerized to its more thermodynamically stable epimer, the 19(S)-alcohol **2.5**.

Ring A conformational analysis of compounds 19(R)-hydroxy--5β,19-cycloandrostane-3,17-dione **2.4** and 19(R)-acetoxy-5β,19-cycloandrost-3,17-dione **2.10** were performed based on the coupling constants and nuclear Overhauser effect (Marat, 1995). The analysis data showed

that there are no clear axial or equatorial environments for the C-1 and C-2 protons. Therefore, Marat (Marat, 1995) concluded that ring A, of the 19(R)-hydroxy **2.4**, exists in the equilibrium between a boat conformation with H-1α, 2β, and H-4α- axial (conformer **A**), (Scheme 2.5) and an inverted boat conformation with H-1β, H-2α, and H-4β-axial (conformer **B**). As shown in Scheme 2.5, MMX force field calculations of the minimum energies of compound **2.4** for both conformers, **A** and **B**, are comparable (ΔE = 1.25 kcal mol^{-1}) which could be consistent with the suggested conformational equilibria. The proposed mechanism of epimerization, of the 19(R)-alcohol **2.4** to **2.5** 19(S)-alcohol, under basic conditions, is shown in Scheme 2.5.

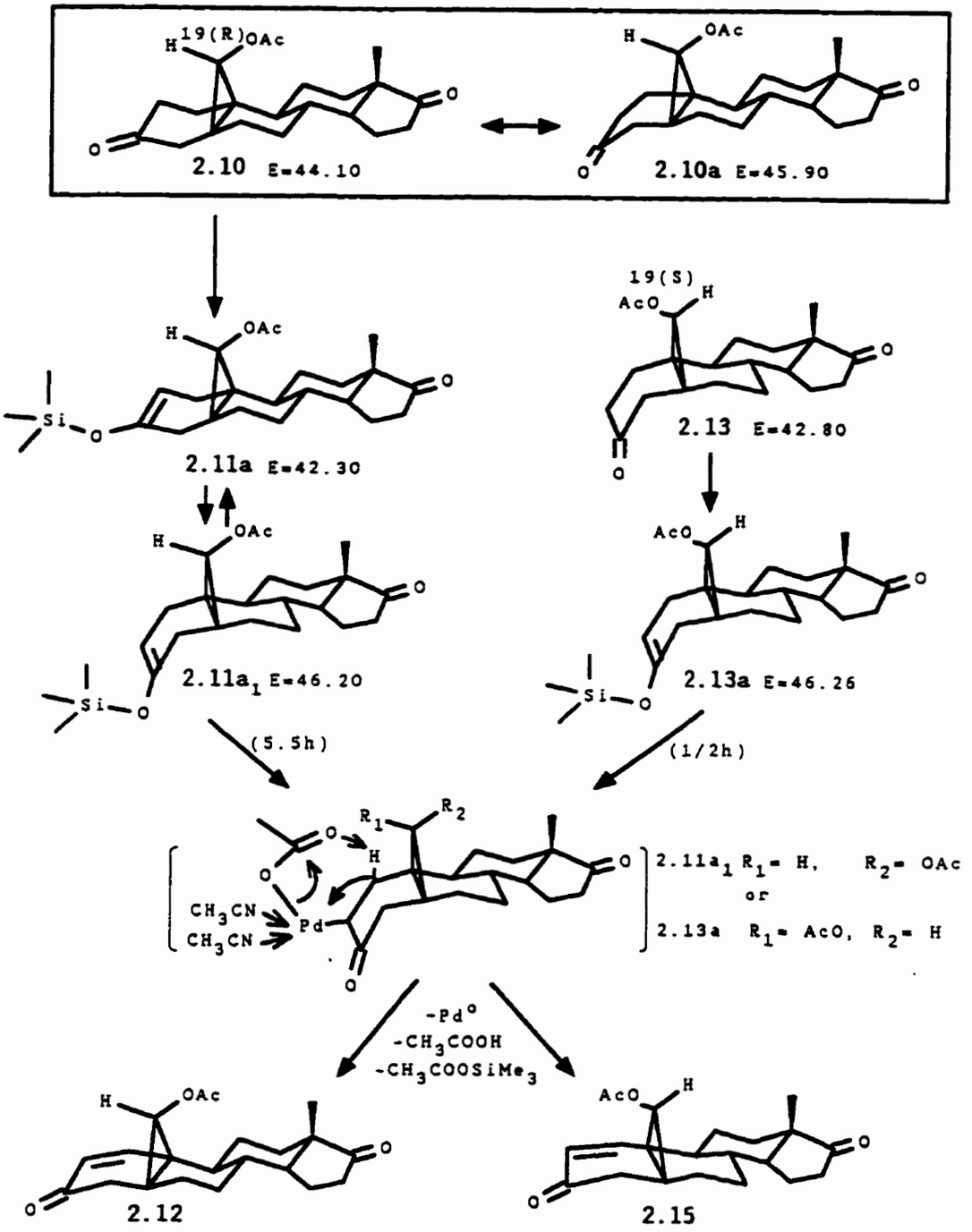
Separate acetylation of the 19(R)- **2.4** and 19(S)-alcohols **2.5/2.6** gave the corresponding acetates, **2.10** and **2.13**, respectively (Scheme 2.4). Production of an enol form of the saturated 19(R)-acetoxy-3,17-dione, under kinetically controlled conditions ("reverse" addition), with TMSiOTf in the presence of hindered base, iPr_2EtN, in CH_2Cl_2 at low temperature (-78 °C) gave an inseparable mixture of the 3-siloxy 2-ene/3-ene **2.11a** and **2.11b** (H-2:H-4; 6.1:1) in 70%. It was noted that TMSiOTf in the presence of iPr_2EtN is regioselective in a kinetic sense. Using TBDMSiOTf or TMSiOTf together with Et_3N, as a base, was not fully successful. Not only enolization of ring A, at C-2 and C-3 occured but also at ring D lowering the product yields.

Treatment of the isomeric 19(S)-acetoxy-3,17-dione **2.13**, under thermodynamically controlled conditions, with TMSiOTf and Et_3N in DMF at room temperature gave an inseparable mixture of the 3-siloxy 2-ene/3-ene, **2.14a** and **2.14b**, (H-2:H-4;2.3:1), but in a different ratio. The yield of the products **2.14a/2.14b** was lowered, because the enolization also occurred in ring D, as determined by the ^1H NMR spec-



Scheme 2.5 Proposed mechanism of epimeriaztion of 19(R)-hydroxy-5β,-19-cycloandrostane-3,17-dione **2.4** to 19(S)-hydroxy-5β,19-cycloandrostane-3,17-dione **2.5/2.6** under basic conditions. E (kcal/mol) is minimized energy of the structure determined by the MMX force field.

trum. On chromatographic separation of the enolization products, a mixture of the 3-siloxy-2-en/3-en **2.14a/2.14b** was obtained in only 46% yield. Preliminary trials to introduce, directly or indirectly, 1,2-unsaturation into ring A, using small scale experiments monitored by ^1H NMR spectra, proved to be unsuccessful. Treatment of the 19(R)--acetoxy **2.10** with $(PhSe)_2$, CSA, and $3\text{-}IO_2C_6H_4COOH$ yielded products which did not indicate the formation of the 1,2-double bond, and they were not further identified. Similarly, reaction of the silyl enol ethers **2.11a/2.11b** with PhSeCl in ethyl acetate, followed by selenium oxidation with hydrogen peroxide was also unsuccessful (Sharpless *et al.*, 1973). Similar treatment of the silyl enols **2.11a/2.11b** with DDQ-Et_3N in room temperature led to recovery of the starting material **2.10**. On the other hand, oxidation of the silyl enols **2.11a/2.11b**, with NBS in THF at 0°C (Blanco *et al.*, 1976), led to substitution of bromine at C-2 and C-4, as shown by the ^1H NMR spectrum, but the compound was unstable. Even treatment of a mixture of the enols **2.11a/2.11b** with a catalytic amount of palladium acetate in the presence of quinone, to reoxidize reduced palladium (Ito *et al.*, 1978), failed. Finally, separate treatment of the mixture of TMsilyl enol ethers **2.11a/2.11b** and **2.14a/2.14b** with equivalent amount of $Pd(AcO)_2$ in CH_3CN for 5.5 h and 30 min, respectively, gave the desired 1,2-unsaturated ketones **2.12** and **2.15**. There is a different reaction rate for **2.11a** and **2.14a**. This again can be explained by the conformational changes of ring A. As mentioned above, the NMR conformational analysis of ring A of both saturated compounds, the 19(R)-acetoxy- **2.4** and the 19(S)-acetoxy-5β,19-cyclosteroid **2.13**, showed that ring A adopts both a boat and inverted boat form for the 19(R)-acetoxy- **2.10** and an inverted boat for the 19(S)-acetoxy- **2.13**



Reagents: i, TMSiOTf, iPr_2EtN, -78 °C; ii, $Pd(OAc)_2$, CH_3CN.

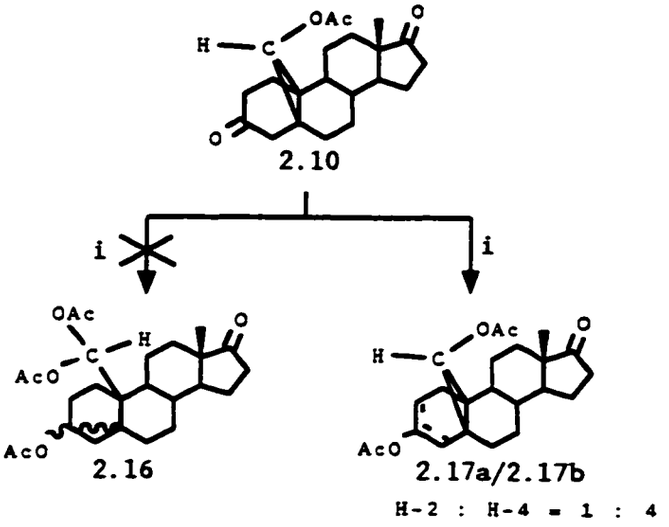
Scheme 2.6 Proposed formation mechanism of the unsaturated compounds **2.12** and **2.15** *via* palladium oxidation (E in kcal mol^{-1}).

(Marat, 1995). Furthermore, the 19(R)-acetoxy **2.10** conformer exists in equlibrium with its conformer **2.10a** having ring A in an inverted boat (Marat, 1995), (Scheme 2.6). MMX force field calculations of the **2.10** and **2.10a** structures showed that the boat-like conformer is only 1.8 kcal mol^{-1} more stable than the corresponding conformer with an "inverted boat". Therefore, an inverted boat-like transition state may well represent the principal path of the reaction as a result of steric interaction between the palladium species and the 19-H proton (above ring A). These conclusions are derived from the observed rate of the reactions.

As outlined in Scheme 2.6, ring A of the 19(R)-acetoxy **2.10** adopts a boat conformation (major conformer) and of the 19(S)-acetoxy **2.13** the inverted boat form. The same conformational structures of ring A could be valid for the silyl enol ethers **2.11a** and **2.13a**, i.e. precursors of unsaturated compounds **2.12** and **2.15**, respectively. As might be expected intuitively, there is a relationship between conformations of ring A and interactions of the palladium active species. In general, for the oxidation reaction of silyl enol ethers **2.11a** and **2.13a** to proceed, a palladium species must form a π-complex (Heck, 1985) with a double bond of the substrate **2.10a**$_1$ or **2.13a** (Scheme 2.6). Because the rate of oxidation of the 19(S)-acetoxy-5β,19-cyclo-3-silyl enol ether **2.13a** is faster (30 min) than the 19(R)-acetoxy analogue **2.10a** (5.5 h), it suggests that a structure with an inverted boat conformation of ring A favours approach of the palladium species to the π-system of the β-face.

For both compounds, **2.11a** and **2.13a**, the oxidation reaction, presumably, proceeds *via* the common intermediate, as shown in Scheme 2.6, with *syn* elimination of the axial 1β-H. A second possible

explanation, for the faster reaction rate, is that reaction of **2.13a** with the palladium species proceeds *via* neighbouring group participation of one of non-bonding pairs of the carbonyl oxygen of the 19(S)-acetoxy substituent which would facilitate delivery of palladium to the β face of the nucleophilic double bond. On the other hand, longer reaction time (5.5 h) required for the 19(R)-acetoxy-5β,19-cycloacetoxy-5β,19-cycloandrost-2-en-3-siloxy **2.11a** implies that



Reagents: i, Ac_2O, $BF_3.OEt_2$, CH_2Cl_2.

Scheme 2.7 Synthesis of a mixture of 3-acetoxy,19(R)-androst-2-en-17-one and 3,19(R)-diacetoxy-5β,19-cycloandrost-3-en-17-one, **2.17a/ 2.17b**.

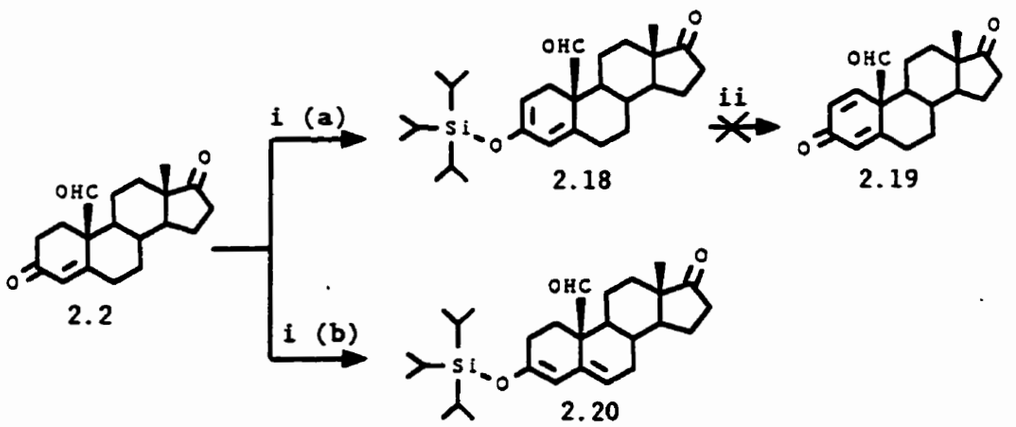
conformational changes of ring A, from a boat form **2.11a** to an inverted boat form **2.11a**, must take place before the reaction occurs (Scheme 2.6). Moreover, the 19(R)-acetoxy group, in compound $\mathbf{2.11a}_1$, is not available to deliver the palladium species to the 2,3-double bond, because of its *trans* orientation. To extrapolate this observation more

studies of the corresponding enols with or without the neighbouring acetoxy group or other functional group could be undertaken.

In a separate reaction (Scheme 2.7) an attempt to intercept isomerisation intermediates, **E** or **F** (Scheme 2.6), by treatment of the 19(R)-acetate cyclosteroid **2.10** with acetic anhydride and $BF_3.OEt_2$ in CH_2Cl_2 gave, instead of the expected 19,19-acetates **2.16**, an inseparable mixture of the 2,3- and 3,4-acetoxy enols, **2.17a/2.17b**, in the reverse ratio (H-2:H-4;1:4) to the analogous silyl enols, **2.11a/2.11b** (H-2:H-4; 4:1). Because the ring opening did not occur, it means that the reagent employed, $BF_3.OEt_2$, was not strong enough to initiate the isomerization process. The advantage of this new synthetic method is the formation of the enol acetates, selectively, in ring A. So far, the reported syntheses of enol acetates involved acetic anhydride in the presence of base, such as NaOAc, KOAc, K_2CO_3, Et_3N and catalytic amount of DMAP, $LiN(SiMe_3)_2$ or a catalytic amount of strong acids such as TsOH and $HClO_4$ (Larock, 1989). Other reagents have been used, namely, $H_2C{=}C(Me)OAc$/cat TsOH, $H_2C{=}C(Me)OAc$/cat TsOH/-$Cu(OAc)_2$, AcCl/KH and DMAP (Larock, 1989). Other methods to produce enol acetates involved: (1) acid-catalized ring opening and elimination of α,β-epoxysilanes formed by hydrosilylation and epoxidation of terminal acetylenes (Nesmeyanov, 1954) and (2) vinylic mercuric acetates decomposition upon palladium acetate (Larock, 1980). Reported synthesis of geminal acetates of aldehydes involved acetic anhydride and ferric chloride (Kochbar *et al.*, 1983).

A trial reaction to simultaneously synthesize both 19ξ-hydroxy-1β,19-cycloandrost-4-ene-3,17-dione and 19ξ-hydroxy-5β,19-cycloandrost-1-ene-3,17-dione by employing Zn-50% aqueous CH_3COOH (Lin, 1994) or Li-NH_3 required androsta-1,4-diene-3,17-dion-19-al as the intermediate,

Scheme 2.8. However, attempted synthesis of androst-1,4-dien-3,17-on-19-al **2.19** *via* palladium oxidation of the 2,4-homoannular dienol triisopropylsilyl ether **2.18** failed. Although treatment of androst-4-ene-3,17-dion-19-al **2.2** with iPr_3SiOTf and Et_3N in CH_2Cl_2 at -78 °C ("reverse addition") gave the expected homoannular 2,4-dienol triisopropylsilyl ether **2.18**, subsequent palladium acetate oxidation of **2.18** yielded a mixture of products which did not indicate the formation of 1,4-diene bond, and they were not further identified. When the reaction was repeated, under the same conditions but with the faster addition of **2.2** (5 min vs 20 min.), a more stable structure, the 3,5-dienol silyl ether **2.20**, was obtained as determined by the ^1H and ^{13}C NMR spectra (see the experimental part).

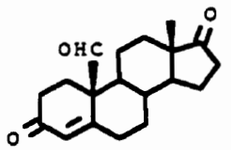
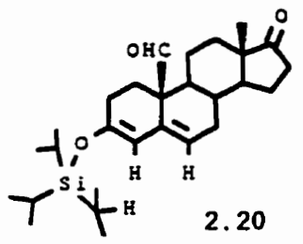


Reagents: **i**, *i*Pr_3SiOTf, *i*Pr_2EtN, CH_2Cl_2, -78 °C (a) 20 min.; (b) 5 min. addition; **ii**, $Pd(OAc)_2$, CH_3CN.

Scheme 2.8 Synthesis of 3-O-(triisopropylsilyl)-androst-2,4-dien-3,17-on-19-al **2.19** and 3-O-(triisopropylsilyl)-androst-3,5-dien-17-on-19-al **2.20**.

Korenchuk *et al.* (Korenchuk *et al.*, 1985) as well as Cole and Robinson (Cole and Robinson, 1991) reported selective kinetic enolization of 17β-O-(terahydropyranyl)-androst-4-en-3-on-19-al toward C-2 to afford 3-O-(tert-butyldimethylsilyl)-17β-O'-(tetrahydropyranyl)-androsta-2,4-diene-3,17-diol-19-al: the synthesis was accomplished with TBDMSiOTf and collidine at 0° C.

Table 2.1 Conformations of the 19-CHO group, in compounds **2.2** and **2.20** determined by the NOE experiments.

Compound	H's Irradiated	H's Enhancement(%)
 2.2	19-H	1β-H (1.2)
		2β-H (1.42)
		6α-H (1.33)
		6β-H (4.57)
		8β-H (1.9o)
		11β-H (1.83)
 2.20	19-H	2-H (1.2)
		4-H (0.43)
		8β-H (6.26)
		11β-H (1.3)
	4-H	6-H (11.96)
		$Si[H(CH_3)_2]_3$ (3.22)
		$Si[H(CH_3)_2]_3$ (5.96)

Tanable and Crowe (1973) also synthesized a 2,4-dienol silyl derivative, under kinetically controlled conditions, by treatment of 17β-O-(tetrahydropyranyl)-androst-4-ene-3-one with lithium hexamethyldisilizane followed by trapping the lithium 2,4-dienolate ions with *tert*-butyldimethylchlorosilane.

Conformations of the 19-aldehyde group in androst-4-ene-3,17-dion-19-al **2.2** and 3-O-(triisopropylsilyl)-androsta-3,5-dien-17-on-19-al **2.20** have been determined by NOE experiments. The results are given in Table 2.1. For both compounds, **2.2** and **2.20**, rotation of the 19-CHO group around the C(10)-C(19) bond is observed.

2.1.1 Summary

1. Unsaturated compounds, 19(R)-hydroxy-5β,19-cycloandrost-1-ene-3,-17-dione acetate **2.12** and 19(S)-hydroxy-5β,19-cycloandrost-1-ene-3,17-dione acetate **2.15**, have been synthesized for biological evaluation in terms of aromatase inhibition.
2. Dissolving metal reduction (Li-NH_3) of androst-4-en-3,17-dion-19-al **2.2** was designed to determine the ratio of the 19(R)-hydroxy- **2.4** and 19(S)-hydroxy- **2.5** products. Results of two experiments of the reductive cyclization of androst-4-ene-3,17-dion-19-al **2.2** both with zinc metal in 50% aqueous acetic acid solution and with lithium metal in liquid ammonia were compared (Scheme 2.3). The ratio of the 19(R/S)-hydroxy groups above rings A and B for both cyclizations was determined.
3. A new synthetic method to produce selectively, in ring A, 3-enol acetates of steroid derivatives by employing Ac_2O and $BF_3.OEt_2$ in CH_2Cl_2 was developed.
4. Suggestions have been made that: (1) the 3-siloxy-19(R)-acetoxy

2-ene **2.11a** might have reacted with a palladium species *via* conformational changes of ring A; and (2) the 3-siloxy-19(S)-acetoxy 2-ene **2.13a** may react with a palladium species *via* neighbouring group participation of one of lone pairs of the carbonyl oxygen of the 19(S)-acetoxy group.

2.1.2 Experimental

Androst-4-ene-3,17-dion-19-al 2.2

The mixture of 19-hydroxyandrost-4-ene-3,17-dione **2.1** (10 g, 33.1 mmol), pyridinium chlorochromate (10.7 g, 50 mmol, 1.5 eq.) in CH_2Cl_2 (80 mL) and molecular sieves 4A (1g, 10% of **2.1**) was stirred at 20 °C, under a positive pressure of argon, until TLC indicated no starting material (ca 24 h). Diethyl ether (100 mL) was added and the mixture filtered, through a silica pad and $MgSO_4$ ($MgSO_4$ added to remove Cr^{3+}), with excess of diethyl ether to give a filtrate, which was washed with saturated aqueous $NaHCO_3$, water, dried, filtered, and evaporated to give a crude product (9.95 g). The product after crystallization, from CH_2Cl_2-EtOAc, gave the 19-aldehyde **2.2** (8.5 g, 28 mmol, 85%), mp 134-136 °C (lit., 129-133 °C; Hagiwara *et al.*, 1960).

^{1}H NMR of **2.2** ($CDCl_3$); δ 9.88 (s, 1H, 10-CHO), 5.93 (s, 1H, 4-H), 0.83 (s, 3H, 18-CH_3).

^{13}C NMR of **2.2** ($CDCl_3$): δ 34.09 (1), 29.61 (2), 197.54 (3), 127.53 (4), 160.35 (5), 33.67 (6), 31.41 (7), 36.38 (8), 53.54 (9), 54.97 (10), 21.09 (11), 30.27 (12), 47.32 (13), 50.91 (14), 21.57 (15), 35.58 (16), 219.51 (17), 13.65 (18), 200.71 (19).

Estr-5(10)-ene-3,17-dione 2.3, 19(R)-Hydroxy-5β,19-cycloandrostane-3,-17-dione 2.4, 19(S)-Hydroxy-5β,19-cycloandrostane-3,17-dione/3-Hydroxy-3β,19(S)-epoxy-5β,19-cycloandrostan-17-one 2.5/2.6

Reductive cyclization with Zn in 50% CH_3COOH/H_2O

To a solution of the 19-aldehyde-4-ene **2.2** (8.5 g, 16.8 mmol) in 50% aqueous acetic acid (125 mL) was added Zn powder (41.5 g; the reaction is exothermic) and the heterogenous mixture stirred for 3.5 h. The reaction mixture was diluted with CH_2Cl_2 (150 mL) and filtered through celite. The organic filtrate was washed with water, saturated $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated to give a crude product, which on FCC, on elution with 10-40% acetone-LP gave fractions which yielded ester-5(10)-3,17-dione **2.3** (153 mg, 0.55 mmol, 3%) and a mixture of the 19(R/S)-alcohols **2.4/2.6**. The 19(R/S)-alcohols on crystallization in CH_2Cl_2-EtOAc gave a pure sample **2.4** (3.14 g, 10.4 mmol, 37%), mp 160-167 °C (decomp.), (from CH_2Cl_2-EtOAc), (lit,. 161-164 °C, from CH_2Cl_2-Et_2O; Lin 1994).

^{1}**H NMR** of **2.4** ($CDCl_3$); δ 3.30 (s, 1H, 19-H), 2.49 (d, J 17.1 Hz, 1H, 4α-H), 2.40 (dd, J 19.2, 10.3 Hz, 1H, 16β-H), 2.31 (d, J 17.2 Hz, 1H, 4β-H).

^{13}**C NMR** of **2.4** ($CDCl_3$): δ 27.61 (1), 36.28 (2), 212.31 (3), 47.89 (4), 21.19 (5), 25.71 (6), 26.23 (7), 36.83 (8), 46.47 (9), 25.33 (10), 24.23 (11), 32.20 (12), 48.68 (13), 51.16 (14), 21.62 (15), 35.63 (16), 221.22 (17), 14.35 (18), 63.40 (19).

Epimerization of **2.4** *to* **2.5/2.6** *under basic conditions*:

The mother liquor, from the above crystallization of products obtained from the reductive cyclization, was evaporated and the oily residue (5.36 g) treated with 0.5 M methanolic KOH (85 mL). The mixture was stirred at room temperature for 1.5 h and then diluted with CH_2Cl_2 (300

mL), washed with water, dried, filtered, and evaporated to give a crude product (5.02 g), which on FCC, on elution with 20% acetone-LP, yielded the 19(S)-alcohol/hemiacetal mixture **2.5/2.6** (2.47 g, 8.87 mmol, 53%), mp 160-165 °C (decomp.), (from CH_2Cl_2-EtOAc), (lit., 161-164 °C, from CH_2Cl_2-Et_2O; Lin 1994).

^1H NMR of the 19(S)-alcohol/hemiacetal **2.5/2.6** ($CDCl_3$); δ 3.43 (bs, 1H, 19(S)-H of **2.5**), 2.47 (d, J 8.3, 1H, 16-H), 2.40 (d, J 8.4, iH, 16-H), 0.89 (s,3H, 18-CH_3).

^1H NMR of the 19(S)-alcohol/hemiacetal **2.5/2.6** (acetone-d_6): δ 5.07 (bs, 1H, 3α-OH of **2.6**), 4.5 (s, 1H, 19(S)-H of **2.6**), 3.77 (s, 1H, 19(S)-H of **2.5**), 0.86 (s, 3H, 18-CH_3).

Estr-5(10)-ene-3,17-dione 2.3, 19(R)-Hydroxy-5β,19-cycloandrostane-3,17-dione 2.4, 17β-Hydroxyestr-5(10)-en-3-one 2.7, and 17β,19-dihydroxy-5β,19-cycloandrostane-3-one 2.8

Reductive cyclization with Li in liquid NH_3

A solution of the 19-aldehyde **2.2** (500 mg, 1.66 mmol) in tetrahydrofuran (25 mL) was added over a period of 1h to a stirred mixture of liquid ammonia (100 mL) and THF (10 mL) containing lithium metal (681 mg, 98 mmol). Stirring was continued for a further 30 min at which time solid NH_4Cl (7.0 g, 130 mmol) and Et_2O (100 mL) were added. Evaporation of the ammonia yielded an organic solution, which was washed with water, dried, filtered, and evaporated to give a residue. The residue on FCC, on elution with 10-40% acetone-LP, gave fractions which yielded the estra-5(10)-diketone **2.3**, (50 mg, 0.18 mmol, 11%), mp 147-149 °C (from acetone-LP), (lit., 144-146 °C; Ueberwasser *et al.*, 1963), the 19(R)-cycloalcohol **2.8** (55 mg, 0.18 mmol, 11%), mp 166-168 °C (from CH_2Cl_2-LP), and the 17β,19(R)-cyclodiol **2.8** (173 mg, 0.57 mmol, 34.3%), mp 168-170 °C (from acetone-EtOAc),

(lit., 165-168 °C, form CH_2Cl_2-Et_2O, Templeton *et al.*, 1996) the 17β-hydroxy estr-5(10)-3-one **2.7** (53 mg, 0.19 mmol, 11.6%), mp 192-196 °C (from acetone-LP), (lit., 193-196 °C; Wilds and Nelson, 1953), and the 17β,19(R)-diol **2.8** (173 mg, 0.57 mmol, 34%), mp 168-170 °C (from acetone-EtOAc-LP).

^{1}H NMR of 2.3 ($CDCl_3$): δ 2.81 and 2.70 (d, J_{AB} 21.2, 4-H_2), 0,91 (s. 1H, 18-CH_3).

^{13}C NMR of 2.3 ($CDCl_3$): δ 24.80 (1), 38.97 (2), 207.91 (3), 44.67 (4), 126.66 (5), 32.39 (6), 27.54 (7), 38.66 (8), 46.24 (9), 130.60 (10), 25.93 (11), 30.60 (12), 47.98 (13), 49.98 (14), 21.43 (15), 35.61 (16), 217.50 (17), 13.98 (18).

^{1}H NMR of 2.7 ($CDCl_3$): δ 3.69 (dd, J 14.44, 8.02 Hz, 1H, 17β-H), 2.78 (d, J 21.4 Hz, 1H, 4α-H), 2.68 (d, J 20.6 Hz, 1H, 4β-H), 2.46 (m, 16β-H), 0.77 (s, 3H, 18-CH_3).

^{13}C NMR of 2.7 ($CDCl_3$): δ 22.99 (1), 39.09 (2), 211.23 (3), 44.64 (4), 126.40 (5), 30.70 (6), 27.44 (7), 39.08 (8), 46.16 (9), 131.00 (10), 26.43 (11), 30.70 (12), 43.56 (13), 49.68 (14), 25.11 (15), 36.98 (16), 81.84 (17), 11.28 (18).

^{1}H NMR of 2.8 ($CDCl_3$); δ 3.67 (t, 1H, 17β-H), 3.3 (s, 1H, 19-H), 2.49 (d, J 17.07. 1H, 4α-H), 2.32 (d, J 17.07 Hz, 1H, 4β-H), 0.77 (s, 1H, 18-CH_3).

^{13}C NMR of 2.8 ($CDCl_3$): δ 28.05 (1), 36.64 (2), 215.17 (3), 48.43 (4), 21.02 (5), 25.95 (6), 27.33 (7), 37.33 (8), 46.77 (9), 25.64 (10), 24.62 (11), 37.64 (12), 44.12 (13), 51.10 (14), 23.37 (15), 30.13 (16), 81.68 (17), 11.55 (18), 63.58 (19).

17β,19(R)-Diacetoxy-5β,19-cycloandrostane-3-one 2.9

The 17β,19(R)-cyclodiol **2.8** (153 mg, 0.502 mmol) in CH_2Cl_2 (5 mL) was treated with Et_3N (200 μL) and Ac_2O (500 μL) at 20 °C for 1 h. CH_2Cl_2

(20 mL) was added and the organic layer washed with 3% HCl, water, saturated aqueous $NaHCO_3$, water, dried, filtered, and evaporated to give a residue which on FCC, on elution with 10% acetone-LP, gave the 17β,19(R)-diacetate **2.9** (130 mg, 0.34 mmol, 67%), mp 139.5-142 °C (from EtOAc-LP) (Found: C, 70.10; H, 8.30. $C_{23}H_{32}O_5$ requires C, 71.09; H, 8.31%).

^{1}H NMR of 2.9 ($CDCl_3$); δ 4.63 (dd, J 9.0, 7.8 Hz, 3H, 17-CH_3COO), 4.00 (s, 1H, 19-H), 2.55 and 2.48 (d, J_{AB} 17.9 Hz, 1H, 4β-H and 4α-H), 2.14 (s, 3H, 19-CH_3COO), 2.04 (s, 17-CH_3COO), 0.82 (s, 3H, 18-CH_3).

^{13}C NMR of 2.9 ($CDCl_3$): δ 28.05 (1), 36.64 (2), 215.17 (3), 48.43 (4), 21.02 (5), 25.95 (6), 27.33 (7), 37.33 (8), 46.77 (9), 25.64 (10), 24.62 (11), 37.64 (12), 44.12 (13), 51.10 (14), 23.37 (15), 30.13 (16), 81.68 (17), 11.55 (18), 63.58 (19), 20.53 (19-OCO$\underline{C}H_3$), 171.14 (19-O$\underline{C}OCH_3$).

19(R)-Acetoxy-5β,19-cycloandrostane-3,17-dione 2.10

To a solution of the 19(R)-cycloalcohol **2.4** (3.22 g, 11 mmol) in CH_2Cl_2 (60 mL) was added Ac_2O (11 mL, 11 mmol, 10 eq.) and Et_3N (1.5 mL, 16 mmol, 1.45 eq.) followed by 4-dimethylaminopyridine (DMAP) (132 mg, 1.1 mmol, 0.1 eq.). The mixture was stirred until TLC indicated no starting material (*ca* 2h). Methanol (20 mL) was added and the product extracted with CH_2Cl_2. The organic layer was washed with water, 3% HCl, water, saturated $NaHCO_3$, water, dried, filtered, and evaporated to give a crude product (3.35 g) which on FCC, on elution with 10% acetone-LP yielded the 19(R)-acetate **2.10** (2.99 g, 8.68 mmol, 79%), mp 184-186 °C (from CH_2Cl_2-EtOAc), (lit., 180-183 °C, from CH_2Cl_2-Et_2O; Lin, 1994).

^{1}H NMR of 2.10 ($CDCl_3$); δ 4.03 (s, 1H, 19-H), 2.54 (s, 4-H), 2.46 (dd, J 19.2, 8.3 Hz, 1H, 16β-H), 2.14 s, 19-$OCOCH_3$), 0.91 (s, 3H, 18-CH_3).

^{13}C NMR of 2.10 ($CDCl_3$): δ 22.47 (1), 36.21 (2), 210.59 (3), 47.20 (4),

21.14 (5), 26.60 (6), 25.87 (7), 36.82 (8), 46.32 (9), 24.75 (10), 23.79 (11), 31.97 (12), 48.45 (13), 50.92 (14), 21.62 (15), 35.74 (16), 220.40 (17), 14.11 (18), 64.21 (19), 20.65 (19-OCO<u>C</u>H$_3$), 170.67 (19-O<u>C</u>OCH$_3$).

19(R)-Acetoxy-5β,19-cycloandrost-1-ene-3,17-dione 2.12

A solution of saturated 19(R)-acetoxy-3,17-dione **2.10** (500 mg, 1.45 mmol) in CH_2Cl_2 (2.5 mL) was added in portions over 5 min ("reverse" addition), to a stirred and cooled (acetone/dry-ice bath) mixture of *i*-Pr_2EtN (320 μL, 1.89 mmol, 1.3 eq.) and trimethylsilyl triflate (340 μL, 1.76 mmol, 1.2 eq.) in CH_2Cl_2 (10 mL) under an argon atmosphere. After stirring for a further 1.5 h, MeOH (0.5 mL) was added to destroy excess reagent followed by diethyl ether. The organic layer was washed with brine, dried, filtered, and evaporated to give a residue which on FCC, on elution with 18% Et_2O-LP containing 0.2% Et_3N, gave fractions consisting of a mixture of the 2- and 3-silyl enol ethers **2.11a/2.11b** (464 mg, 1 mmol, 70%), (H-2:H-4;6.1:1; δ ($CDCl_3$): 4.66 ddd, J_{H-2} 6.1, 2.4, 2.4 Hz; 5.30 d, J_{H-4} 2 Hz allylic coupling).

To a mixture of the 3-siloxy 2-ene/3-ene **2.11** (464 mg, 1.0 mmol) in CH_3CN (5 mL) was added solid $Pd(OAc)_2$ (263 mg, 1.2 mmol, 1.2 eq.). The mixture was stirred at 50°C for 5.5 h under an argon atmosphere and then the solvent evaporated to dryness at reduced pressure to give a residue. The residue was dissolved in diethyl ether (5 mL) and activated carbon added. The heterogenous mixture was refluxed for 5 min, filtered through a short column with celite/silica, and evaporated to give a crude product which on FCC, on elution with 5% acetone-LP, gave the 1,2-unsaturated 19(R)-acetoxy-3,17-dione **2.12** (120 mg, 0.35 mmol, 24 %), mp 146-149 °C (from Et_2O-LP), (Found: C, 73.4; H, 7.8. $C_{21}H_{26}O_4$ requires C, 73.6, H, 7.65%).

^{1}H NMR of **2.12** ($CDCl_3$); δ 7.14 (d, J 10.2 Hz, 1H, 1-H) , 5.80 (d, J 10.2 Hz, 1H, 2-H), 3.64 (s, 1H, 19-H), 2.83 (d, J 18.6 Hz, 4α-H), 2.45 (d, J 18.6 Hz, 1H, 4β-H), 2.19 (s, 19-OCOCH$_3$), 0.87 (s, 3H, 18-CH$_3$).

^{13}C NMR of **2.12** ($CDCl_3$): δ 125.99 (1), 152.21 (2), 195.26 (3), 41.92 (4), 23.52 (5), 26.70 (6), 24.97 (7), 36.76 (8), 43.85 (9), 27.76 (10), 23.39 (11), 31.73 (12), 48.33 (13), 50.64 (14), 21.47 (15), 35.57 (16), 219.86 (17), 14.00 (18), 70.12 (19), 20.76 (19-OCO<u>C</u>H$_3$), 169.93 (19-O<u>C</u>OCH$_3$).

19(S)-Acetoxy-5β,19-cycloandrostane-3,17-dione 2.13

To the 19(S)-alcohol/hemiketal mixture **2.5/2.6** (2.04 g, 6.75 mmol) in CH_2Cl_2 (60 mL) was added Ac_2O (3.5 mL, 0.037 mmol, 5.5 eq.), Et_3N (2 mL, 14.3 mmol, 2.1 eq.), and DMAP (82 mg, 0.67 mmol, 0.1 eq.) and the mixture stirred at 20 °C for 12 h. The mixture was poured into water, extracted with CH_2Cl_2 and the organic layer washed with water, saturated $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated to give a residue which on FCC, on elution with 12% acetone-LP, yielded the non-crystalline 19(S)-acetate **2.13** (1.7 g, 4.9 mmol, 73%).

^{1}H NMR of **2.13** ($CDCl_3$); δ 3.88 (s, 1H, 19-H), 2.44 (dd, J 19.6, 8.9 Hz, 1H, 16β-H), 2.38 (d, J 16.6 Hz, 1H, 4α-H), 2.25 (d, J 16.4 Hz, 1H, 4β-H), 2.08 (s, 3H, 19-COOCH$_3$), 2.07 (m, 16α-H), 0.92 (s, 3H, 18-CH$_3$).

^{13}C NMR of **2.13** ($CDCl_3$): δ 23.12 (1), 36.37 (2), 212.02 (3), 43.10 (4), 24.44 (5), 31.68 (6), 25.70 (7), 35.79 (8), 45.53 (9), 27.92 (10), 24.10 (11), 31.48 (12), 48.26 (13), 50.26 (14), 21.51 (15), 35.69 (16), 221.66 (17), 14.14 (18), 62.13 (19), 20.65 (19-OCO<u>C</u>H$_3$), 170.67 (19-O<u>C</u>OCH$_3$).

19(S)-Acetoxy-5β,19-cycloandrost-1-ene-3,17-dione 2.15

Trimethylsilyl triflate (3.5 mL, 18.1 mmol, 8.34 eq.) was added to a stirred solution of 19(S)-acetoxy-5β,19-cycloandrostane-3,17-dione **2.13**

(748, 2.17 mmol) and Et_3N (6 mL, 42 mmol, 19 eq.) in dry DMF cooled in an ice-bath. After 2 hr of stirring at room temperature, the mixture was poured into Et_2O (100 mL) and the organic layer washed with brine, dried, filtered, and evaporated to give a residue which on FCC, on elution with 8% EtOAc-LP, yielded fractions of a mixture of the 3-siloxy 2-ene/3-ene ethers **2.14a/2.14b** (462 mg, 0.994 mmol, 46%), (H-2:H-4;2.3:1).

^{1}H NMR ($CDCl_3$): (δ 3.79 s, 19-H of **2.14a**), (δ 3.70 s, 19-H of **2.14b**).

Fractions containing **2.14** (462 mg) were dissolved in CH_3CN (30 mL) and treated with a solid $Pd(OAc)_2$ (228, 1.02 mmol, 1.03 eq.) at 40 °C for 30 min to give the 1,2-unsaturated 19(S)-acetate **2.15** (238 mg, 0.7 mmol, 32%), mp 203-205 °C (from CH_2Cl_2-EtOAc) (Found C, 73.65; H, 7.9. $C_{21}H_{26}O_4$ requires C, 73.6; H, 7.65%).

^{1}H NMR of 2.15 ($CDCl_3$); δ 6.77 (d, J 10.2 Hz, 1H, 1-H), 5.97 (d, J 10.2 Hz, 1H, 2-H), 3.94 (s, 1H, 19-H), 2.77 (d, J 18.4 Hz, 4α-H), 2.34 (d, J 18.4 Hz, 1H, 4β-H), 1.90 (s, 19-$OCOCH_3$), 0.95 (s, 3H, 18-CH_3).

^{13}C NMR of 2.15 ($CDCl_3$): δ 127.14 (1), 145.00 (2), 195.21 (3), 40.42 (4), 24.69 (5), 31.36 (6), 24.95 (7), 36.24 (8), 44.16 (9), 30.89 (10), 23.96 (11), 31.45 (12), 48.33 (13), 50.28 (14), 21.56 (15), 35.66 (16), 219.90 (17), 14.21 (18), 61.25 (19), 20.32 (19-$OCO\underline{C}H_3$), 170.10 (19-$O\underline{C}OCH_3$).

3,19(R)-Diacetoxy-5β,19-androst-2-en-17-one/3,19(R)-Diacetoxy-5β,19-androst-3-en-17-one 2.17a/2.17b

A solution of the 19(R)-acetoxy-5β,19-cycloandrostane-3,17-dione **2.10** (350 mg, 1.0 mmol), acetic anhydride (2.0 mL, 21 mmol, 21 eq.), and $BF_3.OEt_2$ (150 μL, 1.2 mmol, 1.2 eq) in methylene chloride (8 mL) was strired at 20 °C for 24 hr, when no starting material was indicated by TLC. The organic layer was washed with water, saturated aqueous

$NaHCO_3$, water, dried, filtered, and evaporated to give an inseparable mixture of two compounds; the 3,19(R)-diacetoxy 2-ene/3-ene derivatives **2.17a/2.17b** (230 mg, 88 mmol, 88%), (H-2:H-4; 1:3.2, base on the ^1H NMR spectrum).

3-O-(Triisopropylsilyl)-androsta-2,4-dien-3,17-on-19-al 2.18

Into a flame-dried flask with powdered molecular sieves 4A (35 mg, *ca* 10%) capped with a rubber septum was added dry CH_2Cl_2 (8 mL) and iPr_2EtN (220 μL) at room temperature, under an argon atmosphere. The mixture was cooled to -78 °C (acetone-solid CO_2), and then iPr_3SiOTf (325 μL) was added followed by a dropwise addition (over 20 min) of a solution of the 19-aldehyde **2.2** (300 mg) in dry CH_2Cl_2 (3 mL). After 1.5 h of stirring, methanol (0.5 mL) was added followed by Et_2O (20 mL), (still at -78 °C), and the organic layer washed with brine, dried, filtered, and evaporated to give a noncrystalline residue **2.18** (395 mg, 0.86 mmol, 87%).

^1H NMR of 2.18 ($CDCl_3$): δ 9.73 (s, 1H, 19-CHO), 5.71 (s, 1H, 4-H), 4.73 (d, J 6.54 Hz, 1H, 2-H), 2.97 (dd, J 16.95, 6.63 Hz, 1H, 1β-H), 2.49 (br t, J 13.66 1H, 6β-H), 2.48 (br d, J 13.77 Hz, 1H, 6α-H) 2.29 (dd, J 19.62, 1.92 Hz, 1H, 1α-H), 1.07-1.05 $SiC(C\underline{H}_3)_2$, 0.88 (18-$C\underline{H}_3$).

^{13}NMR of 2.18 ($CDCl_3$): δ 31.65 (1), 98.11 (2), 147.56 (3), 125.05 (4), 137.97 (5), 31.78 (6), 29.57 (7), 36.73 (8), 51.06 (9), 53.24 (10), 21.57 (11), 31.22 (12), 47.62 (13), 53.54 (14), 21.70 (15), 35.70 (16), 220.39 (17), 13.73 (18), 201.63 (19), 17.93 $SiCH(\underline{C}H_3)_2$, 12.47 $Si\underline{C}H(CH_3)_2$.

3-O-(Triisopropylsilyl)-androsta-3,5-dien-3,17-on-19-al 2.20

To a stirred and cooled (- 78 °C; acetone-solid CO_2) mixture of iPr_2EtN (320 μL, 1.9 mmol, 1.3 eq.), iPr_3SiOTf (470 mL, 1.75 mmol, 1.2 eq.), and powdered molecular sieves 4A (45 mg) in CH_2Cl_2 (8 mL) mL), under an

argon atmosphere, was added, by syringe over 5 min, a solution of androst-4-ene-3,17-dion-19-al **2.2** (433 mg, 1.44 mmol) in CH_2Cl_2 (3 mL), "reverse addition". The mixture was allowed to stir for 1 hr and then transferred into a separatory funnel containing brine (25 mL) and CH_2Cl_2 (40 mL). After partitioning, the separated aqueous phase was further extracted with CH_2Cl_2 (10 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and evaporated to give a glassy residue (0.591 g), which on FCC, on elution with 15-20% Et_2O-LP, gave fractions (0.270 g, 0.59 mmol, 41%) identified as the noncrystalline 3-triisopropylsiloxyandrosta-3,5-dien-17-on-19-al **2.20**.

^{1}H NMR of **2.20** ($CDCl_3$): δ 9.64 (d, J 1.28 Hz, 1H, 19-CHO), 5.56 (d, J 3.33 Hz, 1H, 6-H), 5.44 (d, J 1.59 Hz, 1H, 4-H), 1.06-1-04 $SiC(C\underline{H}_3)_2$, 0.83 (s, 3H, 18-$C\underline{H}_3$).

^{13}C NMR of **2.20** ($CDCl_3$): δ 30.55 (1), 27.64 (2), 152.77 (3), 108.27 (4), 133.48 (5), 120.74 (6), 27.64 (7), 32.81 (8), 47.49 (9), 51.85 (10), 21.25 (11), 31.36 (12), 47.49 (13), 52.05 (14), 21.63 (15), 35.71 (16), 220.21 (17), 13.58 (18), 205.22 (19), 17.92 $SiCH(\underline{C}H_3)_2$, 12.63 $Si\underline{C}H(CH_3)_2$.

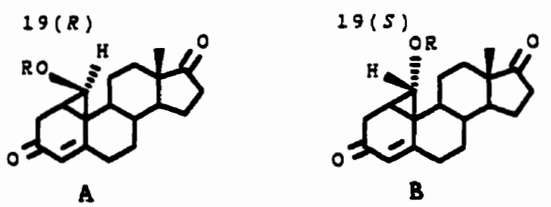
2.2.0 ATTEMPTED SYNTHESIS of 19(R/S)-ACETOXY-1β,19-CYCLOANDROST-4-ENE-3,17-DIONE

Introduction

Synthesis of unsubstituted 1β,19-cyclosteroid derivatives has been reported (Weiland and Anner, 1970). Weiland and Anner attempted to synthesize both 1β,19-cycloandrostane and 5β,19-cycloandrostane derivastives in one reaction by treating a steroid 19-mesylate 1,4-dien-3-one with lithium and biphenyl in tetrahydrofuran (Weiland and Anner, 1968). However, the product formed was only the 5β,19-cycloandrostane derivative. Two years later, they reported successful synthesis of unsubstituted 1β,19-cycloandrostane derivaties by treating a steroid 19-mesylate 1-en-3-one with lithium and biphenyl.

A goal of this project was to synthesize unsaturated 19(R/S)-alcohols and/or 19(R/S)-acetates of the 1β,19-cyclosteroid derivatives, **A** and **B**. as potential mechanism-based aromatase inhibitors (Scheme 2.19).

Saturated analogues, 19(S)-acetoxy- and 19(R)-acetoxy-1β,19-cycloandrostane-3,17-dione **2.32** (Scheme 2.11), were assumed to serve as intermediates for the synthesis of the 4,5-unsaturated



A B

R= H or Ac

Scheme 2.9 Proposed 4,5-unsaturated 19(R/S)-1β,19-cyclosteroid derivatives to be synthesized as potential aromatase inhibitors.

1β,19-cyclosteroid derivatives. The key synthetic steps in the preparation of 4,5-unsaturated 19(R/S)-acetoxy-1β,19-cyclosteroid derivatives, *via* reductive cyclization, involved:

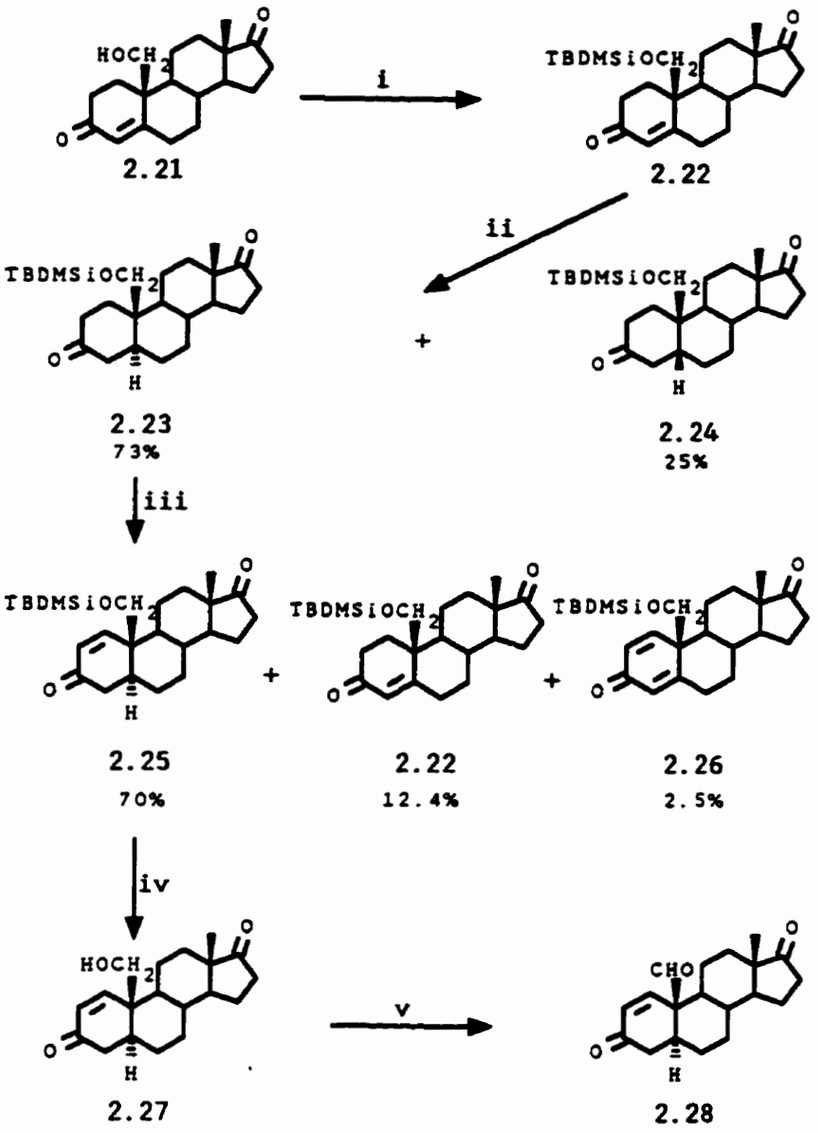
1. synthesis of the 1,2-unsaturated 5α-androst-1-ene-3,17-dion-19-al **2.28** (Scheme 2.10),
2. reductive cyclization of **2.28** with zinc metal in 50% aqueous acetic acid (Scheme 2.11), or
3. reductive cyclization of **2.28** with lithium metal in liquid ammonia (Scheme 2.14), and
4. oxidation of **2.32** to introduce the 4,5-double bond (Scheme 2.15).

Results and Discussion

1. <u>Synthesis of 5α-androst-1-ene-3,17-dione 19-al 2.28 (Scheme 2.10)</u>

The synthesis of 5α-androst-1-ene-3,17-dione 19-al **2.28** was carried out starting from 19-hydroxyandrost-4-ene-3,17-dione **2.21** (Scheme 2.10). Treatment of 19-hydroxyandrost-4-ene-3,17-dione **2.21** with *tert*-butyldimethylsilyl chloride in the presence of imidazole at 50 °C gave the 19-silyloxy derivative **2.23**. Catalytic hydrogenation (10% Pd/C) of 19-*tert*-butyldimetylsiloxyandrost-4-ene-3,17-dione **2.23**, having deliberately introduced a bulky group at C-19 to direct addition of hydrogen from the α-face (Templeton *et al.*, 1966), gave a mixture of two products: the 5α-androstane **2.23** (70%) and the 5β-androstane **2.24** (25%) as determined after FCC. As reported earlier, similar reduction of 19-hydroxyandrost-4-ene-3,17-dione or its 19-acetylated derivatives gave the 5β-androstane as a major product (Knox *et al.*, 1965).

As reported earlier (Lin, 1994; Templeton *et al.*, 1996), introduction of a C-1 double bond through bromination of the 3,17-diketone **2.23** followed by dehydrobromination with LiBr-Li_2CO_3 gave



Reagents: i, TBDMSiCl, imidazole, DMF, 50 °C; ii, 10% Pd/C, H_2, EtOAc; iii, FCC and then $(PhSe)_2$, $3\text{-}IO_2C_6H_4COOH$, CSA, THF; iv, FCC and then nBu_4NF or $SnCl_2.2\ H_2O$; v, PCC.

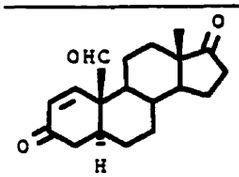
Scheme 2.10 Synthesis of 5α-androst-1-ene-3,17-dion-19-al **2.28**.

a low yield of the desired 1-en-3-one **2.25** (ca 14%), together with the 2β,19-oxide (55%), and the 4-ene-3,17-dione and 1,4-diene-3,17-dione. Similarly, when the 3,17-diketone **2.23** was treated with benzeneselenic anhydride, the 1-en-3-one **2.23** was obtained in 40% yield accompanied by the isomeric 4-ene-3,17-dione **2.22** (35%) and the doubly dehydrogenated 1,4-ene-3,17-dione **2.26** (25%). However, higher yields of the 1-ene-3,17-dione **2.25** were obtained by employing a reported procedure (Barton *et al.*, 1989). Treatment of the ketone **2.23** with a catalytic amount of diphenyl diselenium (Ph_2Se_2), camphorsulfonic acid, and 3-iodylbenzoic acid ($3\text{-}IO_2C_6H_4COOH$) in tetrahydrofuran, under reflux, gave the 1-ene-3,17-dione **2.25** (70%) together with minor by-products, the 4-ene-3,17-one **2.22** (12%) and the 1,4-diene-ketone **2.26** (2.5%). 3-Iodylbenzoic acid was prepared *via* oxidation of 3-iodobenzene acid with Stevens reagent, NaClO, (Stevens *et al.*, 1980) in acetic acid (Barton *et al.*, 1985, 1982).

Deprotection of the 1-en-3-one 19-silyloxy derivative **2.25** either with fluoride ion, nBu_4NF in tetrahydrofuran (Corey and Venkateswaralu, 1972) or with Lewis acids, $ZnCl_2.H_2O$, in acetonitrile (Cort, 1990), yielded the 19-alcohol **2.27**, which was oxidized with pyridinium chlorochromate or tetrapropylammonium perruthenate in CH_2Cl_2-CH_3CN (9:1) in the presence of powdered molecular sieves 4A, to the 19-aldehyde **2.28**. PCC, instead of PDC, was used to minimize formation of by-products. However, higher yields of aldehyde **2.28** (95%), with no by-products, were obtained by using TPAP. Conformers of the 19-aldehyde group in androst-1-ene-3,17-dion-19-al **2.28** have been determined by the NOE experiments. The results are given in Table 2.2. Thus irradiation of a proton of the 19-CHO group in 2.28 showed NOE enhancements of the 1-H (0.45%), 2-H (0.21%), 4β-H (1.94%), 6β-H

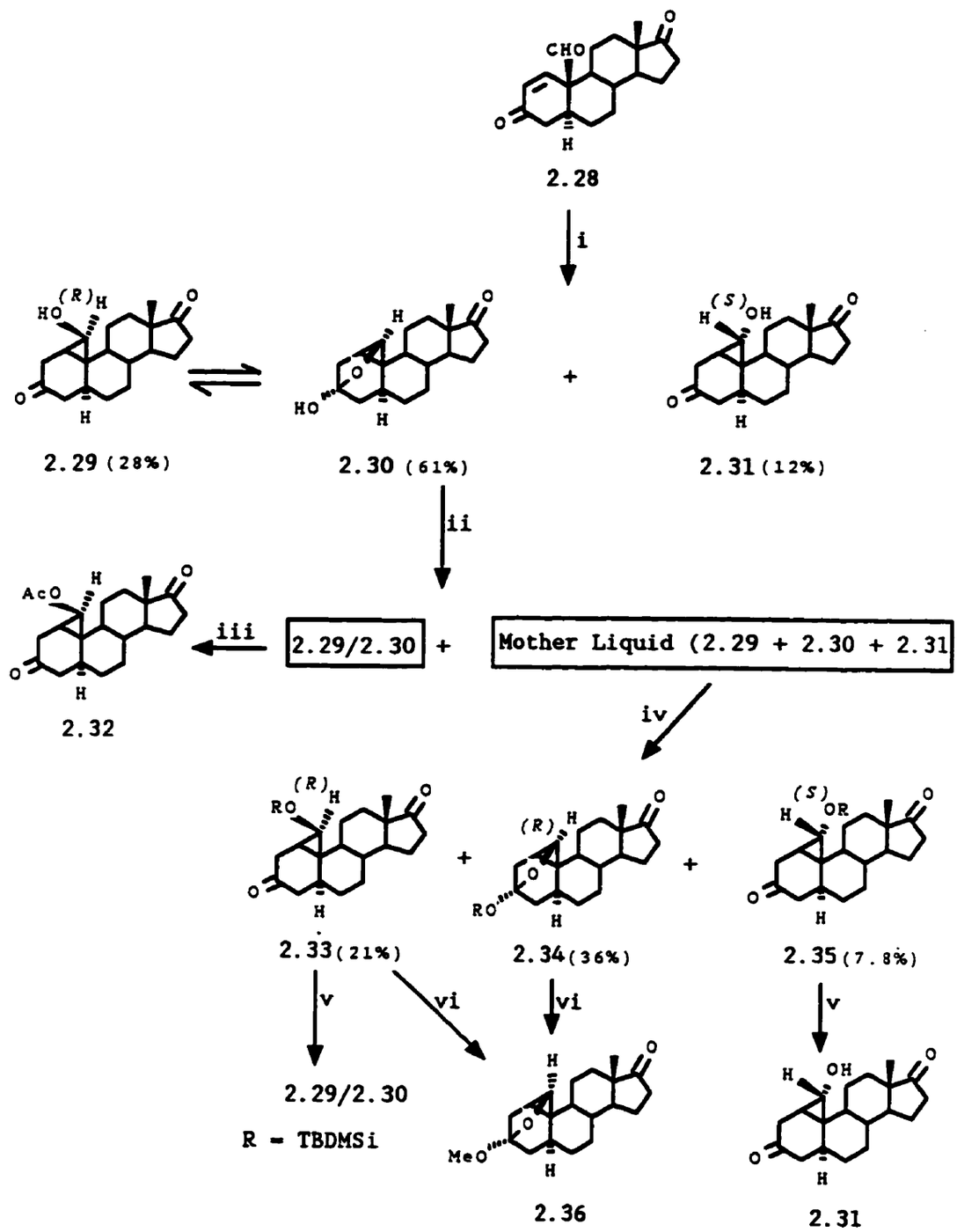
(2.46%) and 8β-H (3.45%). Irradiation of 2-H showed a small enhancement (0.22%) in a proton of the 19-CHO group and a bigger one (7.65%) in the 1-H. Rotation of the 19-CHO group around the C(10)-C(19) bond is observed with the carbonyl group being out-of A and B rings in *ca* 92%.

Table 2.2 The position of the 19-CHO group in compound **2.28** from NOE experiments.

Compound	H's Irradiated	H's Enhancement (%)
 2.28	19-H	1-H (0.45)
		2-H (0.21)
		4-H (1.94)
		6β-H (2.46)
		8β-H (3.45)
	2-H	1-H (7.65)
		19-HCO (0.22)

2. Reductive cyclization of 5α-androst-1-ene-3,17-dione-19-al with zinc in 50% CH_3COOH-H_2O at 20 °C

Treatment of the aldehyde **2.28** with zinc in 50% aqueous acetic acid (Lin 1994; Templeton *et al.*, 1994, 1997) gave a mixture of the following products: the thermodynamically more stable isomer, 19(R)-hydroxy-1β,19-cyclo-5α-androstane-3,17-dione **2.29** was the major product, which was in equilibrium with the hemiketal tautomer, 3α-hydroxy-3β,19-oxido-1β,19-cyclo-5α-androstan-17-one **2.30** (61%), and the less thermodynamically stable isomer, 19(S)-hydroxy-1β,19-cyclo-5α-androstane-3,17-dione **2.31** (12%) (Scheme 2.11). Relative yields were



Reagents: i, Zn, 50% CH_3COOH/H_2O; ii, crystallization; iii, Ac_2O, DMAP; iv, N-(TBDMSi)imidazole, CH_2Cl_2; v, n-Bu_4NF, THF; vi, dil. HCl-MeOH.

Scheme 2.11 Synthesis of the 19(R/S)hydroxy-1β,19-cycloandrostane-3,17-dione, **2.29** and **2.31** and their derivatives.

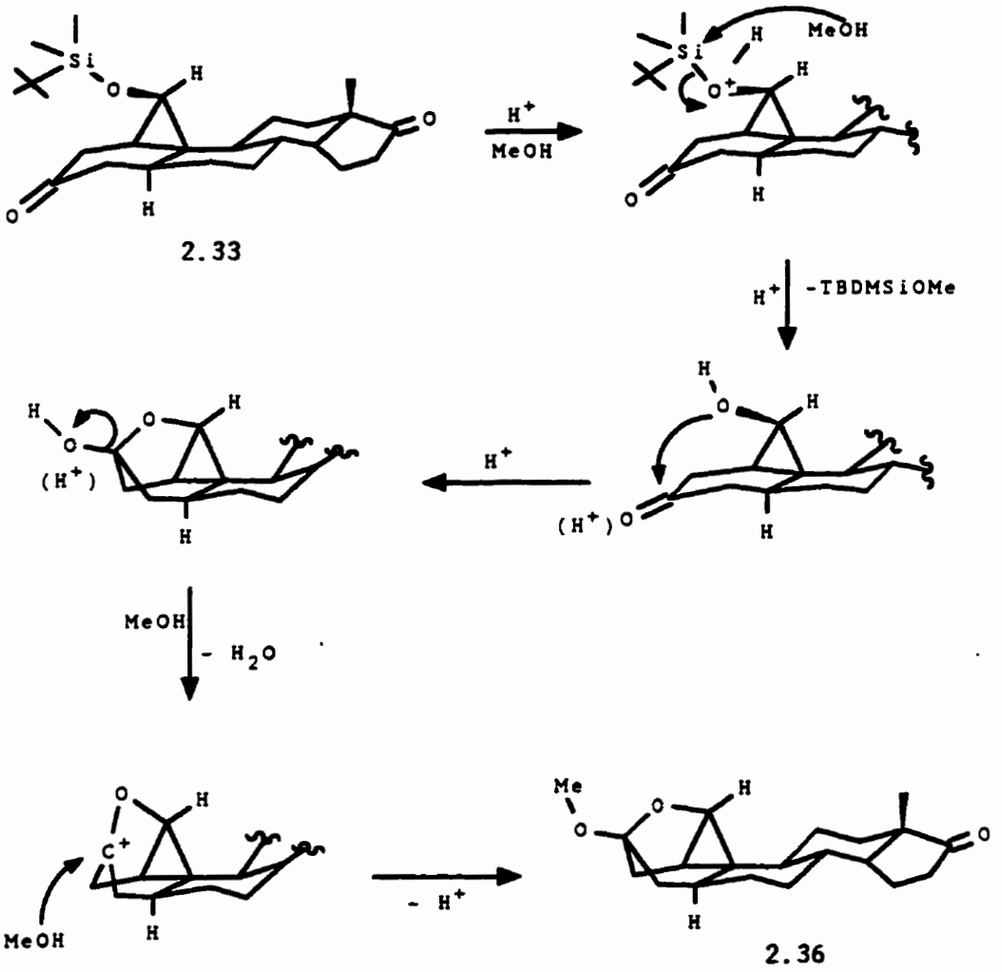
determined based on the ^1H NMR spectrum of the crude product. Crystallization of the crude product, from AcOEt-LP, gave an inseparable mixture of the 19(R)-alcohol/hemiketal compounds **2.29/2.30**, which was subjected to acetylation to produce, solely, the 19(R)-acetate-1β,19-cycloandrostane-3,17-dione **2.32**.

In a separate experiment (Scheme 2.11) the mother liquor was treated with 1-(TBDMSi)imidazole in CH_2Cl_2 for three weeks to yield three silyl derivatives: the 19(R)-*tert*-butyldimethylsilyl- **2.33** (34.5%), the hemiketal 3-silyl- **2.34** (36%), and 19(S)-TBDMsilyl ethers **2.35** (6%): yields were determined after FCC.

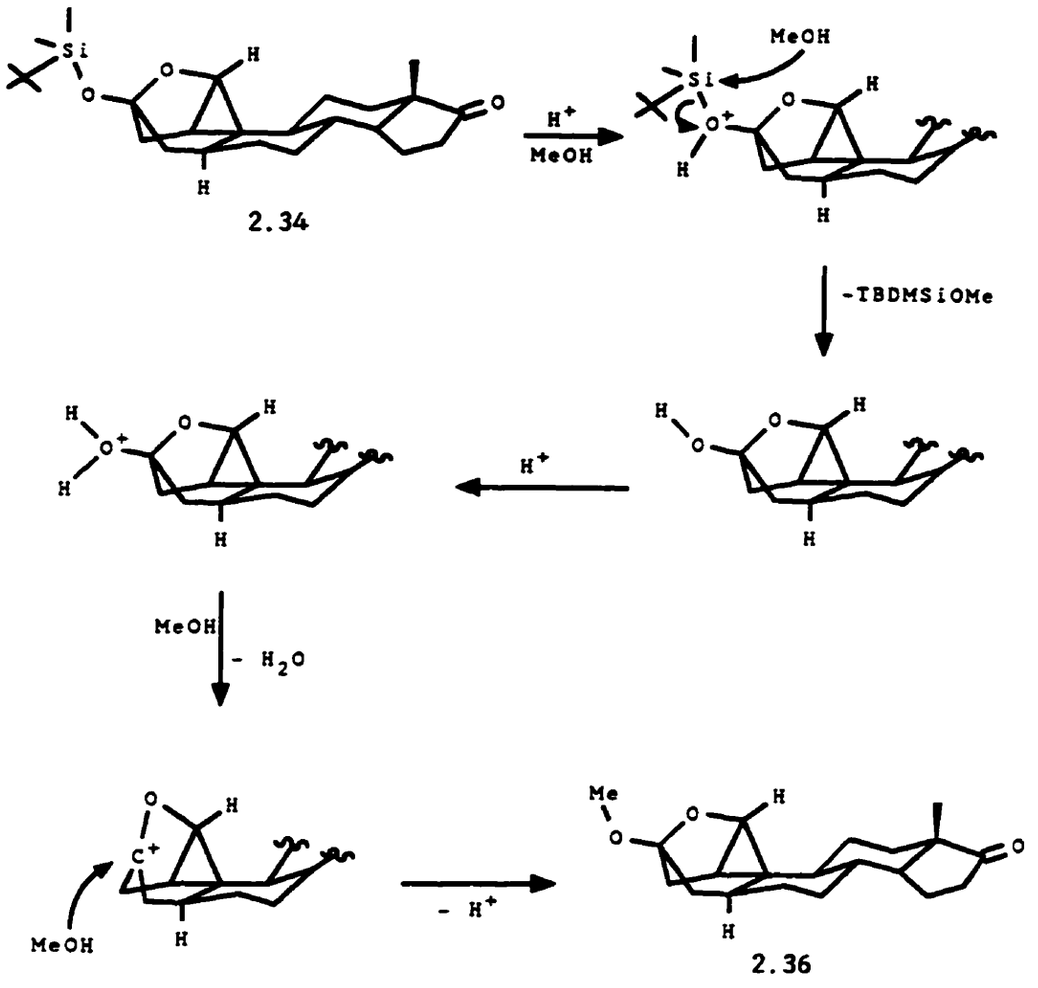
The ^1H NMR spectrum of the 19(S)-TBDMsilyl ether **2.34** showed a doublet at δ = 3.38 ppm (J = 3.1 Hz) which integrated for one proton and corresponded to the 19-cyclopropyl proton. The *trans* coupling, J = 3.1 Hz, between the 19-H and 1α-H confirms the 19(S) stereochemistry, i.e. the 19-H *endo*. Two singlet signals at δ = 0.90 and 0.87, integrated for nine protons and three protons, respectively, were assigned to the C-19 *tert*-butylsiloxy group and the 18-CH_3, respectively. Two singlet signals at δ = 0.12 ppm and δ = 0.9 ppm integrated for 6 protons corresponded to two C-19 dimethylsilyloxy group. The ^{13}C NMR spectrum of the 19(S) TBDMsilyl ether **2.34** showed one methyl signal at 29.51 ppm corresponding to the $Si(CH_3)_3$, a quaternary signal at 18.32 ppm corresponding to a carbon of the *tert*-butylsiloxy group, and two methyl signals at -4.77 ppm and δ = -5.16 ppm corresponding to two methyl groups of the dimethylsilyloxy part at C-19. A methine carbon signal at 56.62 ppm was assigned to the cyclopropyl carbon. Elemental analysis (C,H) was in agreement with this product.

The ^1H NMR spectrum of the 19(R)-TBDMsilyl ether **2.34** is similar to

the 19(S)-TBDMsilyl **2.35** spectrum. A doublet signal at δ = 3.53 ppm (J = 7.09 Hz), integrated for one proton, corresponded to the C-19 cyclopropyl proton. The *cis* coupling (J = 7.09 Hz) between the 19-H and 1α-H confirmed the 19(R) stereochemistry, i.e. 19-H exo. Two single signals at δ = 0.89 ppm and 0.85 ppm, integrated for nine protons and



Scheme 2.12 Proposed formation mechanism of 3α-methoxy-3β,19-oxido-1β,19-cyclo-5α-androstane-3,17-dione **2.36** from the 19(R)-TBDMsiloxy-1β,19-cyclosteroid **2.33**.



Scheme 2.13 Proposed formation mechanism of 3α-methoxy-3β,19-oxido-1β,19-cyclo-5α-androstane-3,17-dione **2.36** from the 3α-TBDMsiloxy-3β,19-epoxy-1β,19-cyclosteroidsteroid **2.34**.

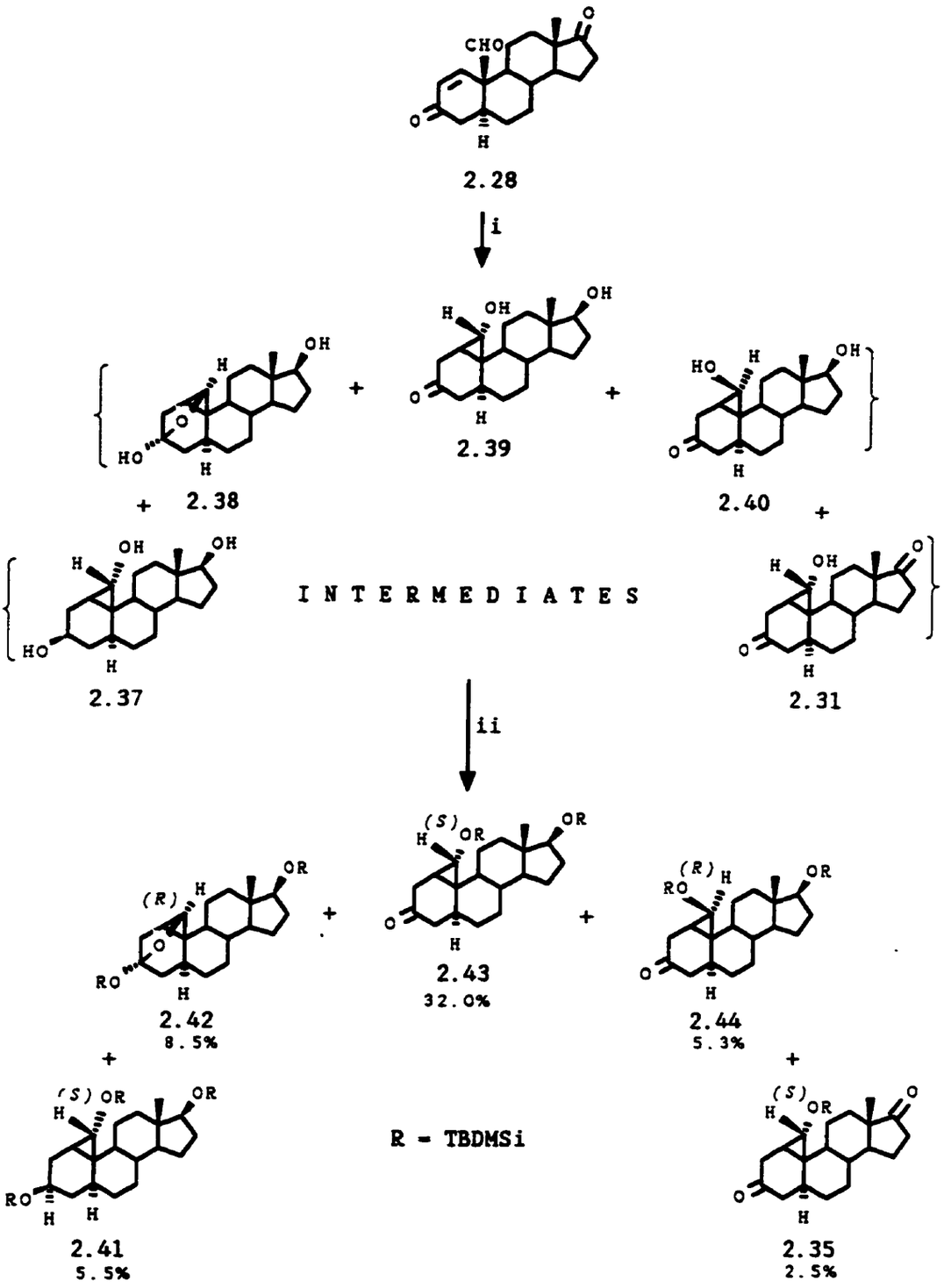
three protons, also respectively, were assigned to the C-19 *tert*-butylsiloxy group and the 18-CH_3, respectively. Two singlet signals at δ = 0.13 ppm and δ =0.95 ppm, each integrated for three protons,

corresponded to the two C-19 dimethylsiloxy groups. Deprotection of the 19(R)-silyl ether with *tert*-butylammonium fluoride in THF gave again the inseparable mixture of **2.29/2.30** as determined by the ^{1}H NMR spectrum. On the other hand, deprotection of the 19(S)-silyl ether with fluoride ion gave a more polar compound, the 19(S)-alcohol **2.30**, which was characterized by its ^{1}H and ^{13}C spectra, and elemental analysis. The 19(S)-alcohol **2.30** was found to be unstable in a $CDCl_3$ solution and partly decomposed to a saturated aldehyde during ^{13}C acquisition, as shown by its ^{1}H NMR spectrum. The aldehyde was not further identified.

Treatment of either the 19(R)-silyl ether **2.33** or the 3α-siloxy-3β,19-ether **2.36** with dilute HCl-MeOH solution at room temperature gave the 3α-methoxy derivative **2.36**. The proposed mechanisms of formation of the 3α-methoxy-3β,19-epoxy-1β,19-cyclo-5α-androstane-3,17-dione **2.36** from the 19(R)-TBDMsiloxy-1β,19-cyclo- **2.33** and of 3α-TBDMsiloxy-3β,19-oxido-1β,19-cyclosteroid **2.34** are shown in Schemes **2.12** and **2.13**, respectively.

3. Reductive cyclization of 5α-androst-1-ene-3,17-dione with lithium in liquid ammonia at -78 °C

Previously, a lithium reduction of androst-4-ene-3,17-dion-19-al **2.2** in liquid ammonia was designed to determine the ratio of products having the 19-hydroxy group above rings A and B. Similarly, in Scheme 2.14, the lithium reduction of the 1-en-19-al **2.28** in liquid ammonia was performed to determine the ratio of products with the 19-hydroxy group out-of or above ring A, i.e. 1β,19(S)-OH : 1β,19(R)-OH. It was found that the Li-NH_3 reduction of the 1-en-19-al **2.28** yielded the less



Reagents: *i*, $Li/NH_{3(liq.)}$, THF, -70 °C; *ii*, TBDMSiCl, iPr_2EtN, DMF, RT.

Scheme 2.14 Products of reductive cyclization of 5α-androst-1-ene-3, 17-dione **2.28** with Li-NH_3 and their TBDMSi derivatives.

thermodynamically stable isomer, 1β,19(S)-cyclopropanol (70%) as the major product, together with the 1β,19(R)-cyclopropanol-hemiacetal mixture (30%), as determined by the ^1H NMR spectrum of the crude product. The result of the dissolving-metal reduction is in contrast with the $Zn/CH_3COOH/H_2O$ reduction, in which the 1β,19(R)-cyclopropanol-/hemiketal mixture **2.29/2.30** (88%) was produced as the major product.

The products of the dissolving-metal reduction were identified as their *tert*-butyldimethylsiloxy derivatives. Treatment of the 1β,19(R/S)-cyclopropanols mixture with TBDMSiCl and iPr_2EtN in DMF for 2 hr at room temperature (Lombardo, 1984), followed by FCC separation, yielded the following compounds in their order of elution: 3β,17β,-19(R)-tri(TBDMsiloxy)-1β,19-cyclo-5α-androstane **2.41** (5.5%), 3α,17β-di(TBDMsiloxy)-3β,19-epoxy-1β,19-cyclo-5α-androstane **2.32** (8.5%), 17β,-19(S)-di(TBDMsiloxy)-1β,19-cyclo-5α-androstan-3-one **2.43** (32%), 17β,-19(R)-di(TBDMsiloxy)-1β,19-cyclo-5α-androstane-3-one **2.44** (5.3%), and 19(R)-TBDMsilyloxy-1β,19-cyclo-5α-androstane-3,17-dione **2.35** (2.5%).

Stereochemistry of 3-H in compound **2.41** (Scheme 2.14) has been established by NOE enhancements. Irradiation of 19-H (the cyclopropyl proton above ring A) in **2.41** resulted in NOE's to 1α-H (1.5%), 2β-H (1.95%), and 4β-H (6.7%); irradiation of 2α-H (2.22 ppm) resulted in NOE's to 1α-H (6.28), 3α-H (7.3%), 2β-H (21%); irradiation of 3α-H resulted in NOE's to 2α-H (7%). Neither irradiation of 19-H nor 3-H resulted in NOE's of 3-H or 19-H, respectively, indicating that these protons are not spatially close to each other. These results are consistent with the assignment of **2.41** having the TBDMsiloxy group at β and 3-H at the α-orientation. Subsequent deprotection of the 17β,19(S)-disilyloxy derivative **2.43** with nBu_4NF afforded the 17β,19-diol **2.39**, which was directly subjected to isomerization trials

under basic and acidic conditions. However, neither treatment with 0.5 M KOH-MeOH nor 1% HCl-THF caused epimerization of the 17β,19(S)-diol **2.39** to the 17β,19(R)-diol **2.40**. This result indicate that the more thermodynamically stable 17β,19(R)-diol **2.40** (MMX, E = 41.22 kcal mol^{-1}) does not originate from the less thermodynamically stable 17β,19-(S)-diol **2.39** (MMX, E = 41.44 kcal mol^{-1}) as observed for the 19(R)-hydroxy-5β,19-cycloandrostane-3,17-dione **2.4**. The epimerization of **2.39** → **3.40**, which was not observed, presumably results from rigidity of ring A and lack of the overlapping orbitals. The results of Li-NH_3 reduction implies that the 1β,19(R)-cyclopropanol **2.39** resulted from direct reductive cyclization. Furthermore, it also suggests that both compounds are genuine products resulting from reductive cyclization. A mechanism of the formation of these compounds is discussed on the pages 123-152.

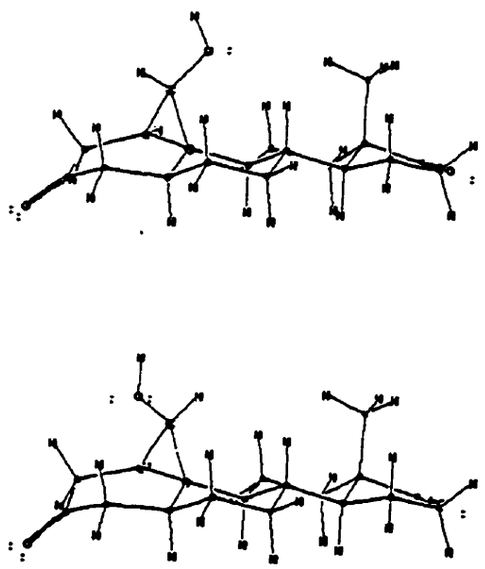
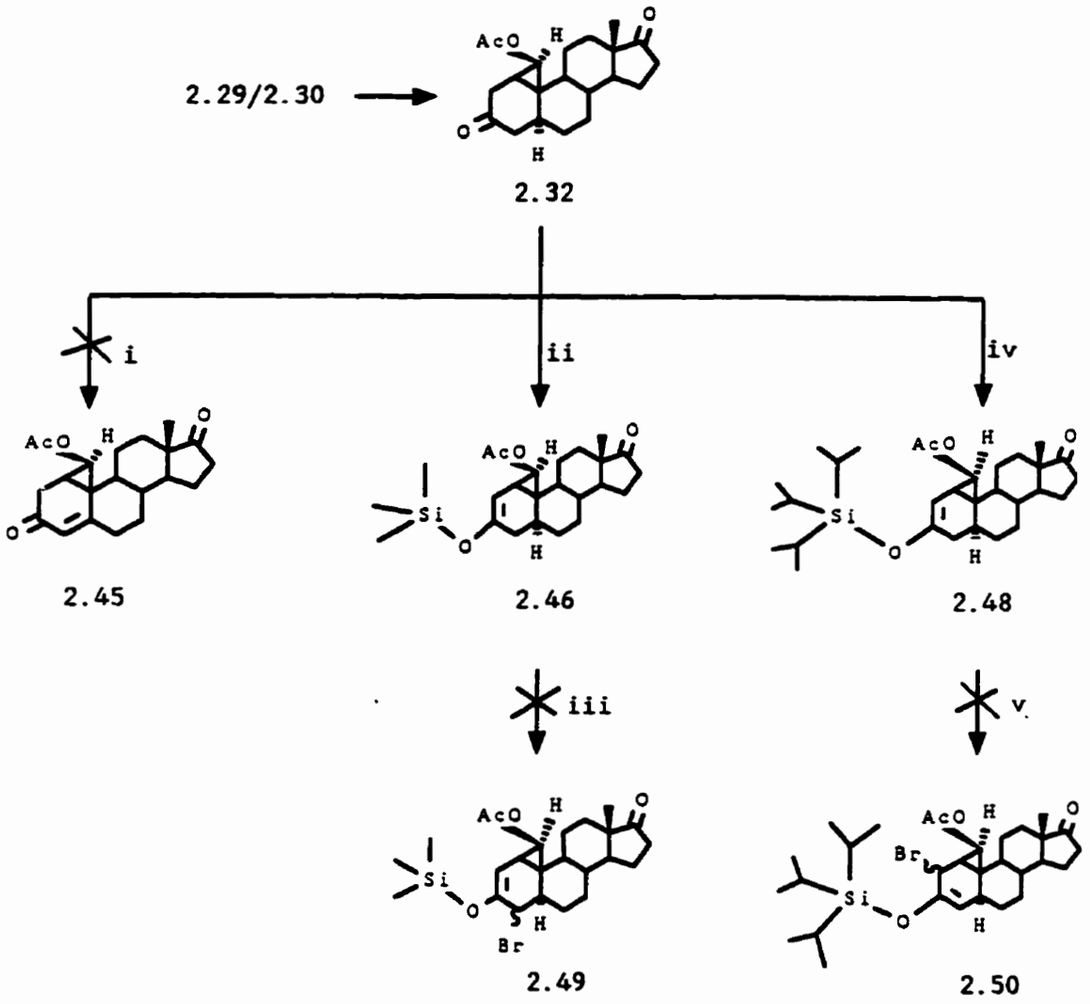


Figure 2.1 Structures of compounds **2.39** and **2.40** determined by MMX geometrical optimization.

4. Oxidation of saturated 19(R)-acetoxy-1β,19-cyclo-5α-androstane-3,17-dione 2.32 and its silyl derivatives: attempt to introduce the 4,5 double bond.

In Scheme **2.15**, the key synthetic step in the preparation of the desired 19-acetoxy-1β,19-cycloandrost-4-ene-3,17-dione **2.45** was



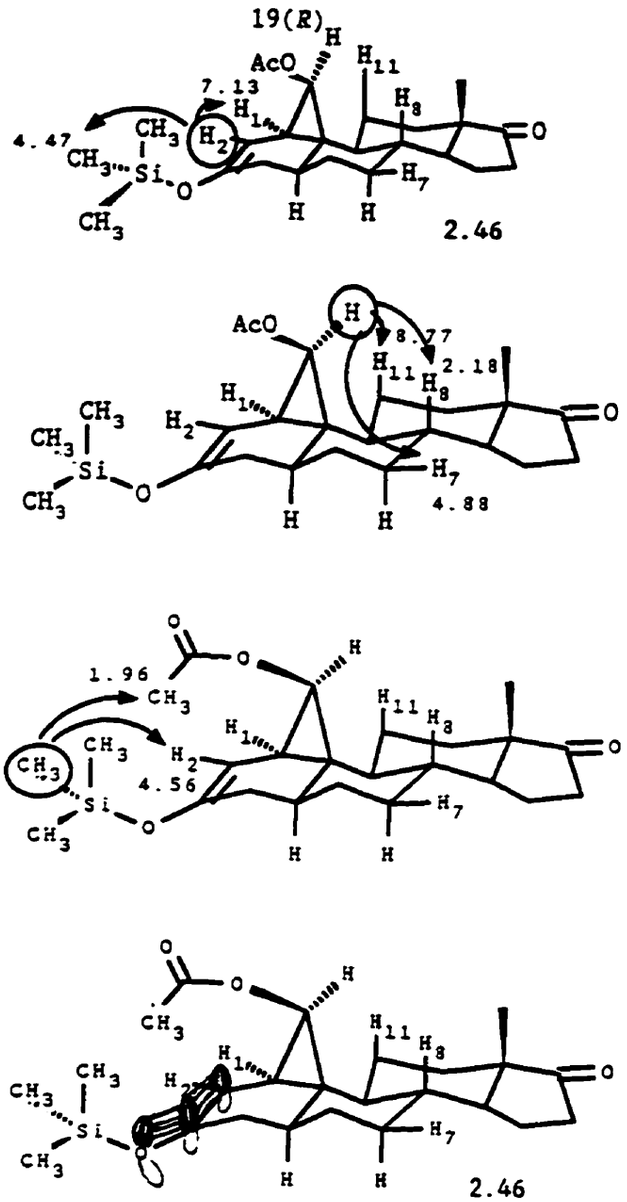
Reagents: **i**, $(PhSeO)_2O$, C_6H_6 or $(PhSe)_2$, $3\text{-}IO_2C_6H_4COOH$, CSA or DDQ, C_6H_6, reflux; **ii**, TMSiOTf, Et_3N, DMF at RT or an ice-water bath; **iii**, NBS, AIBN, CCl_4, reflux; **iv**, *i*-Pr_3SiOTf, Et_3N, Et_2O, reflux; **v**, NBS, CH_2Cl_2, RT or NBS, THF, 0 °C.

Scheme 2.15 Attempted synthesis of 19(R)-acetoxy-1β,19-cycloandrost-4-ene-3,17-dione **2.45**.

introduction of α,β-unsaturation without affecting the strained 1β,19-cyclopropane ring. It was anticipated that very mild reaction conditions must be employed. Therefore, from the very beginning attention was paid to the possibility of introducing a double bond *via* oxidation of the selected silyl enol intermediates, e.g. 3-siloxy,-19(R)-acetoxy-1β,19-cycloandrost-3-en-17-dione.

An attempt to directly introduce the 4,5-double bond by employing $(PhSeO)_2O$ or DDQ was unsuccessful. The 19-acetoxy derivative **2.32** decomposed during the reaction conditions to give by-products which were not further identified. On the other hand, an indirect introduction of the 4,5-unsaturation *via* palladium oxidation of the 3-silyl or 3-acetyl enol ethers required preparation of appropriate 3-enol intermediates. As shown in Scheme 2.15, treatment of the ketone **2.32** with TMSiOTf and Et_3N in DMF, either at room or at an ice bath temperature (*ca* 5 °C), or with *i*-Pr_3SiOTf and Et_3N in diethyl ether under reflux, gave only the undesired 3-siloxy-2-enes **2.46** and **2.48**, respectively.

COSY and HSQC spectra allowed a complete NMR assignment of compound **2.46**. In the ^1H NMR spectrum of compound **2.48**, the doublet of doublets at δ = 4.88 ppm was assigned to the vinylic 2-H. The spin-spin coupling constant between the 2-H and the 1α-H and 4-H was determined to be 6.7 Hz and 1.7 Hz, respectively. A one proton doublet at δ = 4.08 ppm was assigned to the 19(R)-H. The spin-spin coupling constant between the 19(R)-H and the 1α-H was determined to be 7.0 Hz (trans coupling). A proton doublet of doublets at δ = 2.44 ppm is assigned to the 16β-H. The spin-spin coupling constant between 16β-H and the 15α and 15β hydrogens was determined to be J = 19.2 Hz and J = 9.0 Hz, respectively. Two singlet signals of two methyl protons each at



Scheme 2.16 The NOE% of 19(R)-acetoxy-3-trimethylsiloxy-1β,19-cyclo-5α-androst-2-en-17-one **2.46**.

δ = 2.00 ppm and δ = 0.85 ppm are assigned to the two methyl groups of the 19(R)-acetoxy and the C_{13}-CH_3, respectively. Finally, one singlet at δ = 0.14 ppm, integrated for nine protons, corresponded to the 3-trimethylsilyloxy group.

The NOE difference spectra of compound **2.46** supported the 3-TMsiloxy-2-en structure (Scheme 2.16). Irradiation of the vinylic proton at δ = 4.88 enhanced the signals of protons 1-H at δ = 1.73 ppm (7.13%) and the $Si(CH_3)_3$ at δ = 0.16 ppm (4.47%). On the other hand, irradiation of the cyclopropyl proton, 19(R)-H, at δ = 4.08 ppm enhanced the 7β-H signal at δ = 1.77 (4.86%), the 8β-H at δ = 1.66 (2.18%), the 11β-H at δ 0.94 (8.77%), and the 3-$OSi(CH_3)_3$ at δ = 0.16 (0.51%).

Similarly, irradiation of the protons of the 3-trimethylsiloxy group at δ 0.16 ppm enhanced the 2-H signal at 4.88 ppm (4.56%) and the 19(R)-acetate signal at δ = 2.08 (1.96%). Interestingly, no enhancement was observed for protons of the 4α-H and the 4β-H at δ = 1.93 ppm and 1.96 ppm, respectively. Clearly, the 3-trimethysiloxy group is, on average, closer to the 2-H. It suggests that the silyloxy group is constrained to be coplanar with the π-system, with one conformation being preferred. This orientation allows maximum overlap of the π-orbitals and oxygen lone pairs, and is an example of the hyperconjugation effect (Neuhaus and Williamson, 1989).

In the ^{13}C spectrum of the 3-TMsilyl enol **2.46**, a quaternary carbonyl signal of C-3 at 210.09 ppm was substituted by the TMSi-enol quaternary carbon and shifted to lower field at δ = 147.43 ppm; a vinylic signal at 108.04 ppm was assigned to C-2; a methine carbon signal at 59.74 ppm was assigned to the 19 cyclopropyl carbon.

The ^1H NMR spectrum of the 3-iPr_3silyloxy 2-ene **2.48** is similar to

the 3-TMsilyl enol **2.46** spectrum. A doublet at 4.87 ppm was assigned to the vinylic 2-H. The spin-spin coupling constant between the 2-H and the 1α-H and 4-H was determined to be 6.7 Hz and 1.5 Hz, respectively. The two doublet signal of one proton at δ = 4.11 ppm was assigned to the 19(R)-H. The spin-spin coupling constant between the 19(R)-H and the 1α-H was determined to be 7.02 Hz (*trans* coupling). A one proton doublet of doublets at δ = 2.44 ppm is assigned to the 16β-H. The spin-spin coupling constant between 16β-H and the 15α and 15β hydrogens was determined to be 19.1 Hz and 9.0 Hz. Three singlets at δ = 2.00 ppm and δ = 1.06 ppm, and 0.86, integrating for three, eighteen, and three protons, respectively, were assigned to one methyl group of the 19(R)-acetoxy group, the six methyl groups of the triisopropylsiloxy groups, and the C_{13}-CH_3, respectively.

In the ^{13}C spectrum of the 3-triisopropylsiloxy 2-ene **2.48**, a carbonyl signal of the C(3) was substituted by a quaternary carbon and shifted to lower field at 150.74 ppm; a vinylic signal at 95.77 ppm was assigned to C-2; a methine carbon signal at 59.88 ppm was assigned to the cyclopropyl carbon. Signals at 17.98 ppm and 12.61 ppm corresponded to two methyls and a quaternary carbon, respectively, of the 3-iso-propylsilyloxy groups.

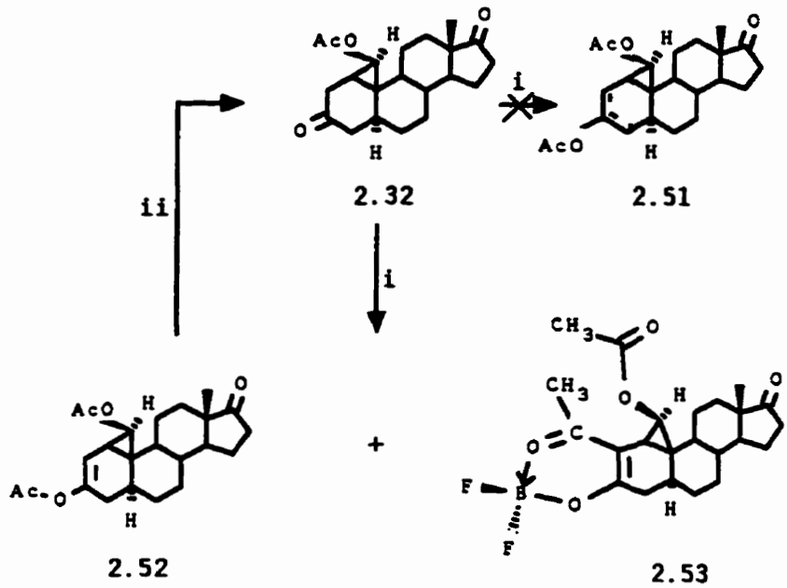
An attempt to introduce 4,5-unsaturation into ring A *via* bromination of C(4) of the 3-TMsiloxy 2-ene **2.46** under a radical-promoted oxidation, using an equimolar amount of NBS in the presence of a catalytic quantity of AIBN as a radical initiator, was made (Scheme 2.15). In another attempt to shift the double bond from C-2,3 to C-3,4, with concomitant introduction of a bromine atom at C-2 while retaining the 3-silyloxy group, by treatment of the 3-triisopropylsiloxy-2-ene **2.48** with NBS in CH_2Cl_2 at room temperature (Magnus and

Mugrage, 1990) was carried out. However, the reactions failed to give the expected derivative **2.49** or **2.50**, respectively. Moreover, in the ^1H NMR spectrum, a one proton doublet corresponding to the 19-cyclopropyl proton was lost indicating loss of the cyclopropane ring.

5. Synthesis of 3,19(R)-diacetoxy-1β,19-cyclo-5α-androst-2-en-17-one 2.52 and the BF_2.cyclopropyl derivative 2.53 (Scheme 2.17).

During a study aimed at the synthesis of geminal 19-diacetates of the 19(R)-acetoxy-5β,19-cycloandrostane-3,17-dione **2.10** instead of the desired compound **2.16** (Scheme 2.7), a mixture of two enol acetates, the 3-acetate-2-ene and the 3-acetate-3-ene, **2.17a/2.17b**, was produced. In retrospect, this is an example of a reaction discovered unintentionally.

When the same reaction conditions were applied to the 19(R)-acetoxy-1β,19-cyclopropane-3,17-dione **2.32** (Scheme 2.17), instead of an expected mixture of the 3-acetoxy-2-ene/3-ene **2.51**, two products were formed: the undesired 2-enol acetate **2.52** and compound **2.53**. Treatment of the ketone **2.32** with an excess of acetic anhydride and $BF_3.OEt_2$ in CH_2Cl_2, for 1 h, gave the 3-acetoxy 2-enol **2.52** (80%) accompanied by a small amount of a more polar product (20%), identified as the BF_2.cyclosteroid **2.53**. Furthermore, compound **2.52** was found to be the intermediate in synthesis of the BF_2-cyclosteroid derivative **2.53**. In a designed experiment, it was shown that the 19(R)-acetate **2.32** was completely converted, *via* the 3,19(R)-diacetoxy-2-ene **2.52**, to the BF_2.cyclosteroid **2.53** by employing an excess of acetic anhydride (50 eq.), more than an equimolar amount of $BF_3.OEt_2$. (2.5 eq.), and a prolonged reaction time (55 h *vs* 1 h). The boron difluoride complex of 17β-hydroxy 2-acylcholestane-3-one and 17β-hydroxy 2-acylandrostanane-



Reagents: i, Ac_2O, $BF_3.OEt_2$, CH_2Cl_2; ii, $BF_3.OEt_2$, CH_2Cl_2.

Scheme 2.17 Synthesis of 3,19(R)-diacetoxy-1β,19-cyclo-5α-androst-2-en-17-one **2.52** and 2-acyl,19-acetoxy-3-(difluoroboronoxy)-1β,19-cyclo-5α-androst-2-en-3,17-dione **2.53**.

3,17-dione has been reported but structures were not given (Youssefyeh, 1963).

The structure of the BF_2.cyclosteroid derivative **2.53** was initially determined by elemental analysis, ^1H and ^{13}C NMR, ^{19}F NMR spectra and supported by X-ray crystallographic analysis (Figure 2.2).

In the ^1H NMR spectrum of the 3-acetoxy-2-ene **2.52**, the one proton doublet of doublets at δ = 5.4 ppm was assigned to the vinylic 2-H. The

Table 2.3 X-Ray crystallographic data for 2-acyl,19-acetoxy-3-(difluoroboronoxy)-1β,19-cyclo-5α-androst-2-en-3,17-dione **2.53**.

	Compound 2.53
Formula	$C_{23}H_{29}O_5BF_2$
M_r	434.29
T(°K)	298
Crystal Size (mm)	0.40 x 0.35 x 0.10
Crystal System	Monoclinic
Space Group	$P2_12_12_1$ (no.19)
Cell Dimensions:	
a/Å	15.077 (3) [a]
b/Å	9.782 (3)
c/Å	15.858 (6)
β	107.02
Cell Volume/Å^3	2236.3 (3)
Z	4
D_{calc}/g cm^{-3}	1.290
F(000)	920.00
λ/A (MoKα)	0.71069
$\mu_{(MoK\alpha)}$/cm^{-1}	0.99
Θ min. and max. (°)	8.74-23.80
2Θ max. (°)	50.1
Total Reflections	4380
Observed Reflections (I>2.00σ(I))	2764
Function Minimized	$\Sigma w(\|F_o\| - \|F_c\|)^2$
Least-square Weights	$4F_o^2/\sigma^2(F_o^2)$
R	0.051
R_w	0.036

[a]Estimated standard deviations in parentheses refer to the last digit.

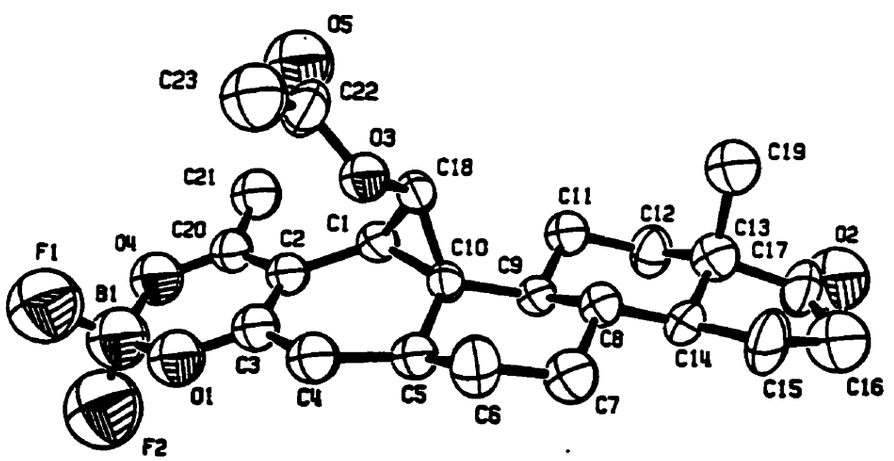


Figure 2.2 Pluto representation of 2-acyl,19-acetoxy-3-(difluoroboronoxy)-1β,19-5α-androst-2-en-3,17-dione **2.53**.

spin-spin coupling constants between the 2-H and the 1α-H and allylic 4-H were determined to be J = 5.43 Hz and J = 2.7 Hz, respectively. The one proton doublet at δ = 4.12 was assigned to the 19-H. The coupling constant between the 19-H and the 1α-H was determined to be J = 7.03 Hz (*cis* coupling). A one proton doublet of doublets at δ = 2.46 ppm was assigned to the 16β-H, and its coupling constant to the 15α- and 15β-hydrogens was determined to be 19.25 Hz and 9.05 Hz, respectively. Finally, the three singlets of three methyl protons each at δ = 2.10 ppm, δ = 2.09 ppm, and δ = 0.86 ppm were assigned to the 3-OCOC$\underline{H}_3$, the 19(R)-OCOC$\underline{H}_3$, and the 18-C$\underline{H}_3$, respectively.

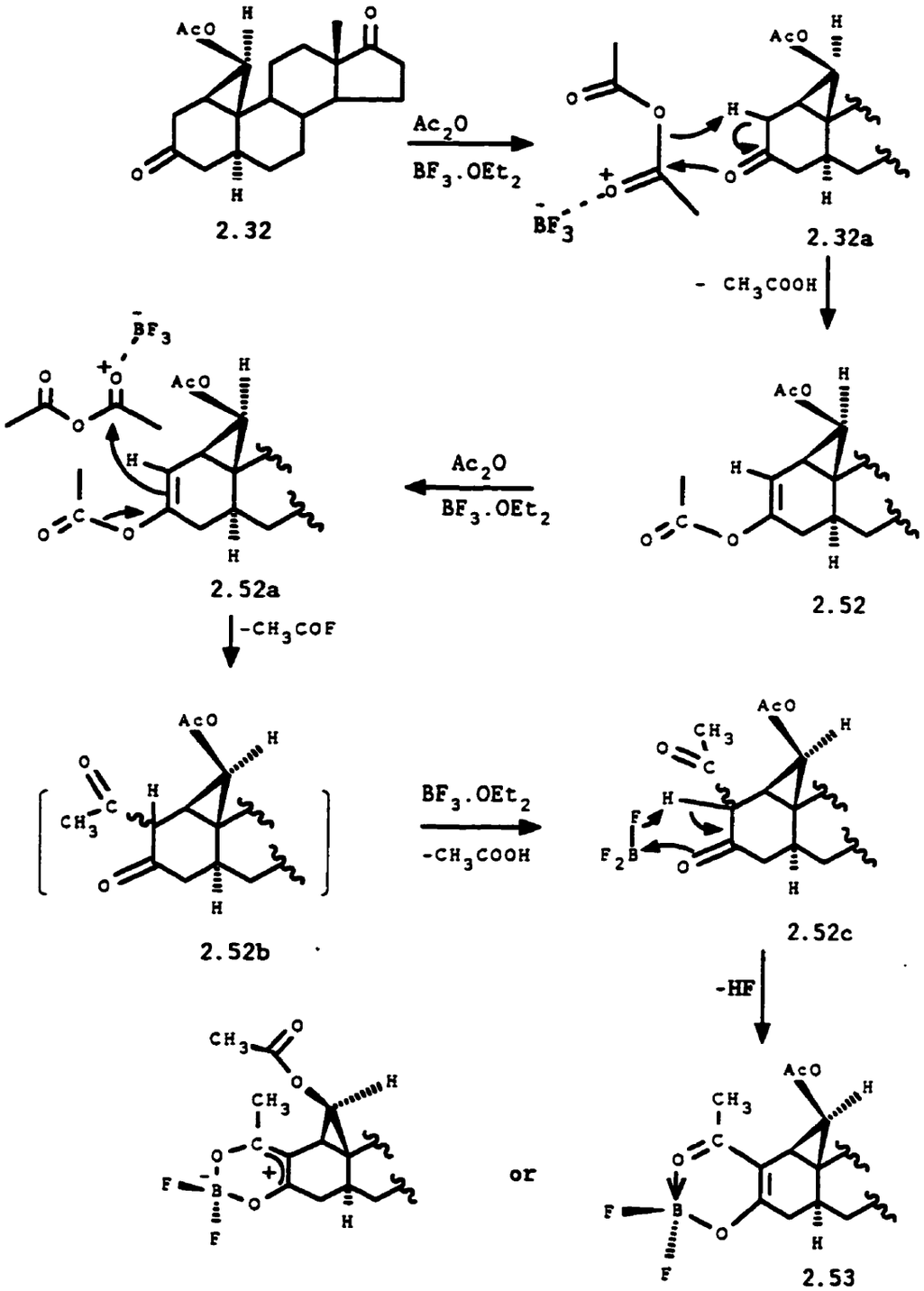
The ^{13}C spectrum of the 3-acetoxy-2-ene **2.52** showed the C-2 vinylic carbon signal at 108.04 ppm and two quaternary carbon signals at 169.29 ppm and 147.43 ppm, which were assigned to two carbonyl carbons: the 3-acetoxy group and the C(3), respectively.

In the ^1H NMR spectrum of the BF_2.cyclosteroid derivative **2.53**, the vinylic 2-H signal, previously observed for **2.52**, was not present. One of the singlet signals of the three methyl protons, previously observed, in the ^1H NMR spectrum of the 3-acetate enol **2.52**, at δ = 2.10 ppm, was shifted to lower field at δ = 2.43, and it has been assigned to the three protons of the 2-CH_3C=O substituent. Two remaining singlet signals of the three methyl protons at δ = 1.99, and δ = 0.88 were assigned to the 19(R)-OCOCH_3, and the 18-CH_3, respectively.

The ^{13}C NMR spectrum of compound **2.53** showed that the C-2 vinylic carbon, observed in the ^{13}C spectrum of the 3-acetoxy-2-ene **2.52** at 108.04 ppm, was replaced by a quaternary carbon and shifted to higher field at 105.15 ppm. This signal was assigned to C-2. Two signals, at 188.42 ppm and 192.57 ppm, were assigned to two quaternary carbons.

The signal at 188.42 was assigned to a quaternary carbon of the acyl group, $CH_3O\underline{C}$, attached to C-2. The signal at 192.57 ppm was assigned to C-3. The presence of the two fluorine atoms in **2.53** was supported by the ^{19}F spectrum. In the ^{19}F NMR spectrum there are two pair of doublets with the ratio 20:80. These pairs corresponded to two fluorine atoms attached to two isotope of boron, ^{10}B and ^{11}B, with their natural abundance $^{10}B:^{11}B$=19.58:80.42. One pair of doublets at δ -143.16 ppm (J 0.26) and δ -143.22 (J 0.26) ppm was assigned to two fluorine atoms attached to ^{10}B. A second pair of doublets at δ -143.22 ppm (J 0.26) and δ -143.84 ppm (J 0.26) was assigned to two fluorine atoms attached to ^{11}B. That there are the two doublet signals indicates that the fluorine atoms are not equivalent.

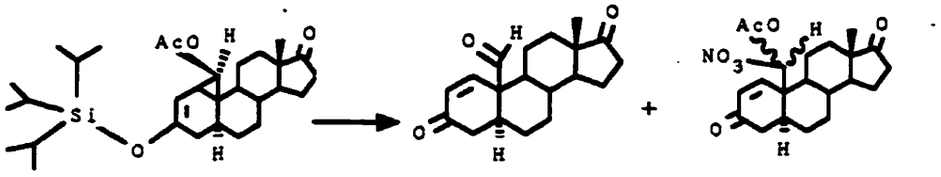
In a separate experiment, considering the acylation mechanism, it was found that the 3-acetoxy-2-ene **2.52** reverted to the starting compound **2.32** upon treatment with $BF_3.OEt_2$ in CH_2Cl_2 (Scheme 2.17). A result of this experiment indicates that rearrangement of the 3-acetoxy group, in compound **2.52**, *via* a four membered transition state, does not occur. Thus, the 2-acyl group in compound **2.53** must originate from acetic anhydride. A proposed mechanism of formation of the enol acetate **2.52** and the acylated BF_2.cyclosteroid **2.53** *via* oxonium intermediates of acetic anhydride is outlined in Scheme 2.18. The enol form of the ketone was suggested as a prerequisite for acylation reactions, but intermediates were not isolated (Hauser and Adams, 1944). In contrast, in a designed experiment, it was possible to isolate and identify an intermediate, which was found to be the enol acetate **2.52**. Formation of the enol ester **2.52** with acetic anhydride in the presence of boron trifluoride may involve interaction of the activated anhydride, and the oxonium ion, with the 3-carbomyl oxygen



Scheme 2.18 Proposed mechanism of the formation of the 3-acetoxy-2-en **2.52** and 2-acyl,19-acetoxy-3-(difluoroboronoxy)-1β,19-cyclo-5α-androst-2-en-3,17-dione **2.53**.

via a six-membered transition state **2.32a**. The activation of the anhydride consists in the coordination of BF_3 with the free pair of electrons of one of the carbonyl oxygens (or two carbonyl groups to form a bidentate "chelate" structure) thereby making the carbonyl carbon a stronger electron acceptor (e.g. increase the electrophilicity of the coordinated complexed functional group). The newly formed enol ester **2.52** may further react with a second oxonium ion, again *via* a six membered transition state, to give the 2-acyl ketone intermediate **2.52b**. Finally, the intermediate, upon reaction with BF_3, through coordination of BF_3 to the 3-carbonyl oxygen (**2.52c**) and elimination of HF, yields the BF_2 cyclosteroid derivative **2.53**. In compound **2.53**, the boron atom is connected both to the 3-oxygen *via* a covalent bond and to the 2-acyl carbonyl *via* a covalent coordinate bond. The BF_2.cyclosteroid derivative can be represented by two structures, **2.53** and **2.53a**, as outlined in Scheme 2.18.

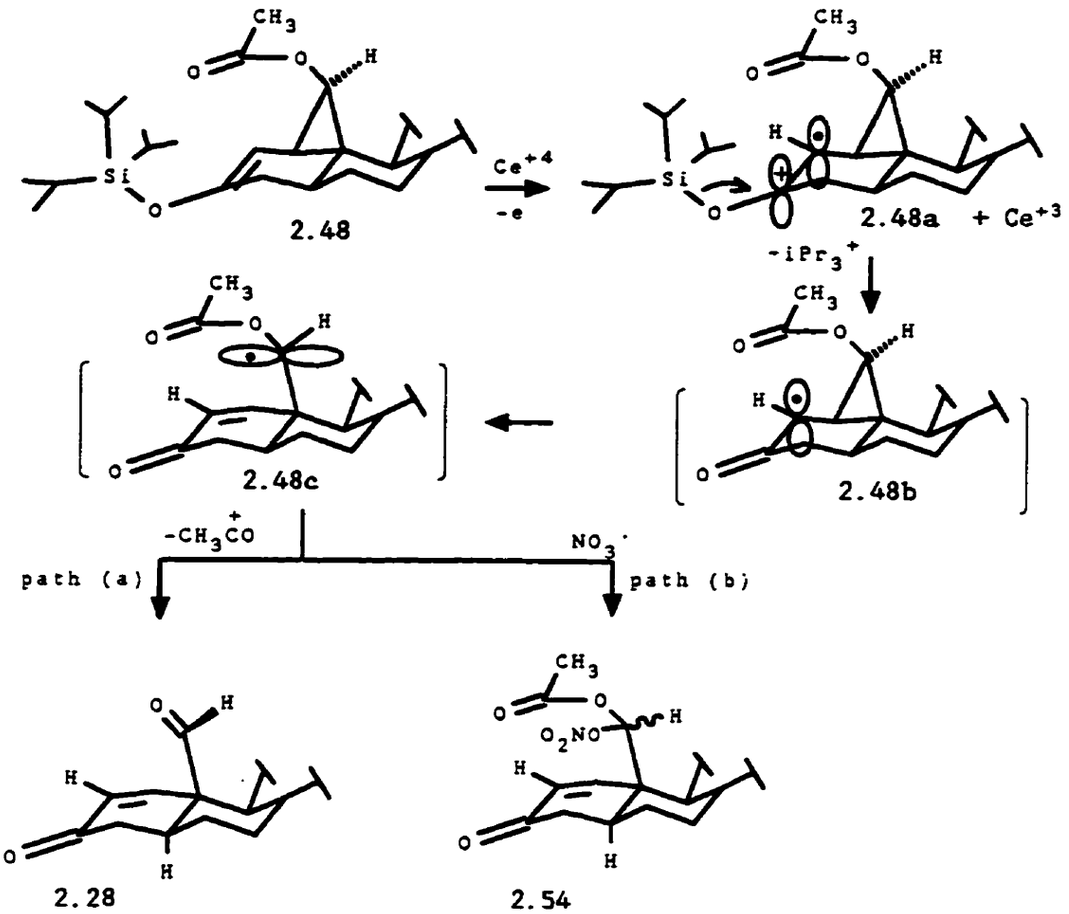
As outlined in Scheme 2.19, one electron oxidation of compound **2.48**



2.48 2.28 2.54

Reagents: **i**, $(NH_4)_2Ce(NO_3)_6$, DMF.

Scheme 2.19 The oxidation products of compound **2.48** by ammonium ceric nitrate: retro-cyclization and substitution at C-19.



Scheme 2.20 Proposed mechanism of oxidation of **2.48** by ammonium ceric nitrate.

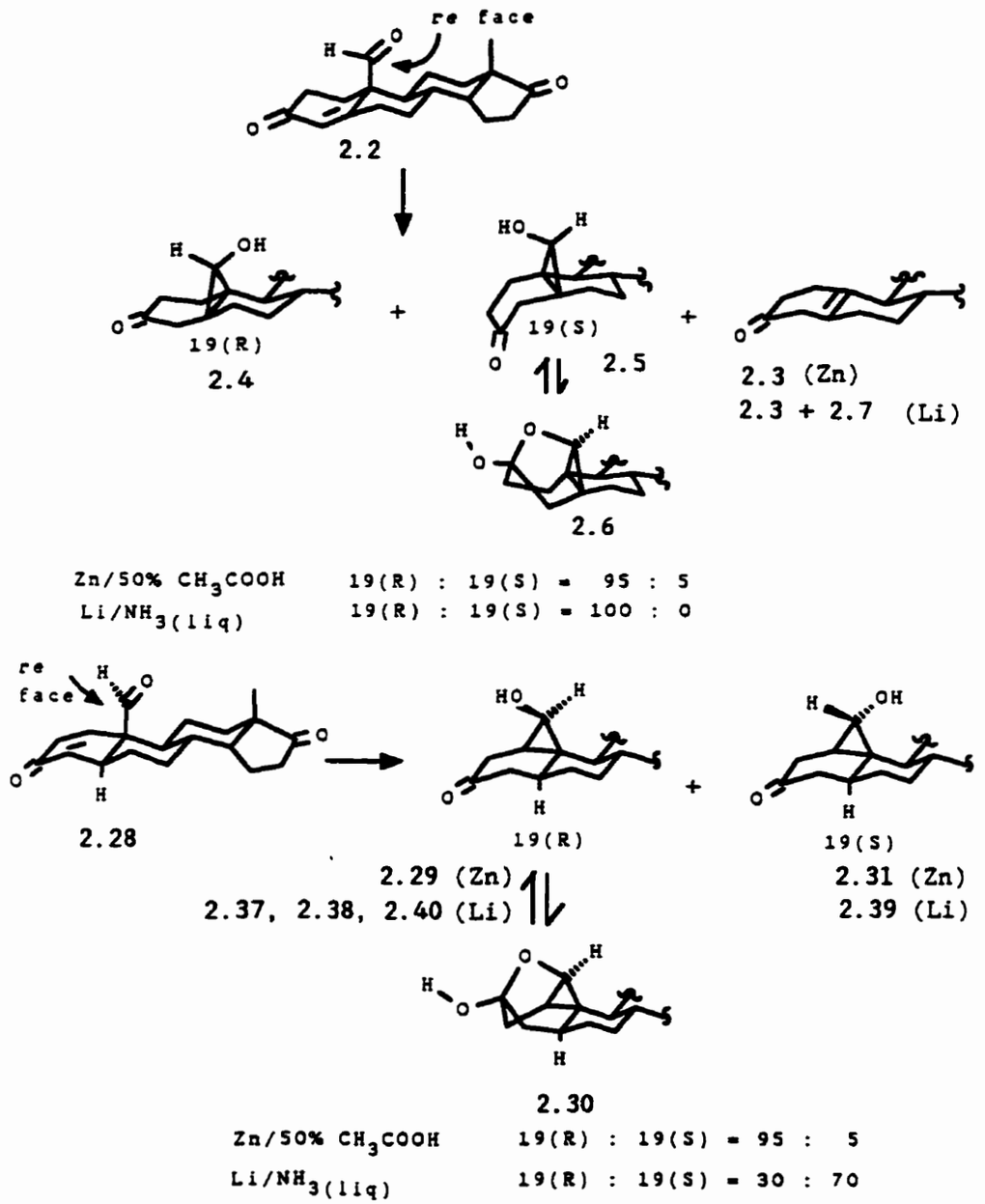
with ammonium ceric(IV) nitrate led to retro-cyclization and gave the starting compound **2.28** accompanied by the 19,19-disubstituted product **2.54** with a ratio **2.28**:**2.54**=1:1.5. Treatment of the 3-triisopropyl-siloxy,19(R)-acetoxy-1β,19-cyclo-5α-androst-2-en-17-one **2.48** with ammonium ceric nitrate in DMF gave a mixture of two compounds with the same polarity as determined by their R_f on TLC. The ^1H NMR spectrum of the mixture showed two patterns of signals. One of the ^1H NMR signal patterns has been assigned to the 1,2-unsaturated 19-aldehyde **2.28** and

the second to compound **2.54**. In the ^1H NMR spectrum of compound **2.54**, the singlet at 7.32 ppm has been assigned to the 19-H. Two doublets at 6.91 ppm and 6.07 ppm (J 10.2 Hz) were assigned to the C-1,2 vinylic protons of the 1,2-double bond. Two singlets at 2.03 ppm and at 0.88 ppm, each integrated for three protons, were assigned to the 19-acetoxy and the 13-Me groups, respectively. The stereochemistry of the 19,19-disubstituted compound **2.54** has not been determined. Even though an effort was made to separate the products by FCC, none has been isolated.

The proposed mechanism of oxidation of **2.48** by ammonium ceric(IV) nitrate is given in Scheme 2.20. A one electron oxidation of **2.48** with ammonium ceric nitrate can result in generation of an odd electron species, **2.48a**. The odd electron species is very reactive and decomposes further with elimination of the triisopropylsilyl group to generate a radical at C-2, **2.38b**. The radical (SOMO) reacts with the LUMO of the 1β,19-cyclopropyl bond forming a new radical at C-19, **2.48c**. The newly formed radical is stabilized by the 19-acetoxy group. However, the 19-radical **2.48c** may react further either with the elimination of the acyl radical (path a) or by the abstraction of ONO or ONO_2 radical (path b), to yield **2.28** or **2.54**, respectively. Surprisingly, the nitronium NO_2 radical was not involved in the reaction. The EIMS spectrum showed the molecular ion at m/z 405 consistent with the introduction of the NO_3 species into the molecule.

It is not known whether compound **2.54** would be susceptible to the reductive cyclization reaction and what products would be formed?

Proposed Mechanism of Reductive Cyclization of Androst-4-ene-3,17-dion-19-al and Androst-1-ene-3,17-dion-19-al with Zinc in 50% Aqueous Acetic Acid and with Lithium in Liquid Ammonia.

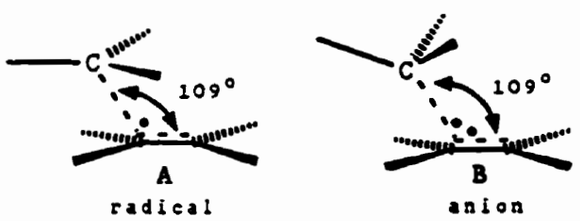


Scheme 2.21 Major products of reductive cyclization of 2.2 and 2.28 with zinc in 50% aqueous acetic acid (25 °C) and lithium in liquid ammonia (- 78 °C).

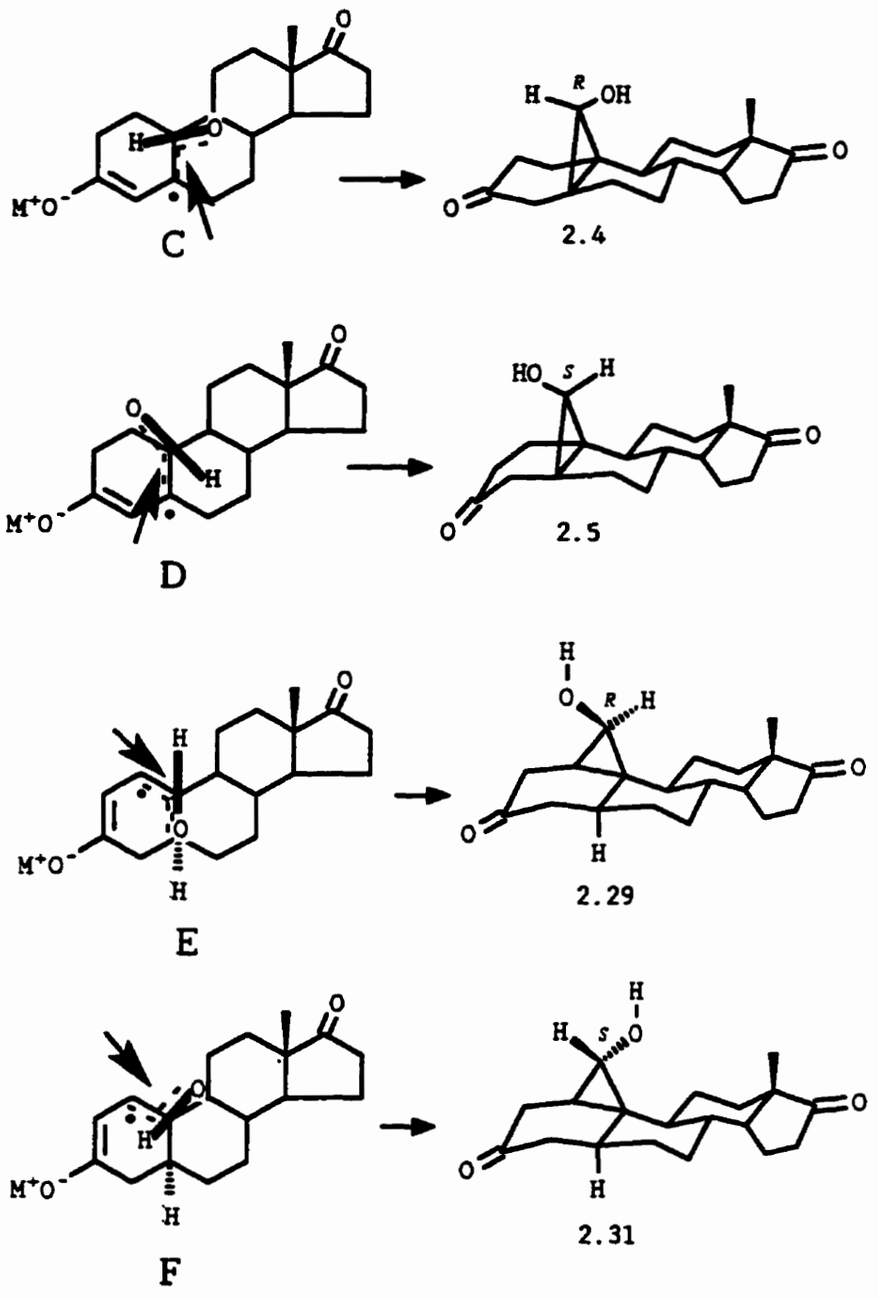
A summary of the results of reductive cyclizations of two unsaturated steroid derivatives, **2.2** and **2.28**, is presented in Scheme 2.21. As we can see, reductive cyclization of androst-4-ene-3,17-dion-19-al **2.21** both with zinc in 50% aqueous acetic acid and lithium in ammonia gave predominantly one major product, the **less thermodynamically** stable 19(R)-alcohol **2.4** (63%).

The products of 5α-androst-1-ene-3,17-dion-19-al **2.28** on reductive cyclization were dependent on the reduction conditions. Unlike reduction of **2.2**, the major product from reductive cyclization of **2.28** with zinc in acetic acid was the **more thermodynamically** stable 19(R)-alcohols, **2.38** and **2.40** (Scheme 2.14). The reverse process was observed upon reduction of **2.28** with Li-NH_3 and the reaction yielded the **less thermodynamically** stable isomer, the 19(S)-alcohols, **2.31**, **2.37**, and **2.39**, (70%), accompanied with the 19(R)-alcohol, **2.38** and **2.40**, (30%). The above results indicate that, although different reduction conditions were employed, conversion of **2.2** → **2.4** involved the same transition structures, whereas the conversion of **2.28** → (**2.38** and **2.40**) or **2.28** → (**2.31** + **2.37** + **2.39**) must involve different structures in the transition state.

There is a general agreement that homolytic addition proceeds *via* transition structures shown in Scheme 2.22 (Beckwith *et al.*, 1983).



Scheme 2.22 Nucleophilic addition to the π system. Transition state structures of a radical **A** and anion **B**.



Scheme 2.23 Orientations of the 19-CHO group in the transition structures **C**, **D**, **E**, and **F** leading to the products **2.4**, **2.5**, **2.29**, and **2.31** respectively. Arrows point to the vertices of the incoming nucleophile (radical or anion) to the π system of the 19-HC=O group: M^+ = Zn^+, Zn^{2+}, Li^+, H^+ or none.

The transition structures have three reactive centers, which are situated at the verticles of a slightly obtuse triangle lying within a plane orthogonal to the nodal plane of the π system. For the intramolecular addition either in a radical anion or anion radical or enolate radical or enolate anion or dianion, this array must be accommodated, as shown in Scheme 2.23, within the gross transition structures, C, D, E, and F leading to the cyclic products 2.4, 2.5, 2.29, and 2.31, respectively.

Mechanism of reductive cyclization of androst-4-ene-3,17-dion-19-al, 2.2.

The results of reductive cyclization of androst-4-ene-3,17-dion-19-al 2.2 have been described in Chapter 2.1.0. A high degree of stereoselectivity has been observed for both the Zn/aqueous acetic acid and Li-NH_3 reductions. In each case, the less thermodynamically stable 19(R)-alcohol 2.4 was the major product. This phenomenon arises because the transition state is shifted towards one of the geometric isomers, in which orientation of the 19-carbonyl oxygen is out-of-ring A with the *re* face exposed to the incoming nucleophile, as shown in conformer C (Scheme 2.23). This situation may be created by the following factors:

(1) structure of the developing radical (sp^2) or anion centers (sp^2 or sp^3);

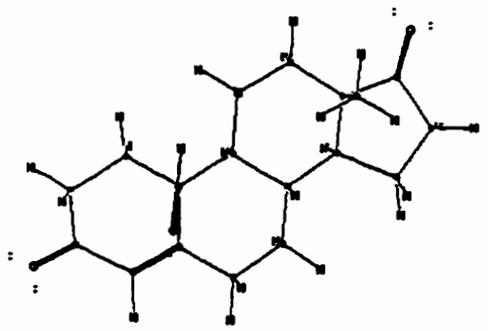
(2) hybridization changes at C-5 and consequently conformational alteration of ring A and/or ring B, when the reaction proceeds *via* a radical or carbanion;

(3) electrostatic repulsion between a radical or a negative charge, localized at C-5, and the lone-pairs of electrons on oxygen of the 19-CHO group;

(4) stabilizing effects of a π orbital of the C-5 anion by a π orbital of the 19-CHO group (homoconjugation effect).

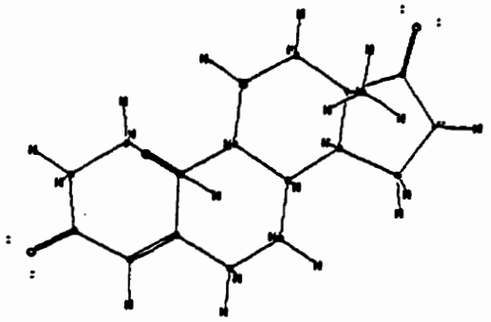
The spatial arrangement of the 19-aldehyde group is one of the most important factors influencing the stereoselectivity of the reaction. Therefore, the conformations of the 19-HC=O moiety, in compound **2.2**, have been studied by NOE experiments. NOEs of the 19-aldehyde proton to the 1β-H (1.2%), 2β-H (1.42%), 6β-H (4.57%), 6α-H (1.33%), 8β-H (1.9%), and 11β-H (1.83%), clearly showed that the 19-proton interacts with all neighbouring protons, except 4-H and 7-H. NOEs established that the 19-CHO group is able to rotate around the C-10-C-19 bond. For these conformers a complete optimization energy was calculated by means of Allinger's MMX molecular mechanics using the standard force-field parameterization including lone pairs of oxygen (Figure 2.3). The MMX program was applied to each conformer as follows: first, the 19-CHO group was rotated manually so the 19-aldehyde proton almost eclipsed the neighbouring proton; second, the 19-proton was fixed and the adjusted position and positions of the remaining atoms were then optimized. Populations of the optimized conformers have been estimated from recalculations of the NOE's results by assuming that a sum of all NOEs is 100%. Energies of the conformers are shown in Figure 2.3. Their energies can be compared with the reference conformers with the 19-HC=O in the lowest energy conformation (Figure 2.3).

To explain the observed diastereoselectivity in the 19(R)-alcohol formation, all conformers must have the 19-carbonyl oxygen oriented *trans* but at about 109° to the developing radical or anion prior to the cyclization. Approximately 60% of the rotational conformers, **G-I**, have the 19-HC=O moiety oriented such that the oxygen of the 19-aldehyde



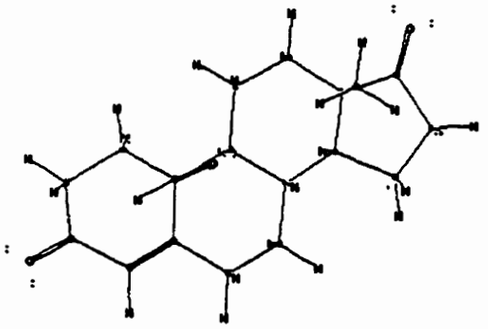
E = 38.83 kcal mol^{-1}

O(19)-C(19)-C(10)-C(5)= - 10.1°



E = = 37.43 kcal mol^{-1}

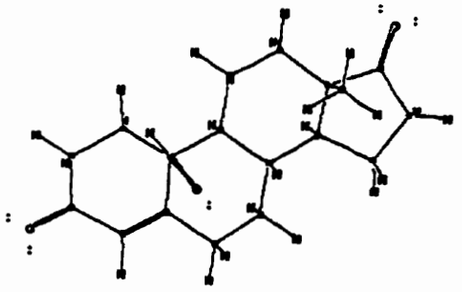
O(19)-C(19)-C(10)-C(5)= - 118.5°



E = 38.0 kcal mol^{-1}

O(19)-C(19)-C(10)-C(5)= - 125.2°

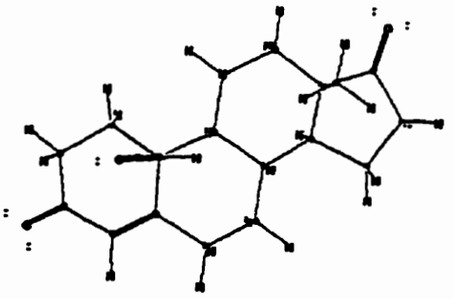
Figure 2.3 The reference structures: MMX geometrical optimization of androst-4-ene-3,17-dion-19-al 2.2: the 19-aldehyde group is in the lowest energy conformations.



G

E = 40.7 kcal mol^{-1}

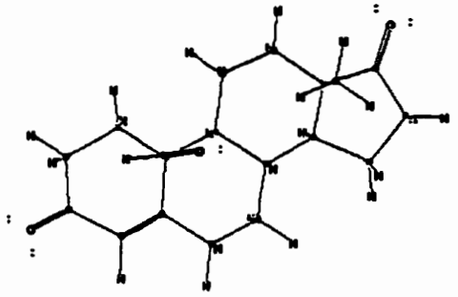
O(19)-C(19)-C(10)-C(5) +43.6°



J

E = 39.58 kcal mol^{-1}

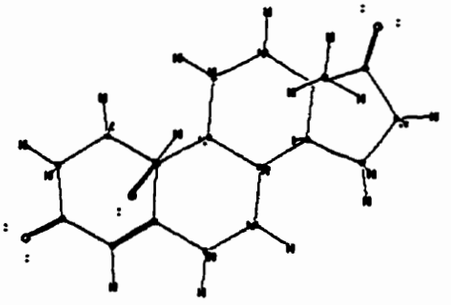
-83.7°



H

E = 38.99 kcal mol^{-1}

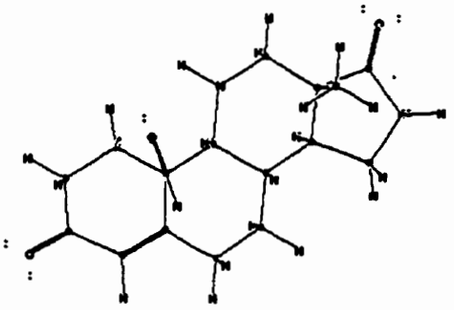
O(19)-C(19)-C(10)-C(5) +98.8°



K

E = 39.48 kcal mol^{-1}

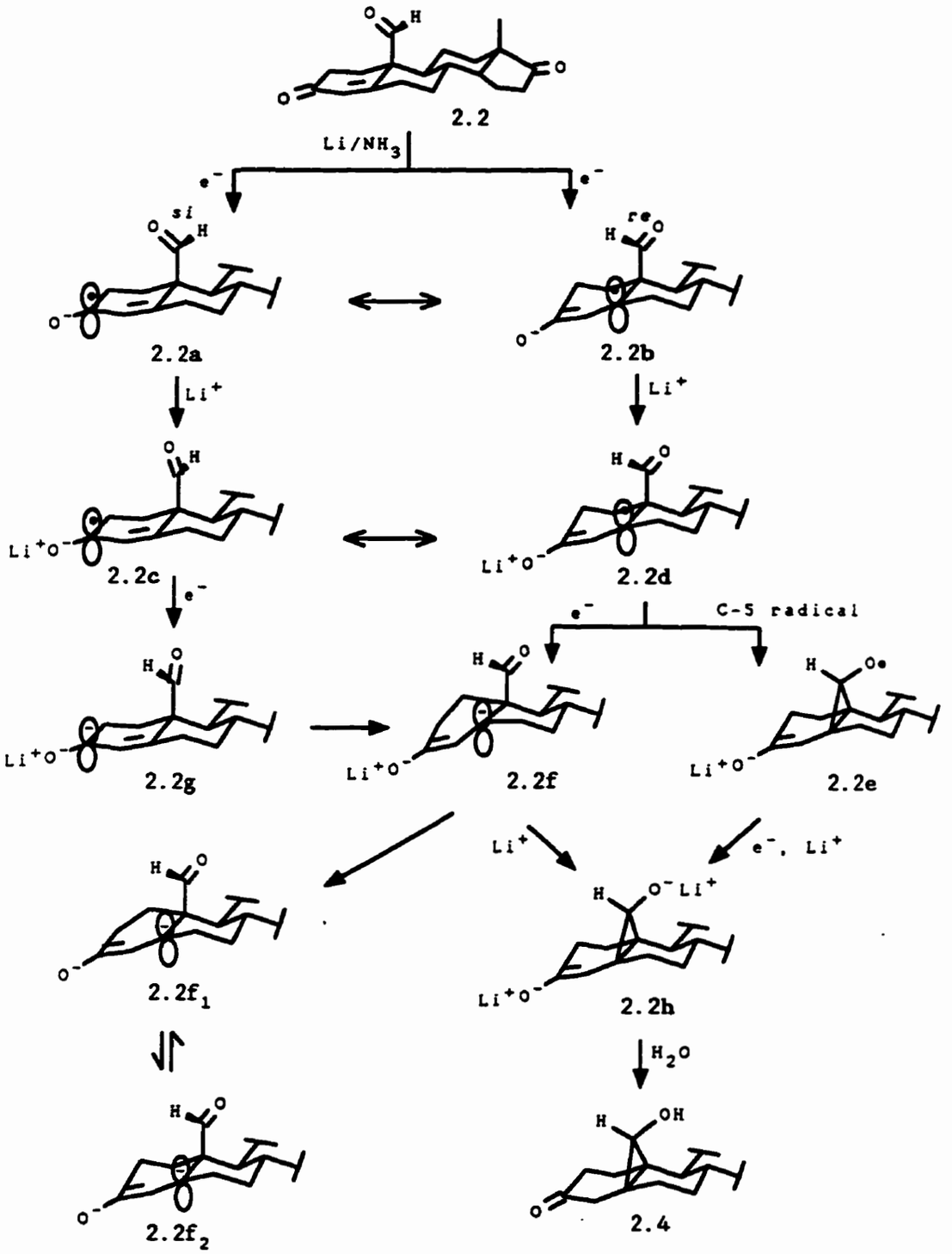
-35.7°



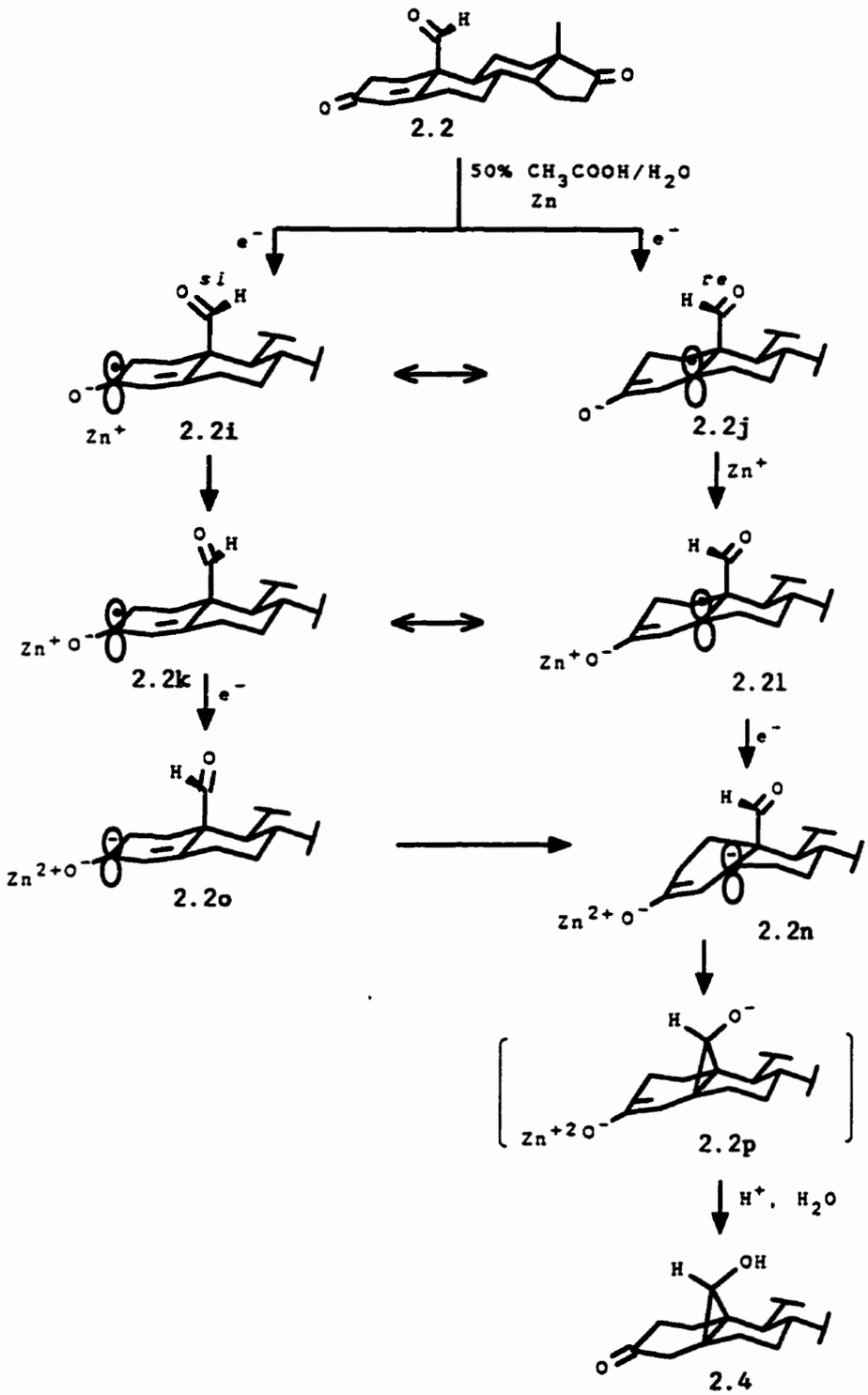
E = 40.93 kcal mol^{-1}

O(19)-C(19)-C(10)-C(5) -158.0°

Figure 2.4 The spatial arrangement of the 19-HC=O group of androst-4-ene-3,17-dion-19-al 2.2: MMX geometry calculations based on NOEs.



Scheme 2.24 Proposed mechanism of reductive cyclization of androst-4-ene-3,17-dion-19-al 2.2 with Li-NH_3.



Scheme 2.25 Proposed mechanism of reductive cyclization of androst-4-ene-3,17-dion-19-al 2.2 with zinc in aqueous acetic acid.

group is above ring B or out-of-ring A. These conformations could be considered as the precursors of the 19(R)-alcohol **2.4**. At least 35% of the remaining rotational conformers, **J** and **K**, must adopt the appropriate orientation of the 19-CHO group.

The proposed mechanism and stereochemistry of reductive cyclization of androst-4-ene-3,17-dion-19-al **2.2** with zinc-aqueous acetic acid and Li-NH_3 are outlined in Schemes 2.4 and 2.5. Numerous studies established several general characteristics of these reactions which suggested a stepwise two-electron reduction involving the formation of organolithium or organozinc intermediates or dianions (Caine and references cited there, 1976; Barton and Robinson, 1954). A one-electron transfer to give a radical anion, and after protonation, the thermodynamically unstable alkoxide, was suggested by House *et al.* (House *et al.*, 1963). The stereochemistry of the reduction of α,β-unsaturated ketones has been clarified (Malhotra *et al.*, 1967, Robinson, 1965, Stork, 1965).

In general, reductive cyclization can be interpreted as the stepwise addition of two electrons at the ends of the conjugated double bonds (Michaelis and Schubert, 1938). In this case, the atoms at the end are oxygen and carbon. Thus, an electron can be added to the end of a π system of the 3-C=O group or to the end of a π system of the 4,5-double bond, i.e. to the C-5 carbon.

Consider the addition of an electron to the antibonding π orbital of the 3-carbonyl group during Li-NH_3 reduction. The reaction could be initiated by reductive activation of the 3-carbonyl group, i.e. transfer of one electron, from the solution, to the lower π^* orbital (LUMO) of the conjugated 3-ketone to generate the radical anion (ketyl) **2.2a**. Reorganization of electrons also gives a radical anion **2.2b**,

however, with the formation of the odd electron at C-5. The stability of the radical anion is due to the formation of a symmetrical resonance system, **2.2a** ↔ **2.2b**. These radical anions can be converted to the enolate radicals, **2.2c** and **2.2d**. The intermediates formed, the C-5 radical anion or O-lithium enolate radical, **2.2b** or **2.2d**, are capable of adding to a π system of the 19-C=O moiety, as the *re* or *si* face of the 19-aldehyde group is oriented to the incoming nucleophile at the dihedral angle, O(19)-C(19)-C(10)-C(1), of *ca* 109°. The intermediate produced could be a reactive 19-alkoxy cyclopropyl radical enolate **2.2e**, which after further one electron reduction would give di-enolates **2.2h**. During the reduction process, a repulsive electrostatic effect on the 19-CHO group first comes from the ketyl **2.2a**. When the 19-carbonyl group is localized above ring A, it is reoriented out-of-ring A toward rings C and B. Second repulsive electrostatic interactions on the 19-HC=O group originate from the C-5 radical. Theoretically, as shown in Scheme 2.24 (conformer **2.2a** and **2.2b**), there are two orientations of the 19-CHO group relative to the C-5 nucleophile. Thus, the nucleophile is available to add to the *re* or *si* face of the 19-aldehyde. The driving force for the reaction is the relief of steric electronic repulsion between the C-5 radical and the free pairs of electrons on the oxygen of the 19-HC=O.

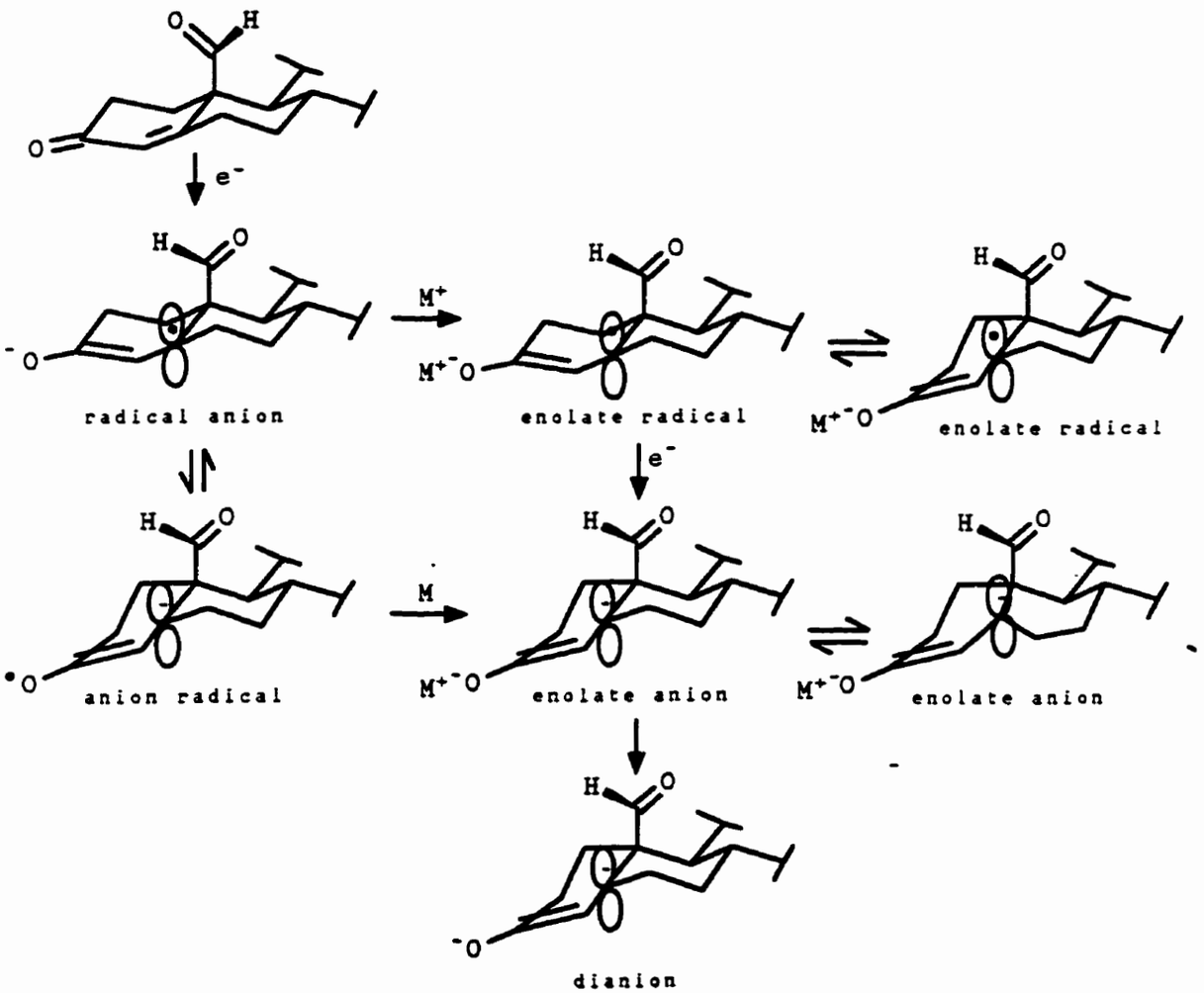
The acceptance of a molecule of a single electron necessarily leads to an electronic rearrangement. Because electrons tend to pair, the resulting molecule may take up a second electron more readily. In this case, the radical anions, **2.2a** or **2.2b**, may form a tight ion pair with the lithium cation and could then be capable of accepting a second electron to yield the O-lithium enolate anion **2.2f** or **2.2g** or di-anions **2.2f**$_1$ and **2.2f**$_2$. It is also important to note that the electrostatic

repulsion between a pair of nonbonded electrons on the oxygen of the 19-HC=O group and the resulting C-5 negative charge leads to the stabilizing effect of the the C-5 anion by the π system of the 19-C=O group. The stabilizing influence of the 19-aldehyde group on the C-5 anion can be explained in terms of orbital and electrostatic interactions. Similarly, a large stabilizing effect of the formyl group on an anion in a formylcyclopropyl anion has been reported (Baschky *et al.*, 1995; Chou *et al.*, 1993).

The recognition of factors which influence particular orientations of the 19-CHO group is important to understand the mechanism of the reaction.

In the reductive cyclization of the 19-CHO, the major product, the less stable isomer **2.4**, results from nucleophilic addition to the *re* face. The qu. 'tative evidence provided by the cyclization of the 19-aldehyde showed that the ratios of products are not determined by the relative stabilities of the most stable conformation **2.5**. This is evidence that stereoelectronic requirements for *re* face addition are more important than the relative stabilities of the products. Furthermore, differences in stereoselectivity correlate with the expected effects of the 19-CHO orientation on the total energy of the potential intermediates. The potential intermediates in the reductive cyclization are expected to be: radical anions, enolate radicals, enolate anions, or di-anions, either with ring A/ring B conformations boat/ chair or half-chair/boat (Scheme 2.26). All intermediates, except of the di-anions, consist of two pairs of conformers, in which both the *re* and *si* faces of the 19-aldehyde group could be exposed to the incoming C-5 nucleophile. These pairs of conformers differ in energy and dihedral angles, O(19)-C(19)-C(10)-C(1). Based on MMX

geometry optimization and energy calculations, it was found that for almost all the *re* conformers, i.e. with the *re* face pointed to the incoming nucleophile, the energy is lower then for the *si* conformers. However, in two cases, the enolate radicals and enolate anions with ring A/B boat/chair or chair/boat, the observed stereoselectivity **exceeds** the probable differences in either the stability of the two potential intermediates or they are not the real intermediates. The



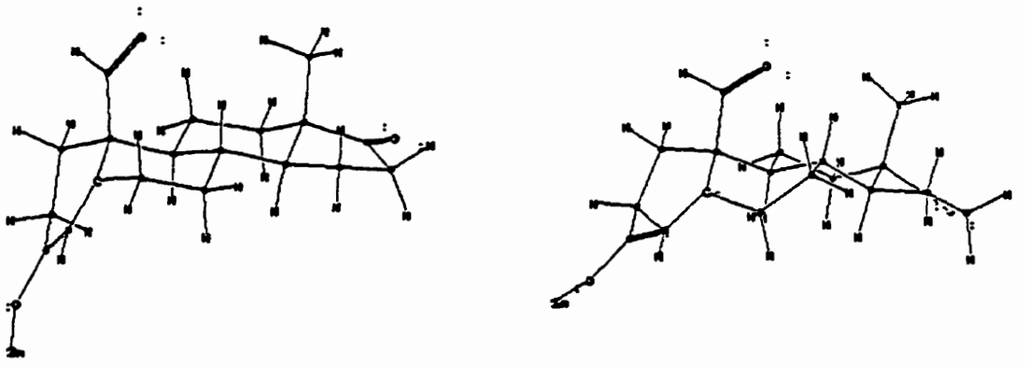
Scheme 2.26 Proposed transition structures during the reductive cyclization reaction.

energy of the *re* conformers is either equal to or lower then the *si* conformers. For the anions, a sole conformer has been established, namely the *re*. Based on the dihedral angle determination, there are two potential intermediates, **2.2b** and **2.2d**, that could be considered as the precursors of the 19(R)-alcohol **2.4** (Scheme 2.24).

The dihedral angles, O(19)-C(19)-C(10)-C(5), of **2.2b** and **2.2d** are 117° and 114°, respectively. Of these two, the radical anion **2.2b** has lower energy (30.85 kcal mol^{-1}) than the enolate radical **2.2d** (34.11 kcal mol^{-1}) in the transition state. However, the most reactive (the least stable) are the two di-anions, $\mathbf{2.2f_1}$ and $\mathbf{2.2f_2}$, and they have only one conformation, the *re* conformation of the 19-aldehyde (Scheme **2.26**; dianion). Their MMX calculated energy is *ca* 80 kcal mol^{-1}. Only one conformation results from the electrostatic interactions between a free pair of electrons on the 19-carbonyl group and the negative charges localized on the 3-oxygen and the C-5 carbon. When the reductive cyclization proceeds *via* the carbanion, one would expect its protonation. The fact that the developed carbanion is not protonated may indicate that the reaction proceeds *via* a radical mechanism or that the developing anion centre is stabilized by the 19-CHO group or the C-5 anion does not have a full sp^3 structure, which is required for protonation, or the rate of its addition to the π system of the 19-carbonyl group is faster then protonation. However, when the reaction proceeds *via* the C-5 carbanion with a full sp^3 hybridization, intermediates are expected to have the structures shown in Figure 2.5. The structure with ring A/B **2.2**, half-chair/boat conformation **2.2f''**, could be required to fulfill the orbital overlap requirements for the reaction to occur.

The governing step in the reductive cyclization is either the

addition of the first or the second electron to the conjugated system. If the crucial step were the addition of the first electron, we should obtain a radical anion. The tertiary radical formed, being the thermodynamically stable species, would lead to the more stable product, the 19(S)-alcohol **2.6** (Scheme 2.21). Hence, the crucial step is the passage from a planar form of the C-5 carbon (sp^2, radical) to a pyramidal form (sp^3, anion). The tertiary anion is the least thermodynamicaly stable species (the most substituted carbon), consequently leading to the reactive species and further to the less stable isomer, the 19(R)-alcohol **2.4** (Scheme 2.21).



2.2f' **2.2f''**

Figure 2.5 The representative structure for the reductive cyclization *via* the C-5 carbanion: **2.2f'**, ring A, half-chair and ring B, chair or **2.2f''**, ring A, half-chair-and ring B, boat.

One can only say that the observed stereoselectivity of the reduction is best explained if **2.2d** or **2.2f** (Scheme 2.24) and **2.21** and **2.2n** (Scheme 2.25) yield **2.4**, respectively. The fact that the NMR conformational analysis of ring A of 19(R)-hydroxy-5β,19-androstane-3,17-dione **2.4** (Marat, 1995) showed that there are structures with ring A being in a boat and "inverted boat" conformation may indicate that

these conformers result from two different precursors and an equlibrium is involved.

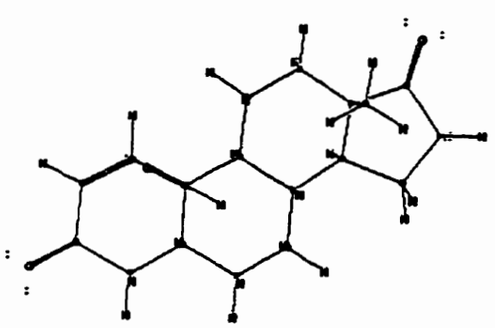
In conclusion, the cyclization reaction is characterized by the following steps:

1. addition of a first electron to give a thermodynamically more stable, C-5 tertiary radical anion species (sp^2) followed by the 3-alkoxy anion protonation or organometal intermediate formation (a tight-ion pair). The electrostatic repulsion effect of the 19-CHO group, e.g. between a lone pair of electrons on the oxygen and the radical.
2. delivery of a second electron to produce a thermodynamically less stable, C-5 tertiary anion (sp^3), consequently leading to ring conformational adjustment (A ring, boat → half-chair) and separation of the 19-carbonyl group from possible interactions with the $3\text{-}O^-(Li^+$, or Zn^+, or Zn^{2+}, or $H^+)$
3. electrostatic repulsion between the C-5 negative charged carbon and a free pair of electrons on the oxygen of the 19-HC=O group, consequently leading to the second orientation of the 19-CHO substituent out-of rings A and B;
4. electrostatic attractive effect between the C-5 negative charge and a π system of the 19-aldehyde group (hyperconjugation effect) leading to the nucleophilic alkylation of the 19-carbon and the formation of the cyclopropanol function.

Mechanism of reductive cyclization of 5α-androst-1-ene-3,17-dion-19-al

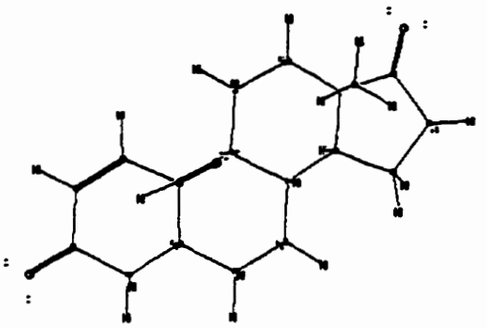
Unlike the reductive cyclization of androst-4-ene-3,17-dion-19-al **2.2**, the major product from the reduction of androst-1-ene-3,17-dion-19-al **2.28**, with zinc in aqueous acetic acid, was the

thermodynamically more stable 19(R)-hydroxy-1β,19-cycloandrostane-3,17-dione **2.29 (95%). Reduction with zinc/aqueous acetic acid showed a dramatic change in the product ratio in comparison with reduction of androst-4-en-3,17-dion-19-al 2.2, for which the thermodynamically less stable** 19(R)-hydroxy-1β,19-cycloandrostane-3,17-dione **(95%) was produced. The reversed ratio of the products was obtained with Li-NH_3 reduction of 2.28.**



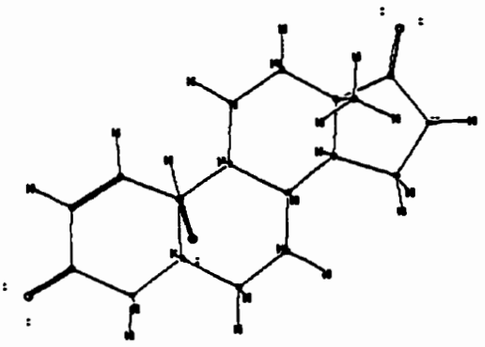
E = 39.7 kcal mol^{-1}

O(19)-C(19)-C(10)-C(1) -0.20°



E = 39.8 kcal mol^{-1}

O(19)-C(19)-C(10)-C(1) -130.6



E = 39.6 kcal mol^{-1}

O(19)-C(19)-C(10)-C(1) +127.8°

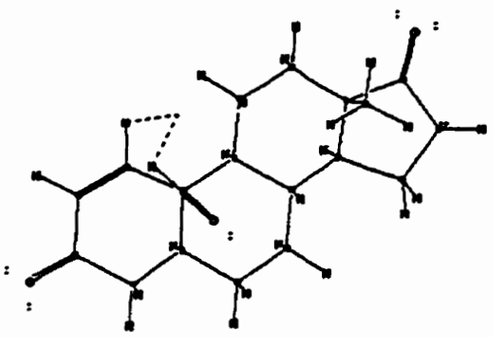
Figure 2.6 MMX geometry optimization of the 19-CHO conformers of androst-1-ene-3,17-dione 2.28.

Both isomers were produced, the thermodynamically less stable 19(S)-alcohols, **2.39** and **2.31**, (70%) and the more stable 19(R)-alcohols (30%), **2.30**, **2.37**, **2.38**, and **2.40**, (Scheme 2.11). These results parallel the reduction of **2.2** with Li-NH_3, where also the thermodynamically more stable isomer **2.4** (95%) was produced (Scheme 2.21).

The results of reductive cyclization, under the different conditions employed, implicate different spacial orientations of the 19-CHO group in the transition structures as shown in Scheme 2.23, **E** → **2.29** and **F** → **2.31**. This difference can be attributed to the difference in populations of the 19-aldehyde rotational conformers. Moreover, the sensitivity of the product ratio to various conditions suggests that change in conformer populations occurred. MMX geometry optimization of the possible conformers is shown in Figure 2.6. In the preferred conformations, π electrons of the 19-HC=O group eclipse either the C-10-C-1 or C-10-C-9 or C-10-C-5 bond. This phenomenon can be explained by the field effect model (FEM), named also the Kirkwood-Westheimer model (Kirkwood and Westheimer, 1938; Backer *et al.*, 1967). The spatial orientation of the 19-HC=O moiety results from electronic effects as a consequence of through-space interactions of the 19-HC=O moiety and π electrons of the 1,2-double bond. In addition to the MMX calculation of the 19-CHO conformers of **2.28**, NOE experiments were performed on **2.28**.

Conformations of the 19-CHO group in the neutral molecule **2.28** have been determined by NOE studies. NOE of the 19-aldehyde proton to 1-H (0.45%), 2-H (0.21%), 4βH (1.94%), 6β-4 (2.46%), and 8β-H (3.45%) show the presence of three major conformers **N**, **O**, and **P** (Figure 2.7).

For these conformers, MMX calculations of their complete

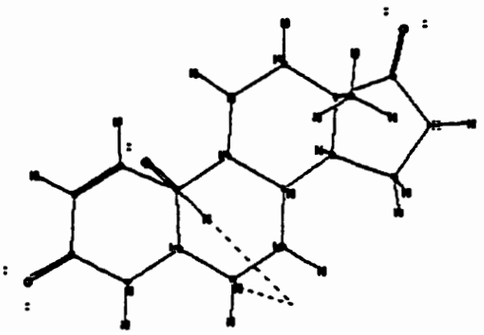


L

E = 40.74 kcal mol^{-1}

O(19)-C(19)-C(10)-C(1) +162.5°

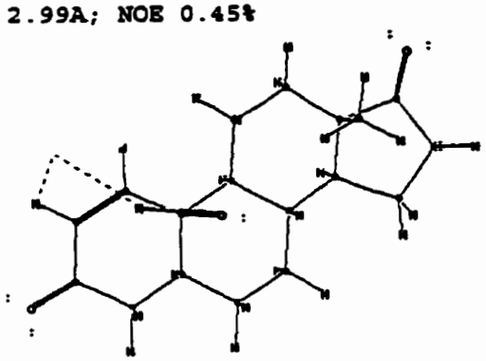
d= 2.99A; NOE 0.45%



O E = 40.74 kcal mol^{-1}

-19.51°

d= 2.25A; NOE 2.46%

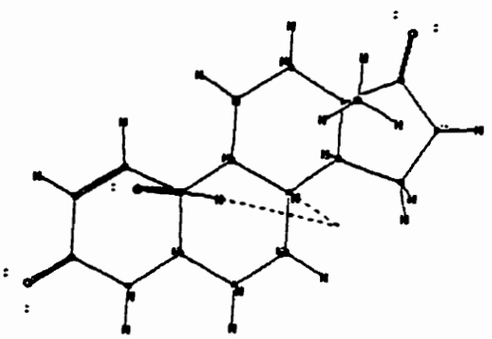


M

E = 40.91 kcal mol^{-1}

O(19)-C(19)-C(10)-C(1) -161.9°

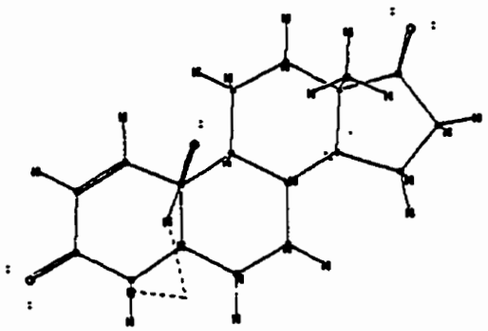
d= 3.79A; NOE 0.21%



P E = 40.74 kcal mol^{-1}

+18.28°

d= 2.19A; NOE 3.45%



N

E = 41.74 kcal mol^{-1}

O(19)-C(19)-C(10)-C(1) -85.5°

d= 2.19A; NOE 1.94%

Figure 2.7 The spatial arrangement of the 19-HC=O group of androst-1-ene-3,17-dion-19-al 2.28: MMX geometrical optimization based on NOE.

optimization energy was performed, while fixing the 19-aldehyde proton as close as possible to 1-H, 2-H, 4-H, 6-H and 8-H (Figure 2.7). Populations of these conformers were estimated as before assuming that the sum of all NOEs is equal to 100%. Approximately, 60% of the rotational conformers (L-O) can be considered as precursors of the 19(S)-alcohol **2.31** and *ca* 40% (conformer P) as precursors the 19(R)-alcohol **2.29**. This estimation is in agreement with the results of reductive cyclization of **2.28** under Li-NH_3 conditions (-78 °C). However, the course of the reduction is not valid for reaction of **2.28** with zinc in aqueous acetic acid (Scheme 2.21) where the more stable isomer, the 19(R)-alcohol/hemiacetal **2.29/2.30**, was the major product. The primary requirement to produce a different product, the 19(R)-alcohol, from the same substrate **2.28**, is to have the 19-CHO group with the *re* face oriented toward the incoming nucleophile at the dihedral angle 109°. This result from the zinc ion (Zn^{+2}, 0.74 A) being large enough to establish the preferred conformation in the transition state (Figure 2.8).

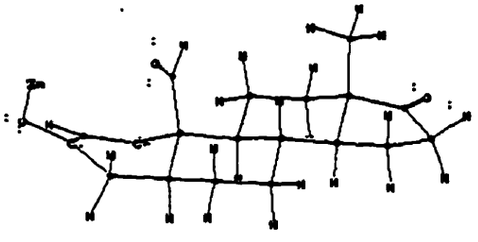
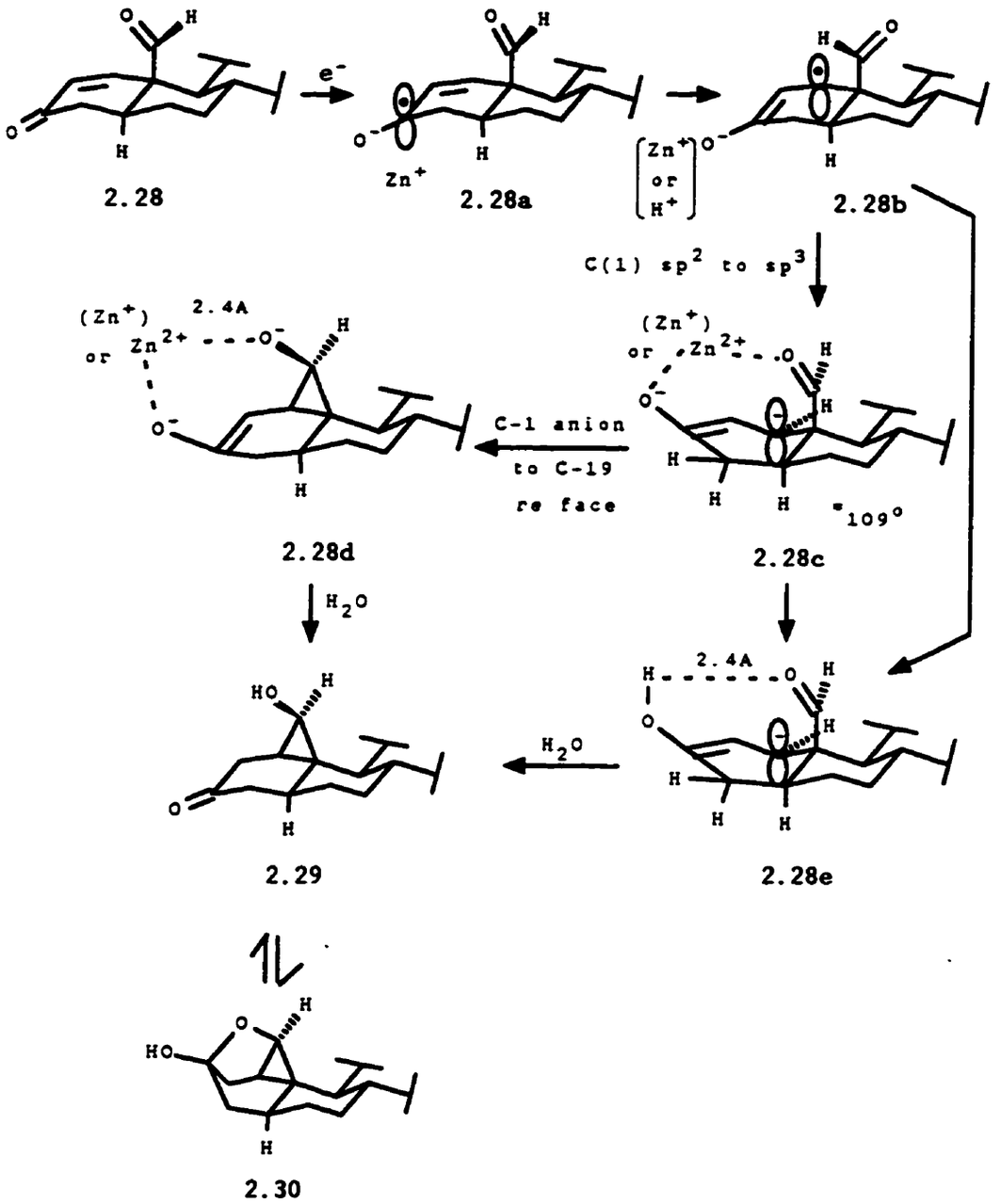


Figure 2.8 Proposed conformation of the O-zinc enolate anion intermediate of **2.28** eith Zn-aqueous acetic acid reduction.



*dihedral angle, O(19)-C(19)-C(10)-C(1)

Scheme 2.27 Proposed mechanism of reductive cyclization of androst-1-ene-3,17-dione **2.28** with zinc in aqueous acetic acid.

These spatial orientations are governed by electrostatic, coordination, and steric interactions. The factors influencing the formation of the less or more thermodynamically stable product are as follows:

(1) effect of electrostatic forces of the radical (or anion) on the free pairs of electrons of the 19-oxygen;

(2) reactivity of the C-1 secondary radical (or anion);

(3) structure of the C-1 radical (sp^2, trigonal, flat) or C-1 anion (fully sp^3, or not fully sp^3);

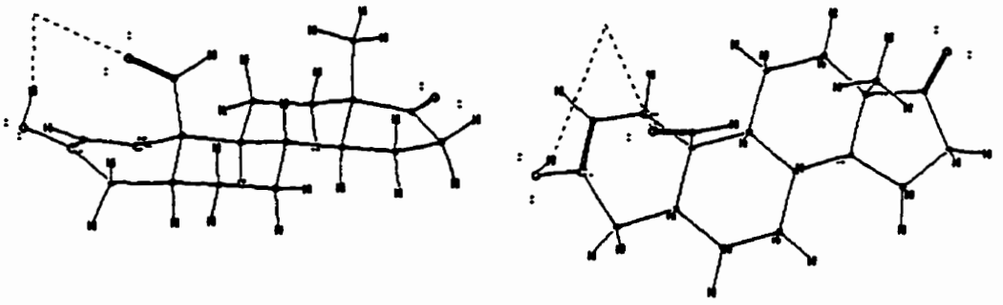
(4) ability of the 19-CHO group to stabilize a developing anion;

(5) distances and affinity of the lithium or zinc cations to coordinate to the 19-carbonyl group:

(6) intramolecular steric interactions of the 19-CHO with the neighbouring protons as well as with the developing radical (or anion) centers.

The proposed mechanism and stereochemistry of reductive cyclization of androst-1-ene-3,17-dion-19-al **2.28** with zinc-aqueous acetic acid is outlined in Scheme 2.27. First, the conjugated ketone reacts with metal (or an appropriate solvated electron) to give a radical anion (ketyl) **2.28a**, followed by reorganization of electrons to yield a radical anion **2.28b**. This radical anion has open to it several competetive reaction paths: first, reaction with a π system of the 19-C=O group; second, reaction with the zinc cations (Zn^+) to form a tight ion pair, i.e. O-zinc enolate radical; third, protonation to give an enol radical; and finally, reduction to a dianion. The radical anion **2.28a** or O-zinc enolate radical **2.28b**, because of its secondary character, is a reactive species. It could react with a π system of the 19-HC=O carbonyl to give the 19(S)-alcohol **2.31** (Scheme 2.21). On the other hand, reaction of the radical anion with Zn^+ or its protonation,

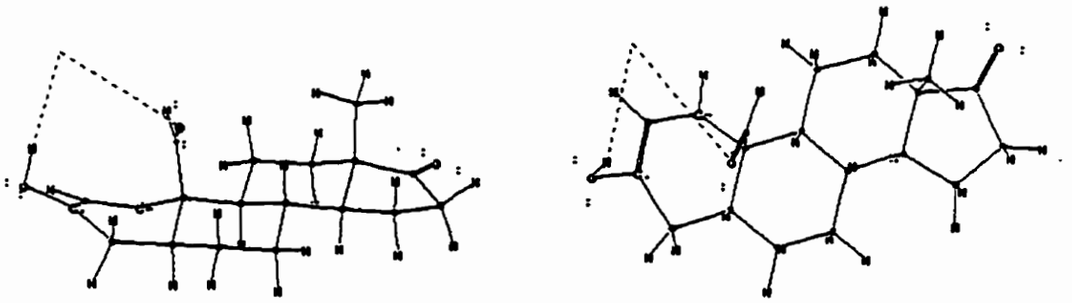
would give rise to the O-zinc enolate **2.28b** or enol radical **2.28b**, which would then be further reduced to an O-zinc enolate anion **2.28c** or enol anion **2.28e**. Reduction of the radical anions either to enolate anions, enol anions, or dianions would give an appropiate tetrahedral species, **2.28c** or **2.28e**, which would give rise to conformers in



E = 41.9 kcal mol^{-1}

O(19)-C(19)-C(10)-C(1) +48.0°

d = 2.43 A (2.4 A)



O(19)-C(19)-C(10)-C(1) +109°

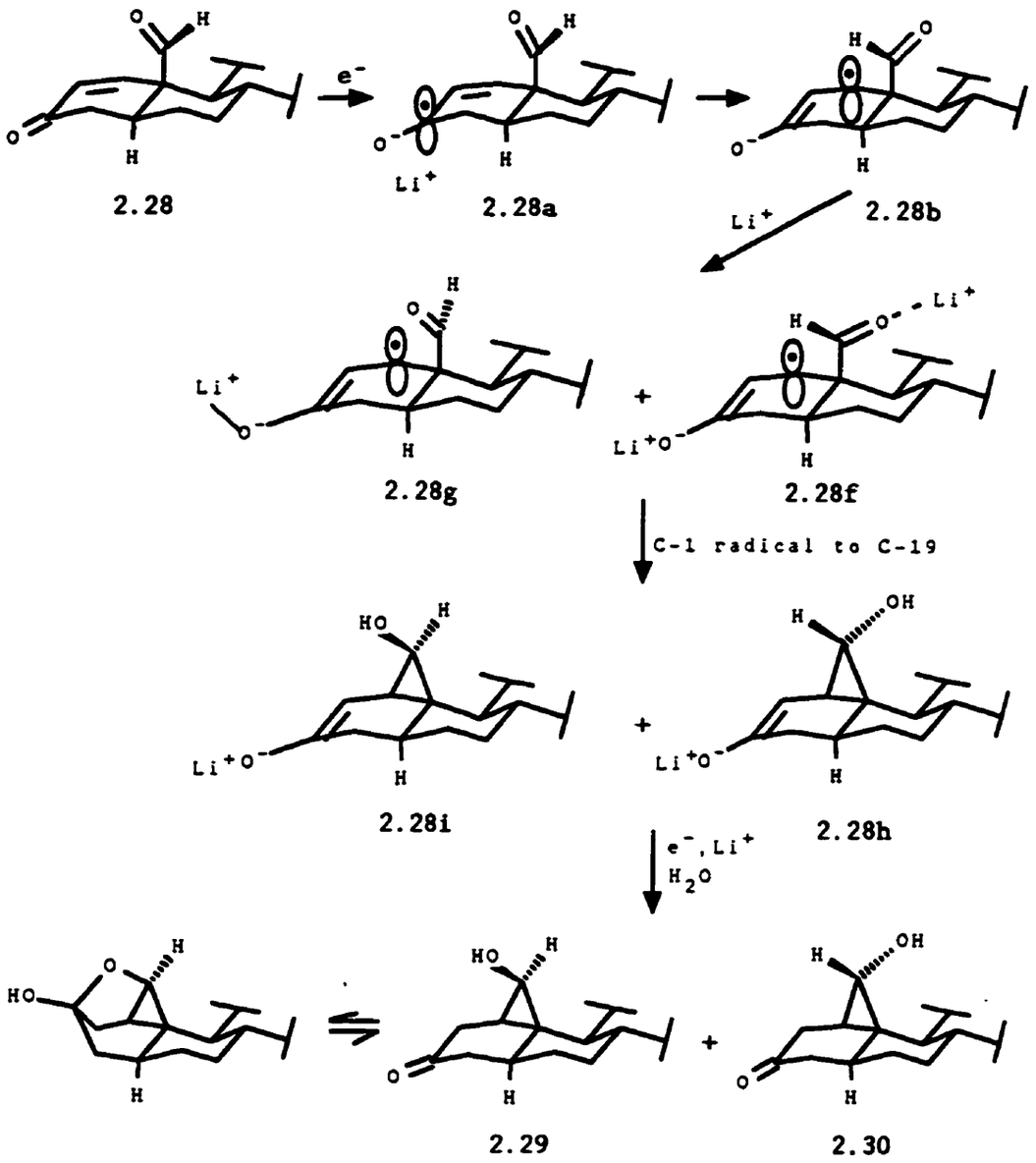
d = 2.9A

Figure 2.9 MMX calculated geometry of the enolate radical **2.28e**. (Distances determined by MMX and Dreiding models (in parentheses)).

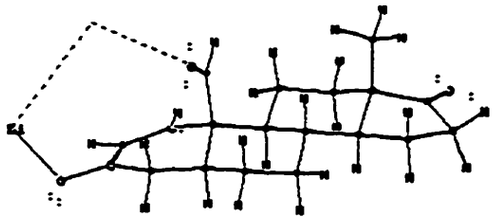
which ring A is more flexible than in the unreduced forms (radicals). Transfer of a second electron during the reduction process presumably, comes from the already oxidized zinc (Zn^+), because it has only one oxidation state, although transfer of a second electron from a neutral zinc is also possible.

Dreiding models showed that substitution of C-1/sp^2 for C-1/sp^3 introduces more flexibility into ring A. Consequently, in the O-zinc enolate anion **2.28c** or enol anion **2.28e** structures, as C-1 changes from sp^2 to sp^3 hybridization, the conformational adjustment occurs in ring A. Formation of the 19(R)-alcohol is evidently favoured by proximity of the 19-carbonyl group to either the zinc cation or a hydrogen. According to Dreiding models separation of zinc or hydrogen and the 19-carbonyl oxygen is in the range, 2.4-2.6 A, as shown in Figure 2.9. Nucleophilic attack of the C-1 anion is at the *re* face of the 19-CHO group to yield **2.28d** (Scheme 2.27).

The proposed mechanism of reductive cyclization of **2.28** with Li-NH_3 is outlined in Scheme 2.28. The conjugated system reacts with an electron to form a radical anion at C-1, which is transformed into the O-lithium enolate radical **2.28f**. The C-1 secondary radical has a planar structure (sp^2), which also imposes a planar conformation of ring A. According to Dreiding models separation of the lithium cation and the 19-carbonyl oxygen greatly exeeds the expected coordination distances (Figure 2.10). One of the intermediates, a radical anion **2.28b**, is capable of adding to a π system of the 19-HC=O moity, as the *re* or *si* face the 19-aldehyde group is oriented under the appropiate angle to the incoming nucleophile, i.e. at a dihedral angle of 109°. Tsang *et al.* (Tsang *et al.*, 1986a, b) also reported reaction of a radical with the aldehyde group to form cycloalcohols. There are two

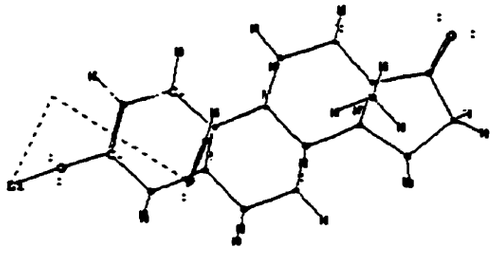


Scheme 2.28 Proposed mechanism of reductive cyclization of androst-1-ene-3,17-dione **2.28** with Li-NH_3.



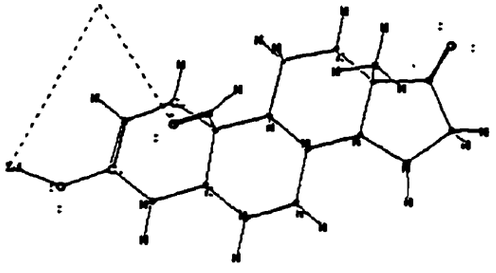
d = 4.9 A

O(19)-C(19)-C(10)-C(1) +109°



d = 4.9 A (4.7 A)

O(19)-C(19)-C(10)-C(1) +109°



d = 4.6 A (4.3 A)

O(19)-C(19)-C(10)-C(1) +68.8°

Figure 2.10 MMX calculated geometry of the O-lithium enolate radical. (Distances determined by MMX and Dreiding models (in parentheses).

available orientations of the 19-CHO group, under which the nucleophile may approach the π system **2.28f** and **2.28g**, as shown in Scheme 2.9. The radical exerts, presumably, a smaller electrostatic effect on the orientation of the 19-CHO group than a negatively charged species. Thus, to explain formation of the 19(S)-alcohol, it is possible to assume that the cyclization reaction upon Li-NH_3 treatment proceeds *via* a radical mechanism. The ratio of the products is in agreement with the observed ratios of conformers established by NOE. Presumably, lower electrostatic repulsive forces of the C-1 radical than the C-1 anion on the lone pairs of the 19-carbonyl oxygen orient the 19-aldeyde group with the *si* face toward the nucleophile. The driving force for the reaction is the relief of steric electronic repulsion between the C-1 radical and the free pair of electrons on oxygen of the 19-HC=O. The reaction occurs, if the rate of addition of the C-1 radical to the 19-CHO is faster than its reduction caused by a capture of a second electron. The product formed would be a reactive 19-oxygen radical **2.28i** and **2.28h** which after a further reduction would give dilithium derivatives.

Similarly to the O-zinc enolate radical, the O-lithium enolate radical is capable of accepting an electron to form the O-lithium enolate anion, which also would give rise to the tetrahedral intermediate. The steric situation in the case of the intermediate would be the same as in the O-zinc enolate anion **2.28c**, consequently leading to the same products. The sharp contrast in the product formation clearly indicates that steric effects (distances) can be important in the transition state, or a different mechanism may operate, for example reaction *via* the enol anion **2.28e** (Scheme 2.27). Upon Li-NH_3 reduction a path *via* the enol radical or enol anion is

excluded, because the reaction was performed without a proton source.

In view of the sensitivity of the product ratio to the preferred conformations of the intermediates (Scheme 2.28; **2.28g** and **2.28f** and Figure 2.10), it invokes the effect of varying the size of the reducing metal to be evaluated.

On the other hand, the reaction may proceed *via* the O-zinc or O-lithium enolate radical (or anion) but instead of considering a developing character of the C-1 carbon (radical or carbanion) as a controlling factor, we should consider a difference in metals size and its coordination properties to the 19-carbonyl group. The smallest atom, lithium (Li^+, 0.60 A), presumably, is not able to establish the preferred conformation during the 19(R)-alcohol formation. As reported, its ability to coordinate to a free electron pair of the carbonyl oxygen (linear coordination) is preferred than to the carbonyl π system. The bigger atom, zinc (Zn^{2+}, 0.74 A), with a smaller tendency than Li^+ to form a tight ion pair with the $3\text{-}O^-$ exerts greater electrostatic interactions to attract the 19-carbonyl group, consequently establishing the desired conformation **2.28c** (Scheme 2.27). As reported, zinc coordinates preferentially to a π system of the carbonyl function. In both cases, whether the reaction proceeds *via* the proposed radical or anion intermediate, the ratio of products depends on populations of the 19-aldehyde rotational conformers.

The addition of an electron to the 19-CHO was considered to be improbable. Because of the lack of symmetry there is a little chance of its stabilization. On the other hand, such an addition would lead to the reduction of the 19-aldehyde function to the 19-alcohol. However, by-products of the 19-alcohol derivatives were not detected.

Summarizing, in the androst-4-ene-3,17-dione **2.2** reductive

cyclization, both with zinc/50% aqueous acetic acid and Li-NH_3, the process is equivalent to the formation of a tertiary anion centre β to the conformationally mobile 19-aldehyde. Steric (19-HC=O/3-O^-M^+ distances), stereoelectronic (19-CHO/C-5 anion repulsion), and the homoconjugation effect (19-HC=O/C-5 anion stabilization) (Werstiuk, 1983) are consistent with the less stable product formation, 19(R)-hydroxy-5β,19-cycloandrostane-3,17-dione **2.4**.

The reductive cyclization of 5β-androst-1-ene-3,17-dion-19-al **2.28**, with zinc in aqueous acetic acid, served to substantiate the concept of a tetragonal structure of the C-1 carbanion. The reaction proceeds *via* the more thermodynamically stable carbanion intermediate (least substituted carbon; March *et al.*, 1992) to yield the more thermodynamically stable product **2.4**. When the C-1 carbon had retained sp^2 hybridization, the predominant products were formed by way of the C-1 radical intermediate. The reaction is kinetically controlled and gives the less thermodynamically stable product **2.30**.

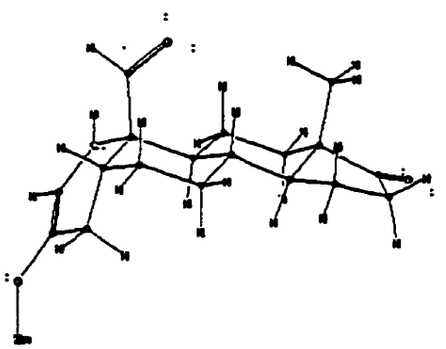
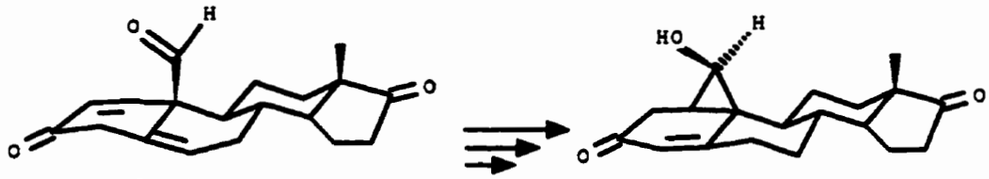


Figure 2.11 The proposed transition structure for the reductive cyclization of 5β-androst-1-ene-3,17-dion-19-al with zinc-aqueous acetic acid and Li-NH_3.

Based on the proposed mechanism, it is possible to predict a course of the reductive cyclization of 5β-androst-1-ene-3,17-dion-19-al (Figure 2.11). Reduction of 5β-androst-1-ene-3,17-dion-19-al both with either zinc in acetic acid or Li-NH_3 is expected to give the less thermodynamically stable isomer, 1β,19(S)-hydroxy-5β-androstane-3,17-dione as the major product. Moreover, it is also possible to design a synthesis of the more stable isomer, the 19(R)-alcohol **2.4**, by selecting an appropriate unsaturated percursor **2.55** and conducting reduction with zinc in 50% aqueous acetic acid (Scheme 2.29).



2.55

Scheme 2.29 The proposed unsaturated precursor of 1β,19(R)-hydroxyandrost-4-ene-3,17-dione: reduction with zinc in 50% aqueous acetic acid, protection of the 19(R)-OH grpup followed by a shift of the 5,6-double bond.

The reaction paths presented here satisfactorily account for the products obtained. However, to understand the proposed mechanism of the reductive cyclizations better and to rationalize the effect of experimental variables on the results observed much additional information is necessary.

2.2.1 Summary

1. 19-*tert*-Butyldimethylsiloxyandrost-1-en-3,17-dione **2.25** has been synthesized by treatment of *tert*-butyldimethylsiloxy-5α-androstane-3,17-dione **2.23** with a catalytic amount of diphenyl diselenide, camphorsulfonic acid, and 3-iodylbenzoic acid, in 70% yield, which is higher than other methods reported. 19-*tert*-Butyldimethylsiloxyandrost-1-ene-3,17-dione **2.25** provides a solid synthetic basis for the preparation of the required intermediate 19(R)-acetoxy-1β,19-cyclo-5α-androstane-3,17-dione **124** which was further used to prepare the 4,5-unsaturated 1β,19-cyclopropyl derivative **A** (Scheme 2.9).
2. Attempts to introduce a C-4 double bond either by direct oxidation of 19(R)-acetate-1β,19-cycloandrostane-3,17-dione **2.32** with $(PhSeO)_2O$ and DDQ or *via* indirect oxidations of the silyl enol ethers, **2.46** and **2.48**, with DDQ-Et_3N or NBS in CCl_4, CH_2Cl_2, or THF were unsuccessful.
3. Lithium-liquid ammonia (-78 °C) reduction of androst-1-ene-3,17-dion-19-al **2.28** gave the less stable isomers of the 19(S)-hydroxy-1β,19-cycloandrostane, **2.39** and **2.31** (70% yield), together with the more stable isomers of the the 19(R)-hydroxy-1β,19-cycloandrostane, **2.31**, **2.37**, **2.38**, and **2.40** (30% yield). However, zinc/50% aqueous acetic acid (25 °C) reductive cyclization of compound **2.28** yielded the more stable isomer, the 19(R)-alcohol **2.29** (95%).
4. A new synthetic method for formation of the acetate enols has been developed. Reaction of 19(R)-acetoxy-1β,19-cycloandrostan-3,17-dione **2.32** with Ac_2O and $BF_3.OEt_2$ in CH_2Cl_2 gave the 3-acetoxy-2-ene **2.52** and the boron difluoride 2-acyl derivative **2.53**. The

structure of compound **2.53** was determined by the ^{1}H, ^{13}C, and ^{19}F NMR spectra and supported by X-ray analysis. Choice of the reaction conditions shifts equilibrium toward either the acetate enol **2.52** or the acyl-BF_2 ketone **2.53**. The mechanism of the formation of the BF_2-steroid derivative **2.53** has been proposed (Scheme 2.18).

5. Oxidation of 3-triisopropylsiloxy,19(R)-acetoxy-1β,19-cycloandrost-2-en-17-one **2.48**, with ammonium ceric nitrate, furnished the 19,19-diester **2.54**, i.e. 19-nitrite ester,19-acetoxy (Scheme 2.19) instead of the expected 19-nitro,19-acetoxy derivative.

2.2.2 Experimental

19-*tert*-Butyldimethylsiloxyandrost-4-ene-3,17-dione 2.22

The mixture of 19-hydroxyandrost-4-ene-3,17-dione **2.21** (12.1 g, 40.0 mmol), imidazole (6.67 g, 98.0 mmol, 2.45 eq.) and *tert*-butyldimethylsilyl chloride (7.336 g, 48.7 mmol, 1.2 eq.) in DMF (75 mL) was stirred at 50 °C under an argon atmosphere for 4 hr, when TLC showed no starting material. Methanol (20 mL) was added to destroy an excess of the silyl reagent, and then the mixture was poured into diethyl ether. The organic layer was washed with cold water, brine, dried, filtered, and evaporated to give a crude product which on FCC, on elution with 20% EtOAc-LP, gave **2.22** (13.4 g, 32.2 mmol, 81%), mp 163-165 °C (from EtOAc-LP), (lit. 161-162 °C; from CH_2Cl_2-Et_2O; Lin, 1994).

^{1}H NMR of 2.22 ($CDCl_3$): δ 5.87 (s, 1H, 4-H), 3.90 (dd, J 10.5, 12.5, 1H, 19-H), 0.92 (s, 3H, 13-CH_3), 0.86 (m, $C(CH_3)_3$), 0.05, 0.05 (m, $SiMe_2$).

^{13}C NMR of 2.22 ($CDCl_3$): δ 33.29 (1), 34.69 (2), 199.65 (3), 126.02 (4), 167.25 (5), 33.58 (6), 30.79 (7), 35.93 (8), 54.07 (9), 43.60

(10), 20.96 (11), 31.73 (12), 47.59 (13), 51.34 (14), 21.71 (15), 35.71 (16), 220.10 (17), 13.89 (18), 65.81 (19).

19-tert-Butyldimethylsiloxy-5α-androstane-3,17-dione 2.23 and 19-tert-Butyldimethylsiloxy-5β-androstane-3,17-dione 2.24

A solution of 19-tert-butyldimethylsiloxyandrost-4-ene-3,17-dione **2.22** (13.41 g, 32.2 mmol) in EtOAc (120 mL) was stirred with 10% Pd/C (1.34 g) in a hydrogen atmosphere for 15 h when no starting material was indicated by TLC. The solution was filtered and evaporated at reduced pressure to give a crude product (13.3 g) which on FCC, on elution with 30-50% Et_2O-LP, gave fractions of the 5α-isomer **2.23** (9.88 g, 23.6 mmol, 73%), mp 136-138 °C (from Et_2O-LP), (from CH_2Cl_2-Et_2O) and the 5β-isomer **2.24** (3.36 g, 8.02 mmol, 25%), mp 153-154 °C (from Et_2O-LP), (lit., mp 151-153 °C, no slovent given, Lin, 1994)

^{1}H NMR of 2.23 ($CDCl_3$): δ 3.97, 3.91 (d, J_{AB} 10.8, 2-H, 19-H,H), 2.45 (m, 16β-H + 4β), 2.07 (m, 16α-H), 1.67 (m, 1H, 5α-H), 0.89 (s, CMe_3), 0.10, 0.08 (m, $SiMe_2$).

^{13}C NMR of 2.23 ($CDCl_3$): δ 33.96 (1), 38.64 (2), 211.91 (3), 44.86 (4), 46.23 (5), 28.32 (6), 30.66 (7), 35.51 (8), 54.33 (9), 39.54 (10), 21.72 (11), 31.93 (12), 47.79 (13), 51.66 (14), 21.78 (15), 35.79 (16), 220.72 (17), 13.92 (18), 60.87 (19).

^{1}H NMR of 2.24 ($CDCl_3$): δ 3.81, 3.60 (d, J_{AB} 9.7, 2H, 19-H,H), 2.63 (dd, J 14.6, 14.6, 1H, 4β-H), 2.47 (dd, J 19.0, 8.6 1H, 16β-H), 2.26 (m, 5β-H), 0.90 (s, 3H, 13-CH_3) 0.89 (s, CMe_3) 0.5, 05 (m, $SiMe_2$).

^{13}C NMR of 2.24 ($CDCl_3$): δ 31.16 (1), 36.91 (2), 212.84 (3), 42.06 (4), 36.38 (5), 24.49 (6), 25.90 (7), 35.21 (8), 41.59 (9), 39.22 (10), 20.59 (11), 32.04 (12), 47.77 (13), 51.92 (14), 21.71 (15), 35.83 (16), 220.47 (17), 13.91 (18), 65.19 (19).

19-*tert*-Butyldimethylsiloxyandrost-4-ene-3,17-dione 2.22, 19-*tert*-Butyldimethylsiloxy-5α-androst-1-ene-3,17-dione 2.25 and 19-*tert*-Butyldimethylsiloxyandrost-1,4-diene-3,17-dione 2.26

Preparation of 3-iodylbenzoic acid ($3\text{-IO}_2\text{C}_6\text{H}_4\text{COOH}$) by hypochlorite oxidation of 3-iodobenzoic acid: (Barton *et al.*, 1982)

Acetic acid (80 mL) was added dropwise to a vigorously stirred suspension of 3-iodobenzoic acid (30 g, 120.9 mmol) in 6% aqueous sodium hypochlorite, Stevens reagent (Stevens *et al.*, 1980), (400 mL, 6% NaClO, *Javex*) at room temperature. The temperature of the solution was kept below 40 °C during addition of acetic acid. An exothermic reaction took place with the formation of a white precipitate (10 min). The mixture was stirred overnight at room temperature, and then the white precipitate was filtered off, washed with water, acetone, ether, and then dried *in vacuo* at room temperature, over P_2O_5, with protection from light, to give white crystals of 3-iodylbenzoic acid (33.4g, 119 mmol, 99%), mp 249-253 °C (decomp.) [lit., mp 243-245 °C (decomp.), Willgerodt (1894)].

A mixture of diphenyl diselenide (0.817 g, 2.62 mmol, 0.1 eq.), camphorsulfonic acid (3.0 g, 12.9 mmol, 0.49), and above prepared 3-iodylbenzoic acid (7.5 g, 26.8 mmol, 1.02 eq.) was heated under reflux in dry tetrahydrofuran (80 mL), until the yellow colour of the diselenide disappeared (10 min). A solution of 19-*tert*-butyldimethylsiloxy-5α-androstane-3,17-dione **2.23** (11 g, 26 mmol) in THF (220 mL) was added and the mixture was refluxed for 2 h, when TLC showed no starting material. After cooling to room temperature, the mixture was poured into saturated sodium bicarbonate and extracted with diethyl ether and ethyl acetate. The organic layer was thoroughly washed with saturated aqueous $NaHCO_3$ and water. The aqueous washings were

extracted with CH_2Cl_2 and the combined organic layers washed with water, brine, dried, filtered, and evaporated to give a crude product (*ca* 11 g) which on FCC, on elution with 30-40% Et_2O-LP, gave fractions of the 1-ene **2.25** (7.64 g, 18.3 mmol, 70%) as a major product, mp 146-148 °C (from Et_2O-LP), (lit., 143-145 °C; from CH_2Cl_2-Et_2O; Lin 1994), the 4-ene **2.22** (1.35 g, 3.23 mmol, 12.4%), mp 157-159 °C (from Et_2O-LP), (lit., 155-158 °C; from CH_2Cl_2-Et_2O; Lin, 1994), and the 1,4-ene **2.26** (180 mg, 0.66 mmol, 2.5%), mp 166.5-167.5 °C (from Et_2O-LP), (lit., 160-163 °C; from CH_2Cl_2-Et_2O, Lin, 1994).

^{1}H NMR of 2.25 ($CDCl_3$): δ 6.82 (d, J 10.23, 1H, 1-H), 6.00 (d, J 10.11, 1H, 2-H), 3.97 (d, J_{AB} 10.62, 1H, 19-H), 3.73 (d, J_{AB} 10.61, 1H, 19-H), 2.69 (dd, J 17.87, 14.18, 1H, ?-H), 2.46 (dd, J 19.21, 8.75, 1H, 16β-H), 0.92 (s, 3H, 13-CH_3), 0.85 [s, 9H, $C(CH_3)_3$], 0.017 [d, J 3.9, 6H, $Si(CH_3)_2$].

^{13}C NMR of 2.25 ($CDCl_3$): δ 130.28 (1), 153.49 (2), 200.20 (3), 41.68 (4), 44.35 (5), 27.37 (6), 30.36 (7), 35.78 (8), 52.05 (9), 43.37 (10), 21.17 (11), 31.80 (12), 47.88 (13), 50.23 (14), 21.69 (15), 35.78 (16), 220.3 (17), 14.10 (18), 62.06 (19), 25.81 [$C(\underline{C}H_3)_3$], 18.11 ($\underline{C}Me_3$), -5.61 and -5.70 [$Si(\underline{C}H_3)_2$].

^{1}H NMR of 2.26 ($CDCl_3$): δ 7.07 (d, J 10.2, 1H, 1-H), 6.33 (dd, J 10.2, 1.9, 1H, 2-H), 6.15 (s, 1H, 4-H), 3.98, 3.87 (d, J_{AB} 9.6, 2H, 19-H,H), 0.95 (s, 3H, 18-CH_3), 0.80 (m, CMe_3), 0.00, -0.01 (d, J 0.01, 6H, $SiMe_2$).

^{13}C NMR of 2.26 ($CDCl_3$): δ 152.49 (1), 130.00 (2), 186.44 (3), 126.04 (4), 165.57 (5), 32.24 (6), 31.64 (7), 35.73 (8), 52.29 (9), 49.60 (10), 22.61 (11), 32.81 (12), 47.72 (13), 50.98 (14), 21.89 (15), 35.62 (16), 219.67 (17), 13.97 (18), 64.36 (19), 25.66 [$C(\underline{C}H_3)_3$], 18.05 ($\underline{C}Me_3$), -5.52 and -5.61 [$Si(\underline{C}H_3)_2$].

19-Hydroxy-5α-androst-1-ene-3,17-dione 2.27

Desilylation with nBu_4NF: To a solution of the silyl ether **2.25** (6.53 g, 15.6 mmol) in freshly distilled (over $LiAlH_4$) THF (80 mL), while cooled in an ice-water bath, was added a solution of 1M n-Bu_4NF in THF (20 mL, 20 mmol, 1.3 eq.), under an argon atmosphere. The mixture was stirred for 4 hr, and then poured into cold water, extracted with EtOAc, and the organic layer washed with saturated aqueous $NaHCO_3$, cold water, brine, dried, filtered, and evaporated to yield a crude product, which on FCC, on elution with 10-20% acetone-LP, gave the unsaturated 19-alcohol **2.27** (3.48 g, 11.5 mmol, 74%), mp 213-216.2 °C (from acetone-LP), [lit., 200-202 °C; from CH_2Cl_2-Et_2O; Lin, 1994)].

Desilylation with a Lewis acid, $SnCl_2.2H_2O$: To a solution of the silyl ether **2.25** (1.9 g, 4.6 mmol) in acetonitrile (30 mL) was added $SnCl_2.H_2O$ (1.34 g, 5.93 mmol, 1.3 eq.). The mixture was stirred for 48 hr, when TLC indicated no starting material. The mixture was poured into water and extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$, water, dried, filtered, and evaporated to give a solid residue (1.30 g) which after crystallization gave the unsaturated 19-alcohol **2.27** (1.25 g, 4.13 mmol, 90.5%), mp 213-216 °C (from acetone-LP).

^{1}H NMR of **2.27** (dry $CDCl_3$): δ 7.05 (d, J = 10.26, 1H, 1-H), 6.12 (d, J 10.15, 1H, 2-H), 2.05 (dd, J 11.45, 7.08, 1H, 19-H), 3.83 (dd, J 11.46, 4.65, 1H, 19-H), 2.77 (dd, J 17.95, 14.28, 1H, 4β-H), 2.47 (dd, J 19.31, 8.8, 1H, 16β-H), 0.92 (s, 3H, 13-CH_3).

^{1}H NMR of **2.27** ($CDCl_3$): δ 7.04 (d, J 10.3, 1H, 1-H), 6.1 (d, J 10.2, 1H, 2-H), 4.1 (d, J = 11.46, 1H, 19-H), 3.81 (d, J 11.47, 1H, 19-H), 2.76 (dd, J 17.95, 14.28, 1H, 4β-H), 2.46 (dd, J 19.31, 8.8, 1H, 16β-H), 0.91 (s, 3H, 13-CH_3).

^{13}C NMR of 2.27 ($CDCl_3$): δ 130.28 (1), 153.49 (2), 200.20 (3), 41.68 (4), 44.35 (5), 27.37 (6), 30.36 (7), 35.78 (8), 52.05 (9), 43.37 (10), 21.17 (11), 31.80 (12), 47.88 (13), 50.23 (14), 21.69 (15), 35.78 (16), 220.30 (17), 14.10 (18), 62.06 (19), 25.81 $C(\underline{C}H_3)_3$, 18.11 $\underline{C}(CH_3)_3$, -5.61 and -5.70 $Si(\underline{C}H_3)_2$.

5α-Androst-1-ene-3,17-dione 19-al 2.28

The mixture of 19-hydroxy-5α-androst-1-ene-3,17-dione **2.27** (4.52 g, 14.9 mmol), pyridinium chlorochromate (5.74 g, 26.63 mmol, 1.8 eq.) in CH_2Cl_2 (70 mL), in the presence of powdered molecular sieves 4A (0.5 g, 10% of **2.28**), was stirred for 2 h, when TLC indicated no starting material. Et_2O (70 mL) was added and the mixture was filtered through a pad of silica:Celite:Na_2SO_4 (1:4:2). The filtrate was washed with saturated aqueous $NaHCO_3$, water, filtered, and evaporated to give a crude product (4.2 g) which on FCC, on elution with 20% acetone-LP, gave the 1-en-19-aldehyde **2.28** (3.6 g, 12.0 mmol, 81%), mp 152-155 °C (from CH_2Cl_2-Et_2O-LP), (lit., 148-150 °C; CH_2Cl_2-Et_2O; Lin, 1994).

^{1}H NMR of 2.28 ($CDCl_3$): δ 9.92 (s, 1H, 19-CHO), 6.98 (d, J 10.21, 1H, 1-H), 6.24 (d, J 10.18, 1H, 2-H), 0.96 (s, 3H, 13-CH_3).

^{13}C NMR of 2.28 ($CDCl_3$): δ 33.96 (1), 38. 64 (2), 211.91 (3), 44.86 (4), 46.23 (5), 28.32 (6), 30.66 (7), 35.51 (8), 54.33 (9), 39.54 (10), 21.72 (11), 31.93 91(12), 47.79 (13), 51.66 (14), 21.78 (15), 35.79 (16), 220.72 (17), 13.92 (18), 60.87(19).

19(R)-Hydroxy-1β,19-cyclo-5α-androstane-3,17-dione/3α-hydroxy-3β,19-oxido-1β,19-cyclo-5α-androstan-17-one 2.29/2.30

<u>From reductive cyclization with zinc in 50% aqueous acetic acid 2.28</u>

The heterogenous mixture of the usaturated 19-aldehyde **2.28** (3.17 g, 10.54 mmol) and Zn powder (25 g) in 50% aqueous acetic acid (80 mL) was stirred at room temperature for 1 h, when TLC indicated no starting

material. The mixture was filtered through sintered glass to remove zinc. Water was added to the filtrate and products extracted with CH_2Cl_2. The organic layer was washed with water, saturated aqueous $NaHCO_3$, water, dried, filtered, and preconcentrated to *ca* 15 mL, and then diethyl ether added: precipitated solids consisted of a mixture of the 19(R)-alcohol/hemiacetal **2.29/2.30** as determined by the ^1H NMR spectrum. Crystallization of the products gave the solid product **2.29/2.30** (2.18 g, 7.2 mmol, 68%), mp 193-196 °C (from CH_2Cl_2-Et_2O-LP), (lit., 186-189 °C; from CH_2Cl_2-Et_2O; Lin, 1994). The mother liquid was dissolved in CH_2Cl_2 and treated with 1-(TBDMSi)imidazole for 3 weeks at room temperature (see compounds **2.33**, **2.34**, and **2.35**).

^1H NMR of 2.29/2.30 (acetone-d_6 or $CDCl_3$): δ 4.02 (d, J = 5.32, 1H, 19-H of **2.39**), 3.68 (d, J = 6.93, 1H, 19-H of **2.29**).

From 19(R)-*tert*-butyldimethylsiloxy-1β,19-cyclo-5α-androstane-3,17-dione **2.33**:

A solution of 19(R)-*tert*-butyldimethylsiloxy-1β,19-cyclo-5α-androstane-3,17-dione **2.33** (197 mg, 0.47 mmol) in THF (1 mL) and 1M *n*Bu_4NF-THF (2 mL, 2 mmol, 4.3 eq.). was stirred at room temperature for 1 h and then diluted with water, extracted with EtOAc (30 mL). The organic layer was washed with water, saturated $NaHCO_3$, water, brine, dried, filtered, and evaporated to give a mixture of the 19(R)-alcohol/hemiacetal **2.29/2.30** (136 mg, 0.45 mmol, 95 %), mp and ^{1}NMR as above.

From 3α-*tert*-butyldimethylsiloxy-3β,19-oxido-1β,19-cyclo-5α-androstane-3,17-dione **2.34**:

A solution of 3α-*tert*-butyldimethylsiloxy-3β,19-oxido-1β,19-cyclo derivative **2.34** (50 mg, 0.12 mmol) in THF (2 mL) and 1M nBu_4NF-THF (0.5 mL, 0.5 mmol, 4 eq.) was stirred at 20 °C for 1 h, poured into water,

extracted with EtOAc (25 mL), washed with saturated aqueous $NaHCO_3$, dried, filtered, and evaporated to give **2.29/2.30** (27 mg, 9 mmol, 75%), mp and ^1H NMR as above.

19(R)-Hydroxy-1β,19-cyclo-5α-androstane-3,17-dione 2.31

From 19(S)-*tert*-butyldimethylsiloxy-1β,19-cyclo-5α-androstane-3,17-dione **2.35**:

To a solution of the 19(S)-TBDMsiloxy-1β,19-cyclo derivative **2.35** (21 mg, 0.050 mmol) in THF (1 mL) was added 1M nBu_4NF in THF (200 μL, 0.2 mmol, 4 eq.). The mixture was stirred at room temperature for 1 h and then diluted with water and extracted with EtOAc (30 mL). The organic layer was washed with water, saturated $NaHCO_3$, water, brine, dried, filtered, and evaporated to give a solid residue (15 mg) which after two crystallizations yielded a pure analytical sample of **2.31** (10 mg, 0.033 mmol, 66%), mp 194-198 °C decomp. (from CH_2Cl_2-EtOAc), (Found: C, 73.40 ; H, 8.84. $C_{19}H_{26}O_3$. 1/2 H_2O (MW 311.423) requires C, 73.28; H, 8.74%).

^1H NMR of **2.31** ($CDCl_3$): δ 3.58 (d, J 2.54 Hz, 1H, 19-H), 2.47 (dd, J 9.23, 19.28, 1H, 16β-H), 0.88 (s, 3H, 18-CH_3).

^{13}C NMR of **2.31** ($CDCl_3$):. δ 20.38 (1), 35.98 (2) 199.42 (3), 43.74 (4), 38.77 (5), 33.02 (6), 31.35 (7), 39.77 (8), 46.55 (9), 30.66 (10), 21.82 (11), 31.68 (12), 47.89 (13), 51.63 (14), 23.04 (15), 37.00 (16), 221.23 (17), 13.76 (18), 55.87 (19)

19(R)-Acetoxy-1β,19-cyclo-5α-androstne-3,17-dione 2.32

From 19(R)-alcohol/hemiacetal **2.29/2.30**:

To a solution of the mixture of the 19(R)-alcohol/hemiacetal **2.29/2.30** (1.91 g, 6.37 mmol) in CH_2Cl_2(30 mL) was added Ac_2O (6.2 mL, 65.6 mmoL, 10.3 eq.) and DMAP (80 mg, 0.65 mmol, 0.1 eq.). The reaction mixture was stirred at 20 °C for 2 h, and then methanol (10 mL) added to

destroy the excess of acetic anhydride. The organic layer was washed with water, saturated aqueous $NaHCO_3$, water, dried, filtered, and evaporated to give a crude product which on FCC, on elution with 50% Et_2O-LP, gave the 19(R)-acetate-1β,19-cyclo **2.32** (1.42 g, 4.12 mmol, 66%), mp 163-166 °C (from Et_2O-LP), (lit. 161-163 °C; from CH_2Cl_2-Et_2O; Lin, 1994).

From 3,19(R)-diacetoxy-1β,19-cyclo-5α-androst-2-en-17-one **2.52**:

The mixture of the 3-acetoxy 2-ene **2.52** (30 mg, 0.08 mmol) in CH_2Cl_2 (1.5 mL) and $BF_3.OEt_2$ (300 μL) was stirred at room temperature for 3 h, until no starting material was indicated on TLC. Into the mixture was added water (1 mL) and the product extracted with CH_2Cl_2 (30 mL). The organic layer was washed with water, saturated aqueous $NaHCO_3$, water, dried, filtered, and evaporated to give a solid residue **2.23** (20 mg, 0.06 mmol, 75%), mp (as above). The residue was identified by its ^1H NMR spectrum.

^1H NMR of 2.23 ($CDCl_3$): δ 4.31 (d, J 7.54 Hz, 1H, 19-H), 2.48 (dd, J 19.09, 2.82 Hz), 2.03 (s, 3H, 19-$OCOCH_3$), 0.89 (s, 3H, 13-CH_3).

^{13}C NMR of 2.23 ($CDCl_3$): δ 17.42 (1), 34.74 (2), 210.09 (3), 43.97 (4), 38.03 (5), 32.69 (6), 30.69 (7), 39.11 (8), 46.23 (9), 26.94 (10), 21.59 (11), 31.04 (12), 47.55 (13), 50.96 (14), 21.65 (15), 35.81 (16), 220.28 (17), 13.69 (18), 56.94 (19), 20.79 ($CO\underline{C}H_3$), 171.23 ($\underline{C}OCH_3$).

19(R)-*tert*-Butyldimethylsiloxy-1β,19-cyclo-5α-androstane-3,17-dione 2.33, 3α-*tert*-Butyldimethylsiloxy-3β,19-epoxy-1β,19-cyclo-5α-androstane-3,17-dione 2.34, and 19(S)-*tert*-Butyldimethylsiloxy-1β,19-cyclo-5α-androstane-3,17-dione 2.35,

The residue from the mother liquid, consisting of the 19(R) hydroxy/-hemiacetal **2.29/2.30** and the 19(S)-hydroxy-1β,19-cyclo **2.31** (*ca* 600 mg, 2 mmol) was dissolved in CH_2Cl_2 (10 mL) and stirred with N-(*tert*-butyl-

dimethylsilyl)imidazole (1.0 g, 5.48 mmol, 3.1 eq.) at room temperature for 3 weeks when TLC indicated no starting material, and then methanol was added to destroy excess reagent. The mixture was poured into water, extracted with CH_2Cl_2, washed with water, dried, filtered, and evaporated at reduced pressure to give a crude product, which on FCC, on elution with 10-50% Et_2O-LP, gave the following fractions given in order of elution (*i*) the ketyl silyl ether **2.34** (300 mg, 0.72 mmol, 36%), mp 150-152 °C (from CH_2Cl_2-EtOAc), (Found: C, 72.16; H, 9.87. $C_{25}H_{40}O_3Si$ requires: C, 72.06; H,9.68); (*ii*) the 19(S)-silyl ether **2.35** (65 mg, 0.156 mmol, 8%), mp 193-196 °C (from CH_2Cl_2-EtOAc), (Found: C, 71.99 ; H, 9.76. $C_{25}H_{40}O_3Si$ requires: C, 72.06; H, 9.68), and (*iii*) the 19(R)-silyl ether **2.33** (288 mg, 0.69 mmol, 34.5%), mp 154-156 °C (from CH_2Cl_2-EtOAc), (Found: C, 72.23 ; H, 9.79. $C_{25}H_{40}O_3Si$ requires: C, 72.06; H, 9.68%).

^{1}H NMR of 19(R)-OTBDMSi **2.33** ($CDCl_3$): δ 3.53 (d, J 7.09, 1H, 19-H), 0.89 [s, 9H, $C(CH_3)_3$], 0.86 (s, 3H, 13-CH_3), 0.13 and 0.9 [s, 6H, $Si(CH_3)_2$].

^{13}C NMR of 19(R)-OTBDMSi **2.33** ($CDCl_3$): δ 17.66 (1), 34.89 (2), 211.75 (3), 44.53 (4), 38.77 (5), 32.70 (6), 30.92 (7), 39.74 (8), 46.60 (9), 26.17 (10), 21.75 (11), 31.11 (12), 47.58 (13), 50.98 (14), 21.83 (15), 35.92 (16), 220.62 (17), 13.56 (18), 55.54 (19), 25.87 $C\underline{Me}_3$, 18.34 $\underline{C}Me_3$, -5.21 and -5.28 $Si\underline{Me}_2$.

^{1}H NMR of **2.34** ($CDCl_3$): δ 4.00 (d, J 5.29, 1H, 19-H), 2.44 (dd, 1H, J 19.18, 8.85, 16β-H), 0.85 (s, 9H, $C(CH_3)_3$), 0.83 (s, 3H, 13-CH_3), 0.11 and 0.10 [(s^{ts}, 6H, $Si(CH_3)_2$].

^{13}C NMR of **2.34** ($CDCl_3$): δ 19.28 (1), 36.29 (2), 104.11 (3), 42.88 (4), 35.70 (5), 35.80 (6), 30.37 (7), 39.19 (8), 42.87 (9), 25.20 (10), 21.50 (11), 31.33 (12), 47.66 (13), 50.73 (14), 21.68 (15), 36.29 (16),

220.86 (17), 13.66 (18), 60.47 (19), 25.81 $C\underline{Me}_3$, 17.86 $\underline{C}Me_3$, - 2.77 and - 2.83 $Si(CH_3)_2$.

^1H NMR of 19(S)-OTBDMSi **2.35** ($CDCl_3$): δ 3.38 (d, J 3.13, 1H, 19-H), 2.46 (dd, J 18.21, 8.20, 1H, 16β-H), 0.90 (s, 9H, Me_3CSi), 0.87 (s, 3H, 13-Me), 0.11, 0.92 (s^{ts}, $SiMe_2$).

^{13}C NMR of 19(S)-OTBDMSi **2.35** ($CDCl_3$): δ 20.17 (1), 35.95 (2), 209.99 (3), 43.80 (4), 38.34 (5), 32.99 (6), 31.34 (7), 39.13 (8), 46.71 (9), 29.51 (10), 21.81 (11), 31.76 (12), 47.86 (13), 51.67 (14), 22.82 (15), 37.31 (16), 221.23 (17), 13.76 (18), 56.62 (19), 18.32 ($\underline{C}Me_3Si$), 25.94 ($\underline{C}H_3CSi$), -4.77 and -5.16 [$Si(\underline{C}H_3)_2$].

3α-Methoxy-3β,19-epoxy-1β,19-cyclo-5α-androstane-3,17-dione 2.36

From 19(R)-*tert*-butyldimethylsiloxy-1β,19-cyclo-5α-androstane-3,17-dione **2.33**:

To a solution of the 19(R)-*tert*-$BuMe_2$siloxy **2.33** (50 mg, 0.12 mmol) in THF (2 mL) was added a diluted HCl-methanol solution (10 mL; 0.5 mL concentrated HCl in 30 mL of MeOH) and the mixture stirred at room temperature for 12 h. The mixture was diluted with CH_2Cl_2 (20 mL) and the organic layer washed with water, saturated $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give a crude product **2.36** (20 mg, 6.2 mmol, 53%), (for ^1H and ^{13}C characteristics of **2.36**, see below)

From 3α-*tert*-butyldimethylsiloxy-3β,19-oxido-1β,19-cyclo-5α-androstane-3,17-dione **2.34**:

To a solution of the 3α-$tBuMe_2SiO$,3β,19-epoxy **2.34** (150 mg, 0.36 mmol) in THF (3 mL) was added a diluted HCl-methanol solution (15 mL; 0.5 mL concentrated HCl in 30 mL of MeOH) and the mixture stirred at room temperature for 12 h. The mixture was diluted with CH_2Cl_2 (30 mL) and the organic layer washed with water, saturated $NaHCO_3$, water, dried

over Na_2SO_4, filtered, and evaporated under reduced pressure to give a solid residue (100 mg), which after crystallization, gave a pure analytical sample **2.36** (74 mg, 0.23 mmol, 64%), mp 207-210 °C (from CH_2Cl_2-MeOH), (Found: C, 76.0; H, 9.2. $C_{20}H_{28}O_3$ (MW 316.444) requires C, 75.9; H, 8.9%).

^{1}H NMR of 2.36 ($CDCl_3$): δ 4.02 (d, J 5.67 Hz, 1H, 19-H), 3.32 (s, 3H, 3α-CH_3O), 2.44 (dd, J 19.39, 9.02, 1H, 16β-H), 0.84 (s, 3H, 13-CH_3).

^{13}C NMR of 2.36 ($CDCl_3$): δ 19.07 (1), 30.28 (2), 105.84 (3), 39.41 (4), 35.35 (5), 35.77 (6), 30.02 (7), 39.16 (8), 44.70 (9), 25.47 (10), 21.49 (11), 31.28 (12), 47.60 (13), 50.68 (14), 21.63 (15), 35.82 (16), 220.70 (17), 13.66 (18), 60.39 (19).

3β,17β,19(S)-tris(*tert*-Butyldimethylsiloxy)-1β,19-cyclo-5α-androstane 2.41, 3α,17β-bis(*tert*-Butyldimethylsiloxy)-3β,19-epoxy-1β,19-cyclo-5α-androstane 2.42, 17β,19(S)-bis(*tert*-Butyldimethylsiloxy-1β,19-cyclo-5α-androstan-3-one 2.34, 17β,19(R)-bis(*tert*-Butyldimethylsiloxy-1β,19-cycloandrostane-3-one 2.44, and 19(S)-*tert*-Butyldimethylsiloxy-1β,19-cycloandrostane-3,17-dione 2.35.

A solution of the unsaturated 19-aldehyde **2.28** (440 mg, 1.47 mmol) in tetrahydrofuran (20 mL, freshly distilled over $LiAlH_4$) was added over a period of 20 min to a stirred mixture of liquid ammonia (100 mL) and THF (10 mL) containing lithium metal (520 mg, 75 mmol) under an argon atmosphere. Stirring was continued for a further 1.40 h at which time solid NH_4Cl (8 g, 150 mmol) was added. Addition of CH_2Cl_2 (150 mL) followed by evaporation of the ammonia, under a gentle stream of argon, left an organic layer which was washed with water, dried over Na_2SO_4, filtered, and evaporated to give a glassy residue (394 mg). The residue was treated with TBDMSiCl (990 mg, 6.57 mmol) and *i*-Pr_2EtN (1.5 mL, 8.6 mmol) in dry DMF (20 mL) for 2 h at room temperature. Et_2O (50

mL) was added and the organic layer washed with cold water, brine, dried, filtered, and evaporated to give a residue (800 mg) which on FCC, on elution with 0.5- 50% Et_2O-LP, yielded fractions given in the order: non-crystalline tri-t$BuMe_2Si$ **2.41** (38 mg, 0.0585 mmol, 5%), 3β,19-epoxy **2.42** (48 mg, 0.09 mmol, 9%), mp 166-170 °C (from Et_2O-MeOH), (Found: C, 70.0 ; H,10.6%. $C_{31}H_{56}O_3Si_2$ requires C, 69.9; H, 10.6%), 17β,19(S)-di-t$BuMe_2Si$ **2.43** (180 mg, 0.338 mmol, 32%), mp 125-127 °C (from Et_2O-MeOH), (Found: C, 69.7; H, 10.8. $C_{31}H_{56}O_3Si_2$ requires C, 69.9; H, 10.6%), 17β,19(R)-di-t$BuMe_2Si$ **2.44** (30 mg, 0,0563 mmol, 5.33%), mp 152-160 °C (from Et_2O-MeOH), (Found: C, 69.9; H,10.75. $C_{31}H_{56}O_3Si_2$ requires C, 9.9; H, 10.6%), and 19(S)-t$BuMe_2Si$ **2.35** (15 mg, 0.036 mmol, 2.5%), mp (as above).

^1H NMR of **2.35** ($CDCl_3$): δ 3.53 (t, J 8.2, 1H, 17α-H), 3.44 (m, 1H, 3α-H), 3.02 (d, J 2.9, 1H, 19-H), 2.22 (m, 2β-H), 0.67 (s, 3H, 13-Me).

^{13}C NMR of **2.35** ($CDCl_3$): δ 20.13 (1), 33.68 (2), 69.20 (3), 37.53 (4), 39.96 (5), 32.33 (6), 32.74 (7), 42.11 (8), 48.36 (9), 31.64 (10), 23.51 (11), 37.47 (12), 43.48 (13), 51.23 (14), 23.59 (15), 31.06 (16), 81.71 (17), 11.55 (18), 60.85 (19).

^1H NMR of **2.42** ($CDCl_3$): .δ 4.00 (d, J 5.5, 1H, 19-H), 3.52 (t, J 8.2, 17α-H), 0.66 (s, 3H, 13-Me).

^{13}C NMR of **2.42** ($CDCl_3$): δ δ 19.10 (1), 36.06 (2), 104.05 (3) 49.94 (4), 35.78 (5), 36.37 (6), 30.98 (7), 39.79 (8), 44.82 (9), 29.73 (10), 21.88 (11), 36.87 (12), 43.24 (13), 49.94 (14), 23.41 (15), 31.13 (16), 81.72 (17), 11.27 (18), 60.68 (19), CMe_3, CMe_3, -2.80, -2.85, -5.27, -5.32 $SiMe_2$.

^1H NMR of **2.43** ($CDCl_3$): δ 3.35 (d, J 3.1, 1H, 19-H), 3.55 (dd, J 7.9, 8.5, 1H, 17α-H), 0.69 (s, 3H, 17α-H).

^{13}C NMR of **2.43** ($CDCl_3$): δ δ 19.97 (1), 37.38 (2), 210.50 (3), 43.93

(4), 38.51 (5), 33.21 (6), 32.13 (7), 39.76 (8), 46.95 (9), 29.70 (10), 23.32 (11), 37.45 (12), 43.35 (13), 50.98 (14), 23.52 (15), 31.03 (16), 81.62 (17), 11.40 (18), 56.720 (19), CMe_3, CMe_3, -4.47, -4.77, -4.77, -5.15 $SiMe_2$.

^{1}H NMR of 2.44 ($CDCl_3$): δ 3.55 (m, 19-H overlaps with 17α-H), 3.55 (m, 17α-H overlaps with 19-H), 0.69 (s, 3H, 13-Me).

^{3}C NMR of 2.44 ($CDCl_3$): δ δ 17.42 (1), 34.94 (2), 212.20 (3) 44.64 (4), 38.87 (5), 32.90 (6), 31.66 (7), 40.40 (8), 46.65 (9), 26.22 (10), 22.16 (11), 36.54 (12), 43.19 (13), 50.14 (4), 23.47 (15), 30.97 (16), 81.61 (17), 11.23 (18), 56.65 (19), CMe_3, CMe_3, -4.44, -4.81, -5.16, -5.32 $SiMe_2$.

19(R)-Acetoxy-3-trimethylsilyl-1β,19-cyclo-5α-androst-2-en-17-one 2.46

To a solution of 19(R)-acetoxy-1β,19-cyclo-5α-androstane-3,17-dione **2.32** (140 mg, 0.406 mmol) and Et_3N (300 μL, 2.08 mmol, 5.1 eq.) in DMF (1 mL) was added TMSiOTf (240 μL, 1.24 mmol, 3.05 eq.) while cooled in an ice-water bath. The mixture was stirred for 3 hr and then poured into diethyl ether. The organic layer was washed with brine, dried, filtered, and evaporated to give a glassy residue which on FCC, on elution with 18% Et_2O-LP and Et_3N (1.5 mL/L of the eluent), gave compound **2.46** (50 mg, mmol, %), which failed to crystallize.

^{1}H NMR of 2.46 ($CDCl_3$): δ 4.88 (dd, J 6.7, 1.7 Hz, 1H, 2-H)), 4.07 (d, J 7.0 1H, 19(s)-H), 2.44 (dd, J 19.15, 2.44 Hz, 1H, 16β-H), 0.85 (s, 3H, 13-CH_3), 0.16 [s, 9H, $Si(CH_3)_3$].

^{13}C NMR of 2.46 ($CDCl_3$): δ 19.17 (1), 97.28 (2), 150.28 (3), 35.92 (4), 36.98 (5), 31.93 (6), 30.95 (7), 39.29 (8), 46.28 (9), 28.76 (10), 21.76 (11)[a], 31.03 (12), 47.55 (13), 51.00 (14), 21.68 (15)[a], 35.79 (16), 220.50 (17), 13.66 (18), 59.77 (19), 0.21 ($Si\underline{C}H_3$), 21.04 ($CO\underline{C}H_3$), 171.39 ($\underline{C}OCH_3$).

19(R)-Acetoxy-3-triisopropylsilyl-1β,19-cyclo-5α-androst-2-en-17-one 2.48

To a solution of 19(R)-acetoxy-1β,19-cyclo-5α-androstane-3,17-dione **2.32** (200 mg, 0.581 mmol) and Et_3N (250 μL, 1.7 mmol, 2.9 eq.) in diethyl ether (25 mL) was added *i*-Pr_3SiOTf (450 μL, 1.3 mmol, 2.2eq.) under an argon atmosphere. The mixture was refluxed for 2 hr and then cooled to room temperature. The organic layer was washed with water, brine, dried, filtered, and evaporated to give a glassy residue which on FCC, on elution with 20% Et_2O-LP, yielded compound **2.48** (280 mg, 0.56 mmol, 96.4%), which failed to crystallize.

^{1}H NMR of **2.48** ($CDCl_3$); δ 4.87 (dd, J 6.71, 1.69, 2-H), 4.12 (d, J 7.05, 1H, 9-H), 2.01 (s, 3H, 19-$OCOCH_3$), 2.46 (dd, J 19.13, 8.95 1H, 16β-H), 1.07, 1.05 (m, $[(CH_3)_2CH]_3Si$), 0.87 (s, 3H, 13-Me).

^{13}C NMR of **2.48** ($CDCl_3$): δ 19.19 (1), 95.77 (2), 150.74 (3), 35.85 (4), 37.21 (5), 31.92 (6), 31.00 (7), 39.26 (8), 46.32 (9), 28.69 (10), 21.75 (11), 31.05 (12), 47.57 (13), 51.03 (14), 21.69 (15), 35.85 (16), 220.55 (17), 13.67 (18), 59.88 (19), 171.52(19-$\underline{C}OCH_3$), 21.19 (19-$CO\underline{C}H_3$), 17.98 [3 x $(\underline{C}H_3)_2CHSi$], 12.61 [3 x $(CH_3)_2\underline{C}HSi$].

3,19(R)-Diacetoxy-1β,19-cyclo-5α-androst-2-en-17-one 2.52 and 2-Acyl,19(R)-acetoxy-1β,19-cyclo-5α-androst-2-en-3-boronoxy difluoride 2.53

To a solution of the 19(R)-acetoxy **2.32** (200 mg, 0.568 mmol) in CH_2Cl_2 (4 mL) was added Ac_2O (1.0 mL, 10.6 mmol, 18.7 eq.) and $BF_3.Et_2O$ (100 μL, 0.81 mmol, 1.43 eq). The reaction mixture was stirred at 20°C for 1 h when no starting material was indicated by TLC, and then methanol (2 mL) was added to destroy an excess of acetic anhydride. Stirring was continued for 1/2 h, and then the mixture was poured into water, extracted with CH_2Cl_2, washed with water, saturated $NaHCO_3$, water,

dried over Na_2SO_4, filtered, and evaporated to give a crude product which on FCC, on elution with 10% acetone-LP, gave fractions of **2.52** (149 mg, 0.385 mmol, 68%), mp 83-86 °C (from Et_2O-hexanes), (Found: C, 71.17; H, 7.95%. $C_{23}H_{30}O_5$ requires: C, 71.47; H, 7.82) and **2.53** (20 mg, 0.052 mmol, 9%), mp (see below).

^{1}H NMR of 2.41 ($CDCl_3$): δ 5.43 (dd, J 6.86, 2.56 Hz 1H, 2-H), 4.12 (d, J 7.03, 1H, 19(S)-H), 2.46 (dd, J 19.25, 9.05 Hz, 1H, 16β-H), 2.10 (s, 3H, 3-$OCOCH_3$), 2.09 (s, 3H, 19(R)-$OCOCH_3$), 0.86 (s, 3H, 13-CH_3).

^{13}C NMR of 2.41 ($CDCl_3$): δ 19.06 (1), 108.04 (2), 147.43 (3), 33.26 (4), 36.47 (5), 30.99 (6), (7), 30.45 (8), 46.16 (9), 29.93 (10), 21.71 (11), 31.88 (12), 47.52 (13), 50.94 (14), 21.71 (15), 35.79 (16), 220.35 (17), 13.67 (18), 59.74 (19), 169.29 (3-$\underline{C}OCH_3$), 20.89 (3-$CO\underline{C}H_3$ and 19-$CO\underline{C}H_3$), 171.80 (19-$\underline{C}OCH_3$).

2-Acyl,19(R)-acetoxy-1β,19-cyclo-5α-androst-2-en-17-one 2.53.

A mixture of the 19(R)-acetoxy **2.32** (150 mg, 0.44 mmol), Ac_2O (2 mL, 21.8 mmol, 50 eq.), and $BF_3.OEt_2$ (180 μL, 1.09 mmol, 2.5 eq.) in CH_2Cl_2 (2 mL) was stirred at room temperature for 55 h, when no starting material **2.32** and no intermediate **2.52** were indicated by TLC. After work-up, as described for **2.52**, a crude product on FCC, on elution with 20% acetone-LP, gave fractions of **2.53** (140 mg), which after crystallization yielded the 3-OBF_2 derivative **2.53** (125 mg, 0.32 mmol, 74%), mp 225-228 °C (from Et_2O-MeOH), (Found: C, 62.66; H, 6.90%. $C_{23}H_{29}O_5BF_2.1/3H_2O$, MW 440.287, requires: C, 62.74, H, 6.79%).

^{1}H NMR of 2.53 ($CDCl_3$): δ 2.42 (d, J 6.72 Hz, 1H, 19(S)-H), 2.43 (s, 3H, C_2-($C\underline{H}_3$)C(OH) O-), 1.98 (s, 3H, 3-$OCOC\underline{H}_3$).

^{19}F NMR of 2.53 ($CDCl_3$): δ -143.22 (d, J 1F, O-^{10}BF), δ -143.16 (d, J 0.26, 1F, O-^{10}BF); δ -143.84 (d, J 0.26, 1F, O-^{11}BF), δ -143.78 (d, J 0.26, 1F, O-^{11}BF).

^{13}C NMR of 2.53 ($CDCl_3$): δ 17.93 (1), 105.19 (2), 188.42 (3), 35.73 (4), 34.94 (5), 30.85 (6), 30.52 (7), 39.16 (8), 46.01 (9), 28.85 (10), 21.64 (11), 31.49 (12), 47.42 (13), 50.76 (14), 21.83 (15), 37.49 (16), 219.82 (17), 13.66 (18), 57.87 (19), 192.57 ($C_2(CH_3)\underline{C}(OH)$-O)), 170.79 (3-$\underline{C}OCH_3$), 20.61 (3-CO$\underline{C}H_3$) 22.53 ($\underline{C}H_3$)C(OH)-O).

19-Nitrite ester,19-acetoxy-1β,19-cyclo-5α-androst-2-ene-3,17-dione 2.54 and androstan-1-en-3,17-dion-19-al 2.28.

The 3-triisopropylsiloxy-19(R)-acetoxy-1β,19-cycloandrost-2-en-17-one **2.48** (67 mg, 0.13 mmol) was dissolved in dry DMF (2 mL) and solid ammonium ceric nitrate, $(NH_4)_2Cr(NO_3)_6$, (170 mg, 0.3 mmol, 2.3 eq.) was added in one portion. After 1/2 h of stirring, no starting material was detected by TLC. The mixture was diluted with diethyl ether (20 mL) and organic layer washed with cold water, saturated aqueous $NaHCO_3$, dried, filtered, and evaporated to give an oily residue (60 mg), which on FCC, on elution 0.5% acetone-CH_2Cl_2, gave a mixture consisting of two compounds, the 1,2-unsaturated 19-aldehyde **2.28** and 19(R/S)-acetoxy,19-nitrite ester-5α-androst-2-en-3,17-dione **2.54**, identified by the ^1H NMR spectrum.

^1H NMR of 2.54 extracted from a mixture of **2.28** and **2.54** ($CDCl_3$): δ 7.32 (s, 1H, 19-H), 6.91 (d, J 10.18, 1H, H-1), 6.07 (d, J 10.21, 1H, H-2), 2.03 (s, 3H, 19-$COOCH_3$), 0.88 (s, 3H, 13-Me).

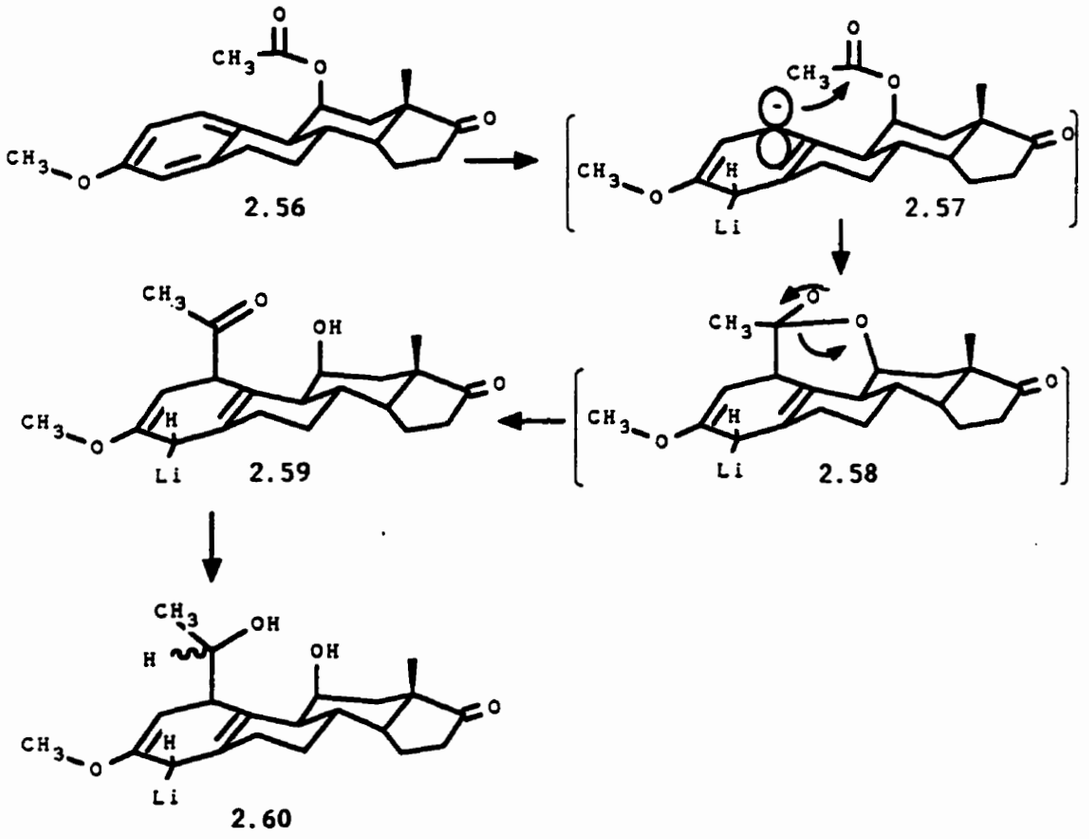
^1H NMR of 2.28 extracted from a mixture of **2.28** and **2.54** ($CDCl_3$): δ 9.91 (s, 1H, 19-CHO), 6.98 (d, J 10.21, 1H, H-1), 6.21 (d, J 10.12, 1H, H-2), 0.94 (s, 3H, 13-Me).

EIMS (70 eV) of **2.54**: $M^{+\cdot}$ 405 (0.5), m/z 344 (13.5), 326 (9), 301 (33), 204 (100), 271 (26), 255 (25), 242 (28).

2.3.0 Synthesis of bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate): Dimer Formation

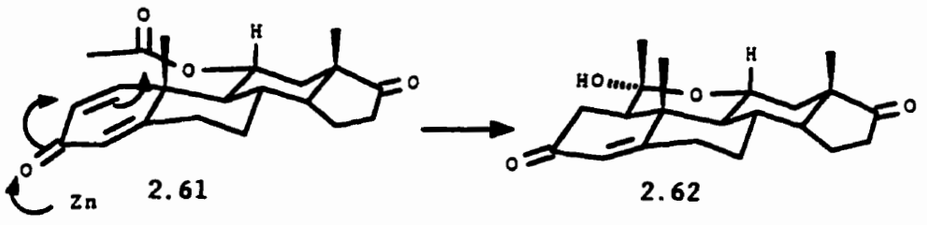
Introduction

The concept (Birch, 1950; Barton and Robinson, 1954) of the two electron addition to α,β-unsaturated ketones or aromatic compounds undergoing reduction by dissolving metals in liquid ammonia led to an understanding of the formation of some unexpected reduction products of



Scheme 2.30 Proposed mechanism of the O→C acyl transfer upon reaction of 11β-acetoxy-1,3,5(10)-estratriene **2.56** with Li-NH_3 (Magerlein *et al.*, 1958).

steroidal derivatives. Reductive cyclization of steroid derivatives having both unsaturation in ring A and an acetoxy group at C-11 were reported. Scheme 2.30 presents the conversion of 11-acetoxy-estra-1,3,5(10)-triene-3,11β-diol-17-one **2.56** to compound **2.60**, in 10% yield, by lithium-ammonia-alcohol reduction (Magerlein and Hogg, 1958). The reaction proceeds *via* a postulated cyclic hemiacetal intermediate **2.57**. The proposed mechanism involves the formation of the dianion **2.57**, formed by the addition of electrons on the aromatic ring. Due to the favourable steric relationship of the carbanion at C-1 and the carbonyl group of the 11β-acetate, nucleophilic addition to the acetate carbonyl occurred giving rise to intermediate **2.58**. The conversion of intermediate **2.58** to a final compound **2.60** involved a Claisen type transfer of the acyl group from C-11 to C-1, and addition of a proton, followed by reduction of the acyl group now located at C-1. On the other hand, reduction with Li-NH_3 of the 1,4-dien-3-one derivative **2.61** containing an 11α-acetoxy substitent, led to intramolecular acylation of the incipient β-carbanion generated at C-1 giving rise to a stable cyclic hemiacetal **2.62** shown in Scheme 2.31 (Tanabe *et al.*, 1961).



2.61 2.62

Scheme 2.31 Proposed mechanism of reductive cyclization of 11α-acetoxyandrost-1,4-diene-3,17-dione **2.61** with Li-NH_3 (Tanabe *et al.*, 1961).

Reduction of other unsaturated steroid derivatives with Li-NH_3, for example cholest-4-en-3-one, led to reduction of the carbon-carbon double bond (Barton *et al.*, 1954). On extended exposure of cholest-4-en-3-one to excess zinc dust in glacial acetic acid at room temperature, 5α-cholest-3-ene was isolated in about 40% yield; mixtures of 5α- and 5β-3-enones were obtained when other 4-en-3-ones were treated similarly (McKenna *et al.*, 1959). In contrast, cholest-4-en-3-one under Clemmensen conditions, i.e. on reflux in toluene with amalgamated zinc and 7 M hydrochloric acid, gave 5β-cholest-3-ene in 48% yield (Davis and Woodgate, 1966). On the other hand, treatment of cholest-4-en-3-one with sodium amalgam in propanol-acetic acid has been used to prepare cholestane pinacol (Squire, 1951). Unsaturated pinacols have been also obtained in the Li-NH_3 reduction of cholest-4-en-3-one (Caine and references cited there, 1976); Jellinek, 1955). Templeton *et al.* (Templeton *et al.*, 1990) also reported formation of a steroid dimer derivative. Treatment of 4-chlorotestosterone acetate with zinc in glacial acetic acid furnished not only a 3-hydroxy-4-chlorotestosterone acetate dimer but also a mixture of two compounds, 17β-acetoxy-4-chloro-5α- and 5β-androst-3-ene.

The nature of the 3-ene product formation is not completely understood. The reaction resembles the Clemenson reduction of ketones and aldehydes to methylene and methyl groups using zinc and hydrochloric acid for which two mechanisms have been proposed. One proposal involved an acid-catalyzed reduction of the carbon-oxygen bond and formation of bis(chloroalkylzinc)alkyl intermediates, followed by their hydrolysis (Nakabayashi, 1960). The second proposal involved organozinc carbenoid intermediates (Motherwell, 1973, 1992;

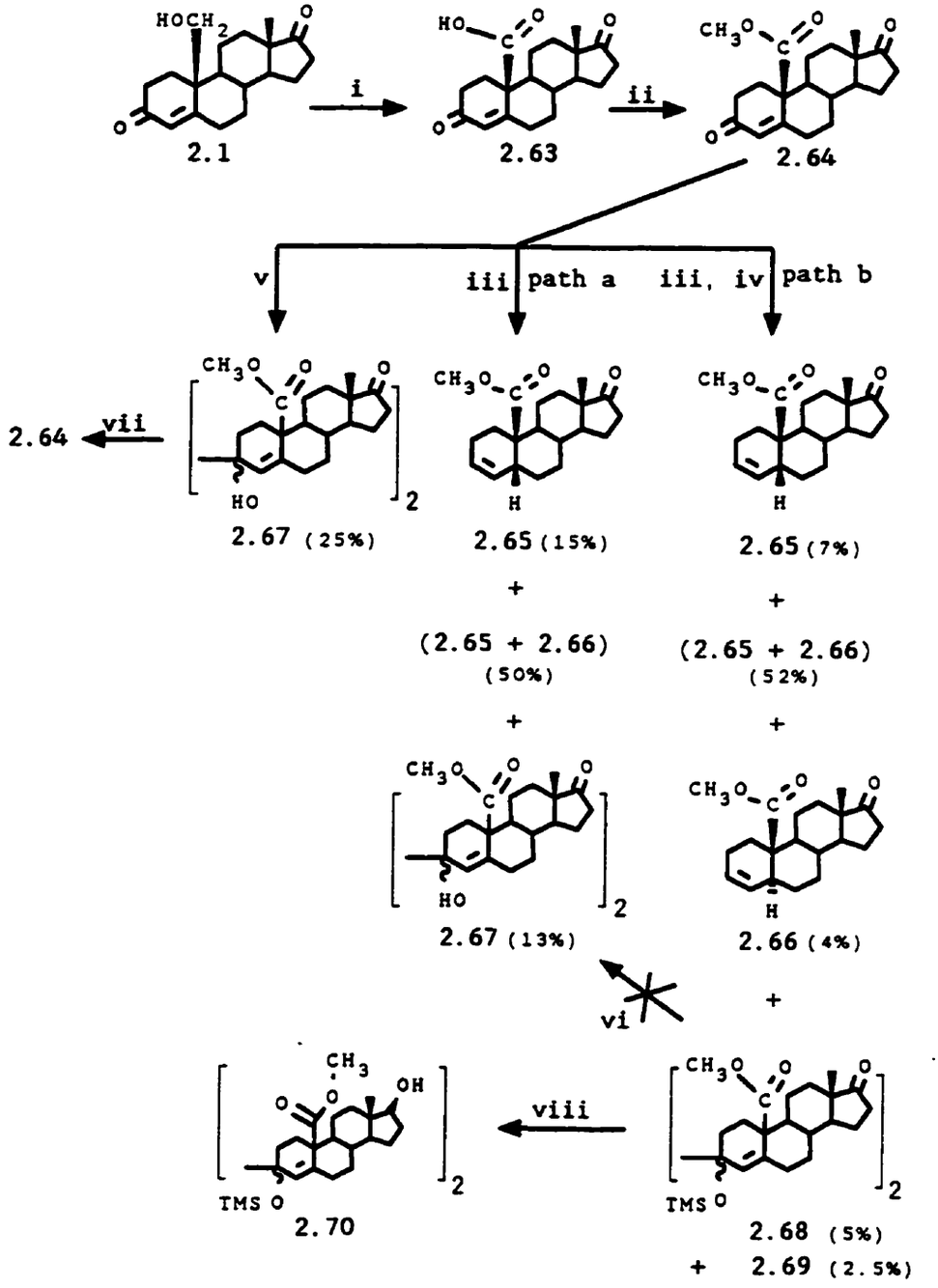
Nakabayashi, 1960).

The purpose of this work was to synthesize 19,19-disubstituted 5β,19-cyclopropane derivatives and subject them to biological evaluation for aromatase inhibition. In this chapter, the results of reduction of the methyl androst-4-en-3,17-dion-19-oate, both with zinc in 50% acetic acid and lithium in liquid ammonia, are described.

Results and Discussion

As shown in Scheme 2.32, 19-hydroxyandrost-4-ene-3,17-dione **2.1** was oxidized with Jones reagent to give the 19-acid **2.63** (Ueberwasser *et al.*, 1963), which, after treatment with diazomethane, produced the 19-methyl ester **2.64** (Dyer and Harrow, 1979a). Treatment of the 19-methyl ester **2.64** with zinc in 50% aqueous acetic acid at 20°C (path a) gave a mixture of products which, after separation by column chromatography, yielded the following fractions: (i) methyl 5β-androst-3-en-17-on-19-oate **2.65** (15%); (ii) a mixture of compounds, **2.65** and methyl 5α-androst-3-en-17-on-19-oate **2.66** (50%); and (iii) the dimeric steroid bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) **2.67** (13%).

However, as previously described in Chapter 2.10, treatment of androst-4-ene-3,17-dione-19-al with zinc in aqueous acetic acid or glacial acetic acid gave the 19(R)-hydroxy-5β,19-cyclo-androstane-3,17-dione **2.4**. The expected C-3 unsaturated isomers were not observed in the analogous reduction reaction with the 19-aldehyde. This observation indicates a faster rate of reaction for C-5,19-cyclopropane cyclization then for carbonyl reduction or dimer formation. The chromatographic separation of compound **2.67** was very tedious. Therefore, in a separate experiment, the crude product from the zinc reduction, was directly treated with the 1-(trimethylsilyl)



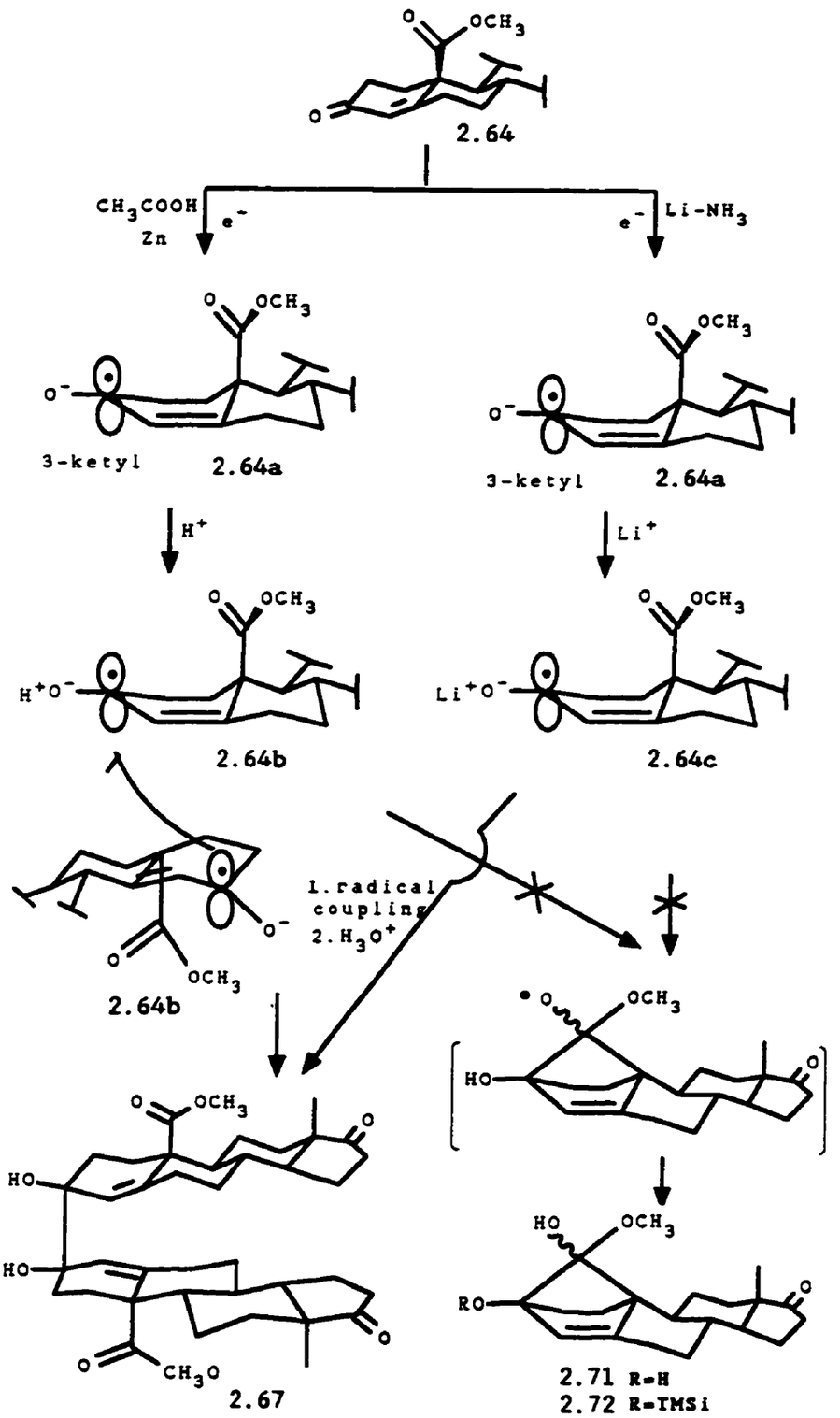
Reagents: i, Jones reagent; ii, CH_2N_2, Et_2O; iii, Zn, 50% H_2O-CH_3COOH, iv, 1-(TMSi)imidazole; v, Li-NH_3; vi, nBu_4NF or CsF; vii, $NaIO_4$, MeOH; viii, $LiAlH_4$, Et_2O-THF, RT.

Scheme 2.32 Synthesis of the dimeric steroids, 2.67, 2.68, 2.69, and 2.70 and related compounds, 2.65 and 2.66.

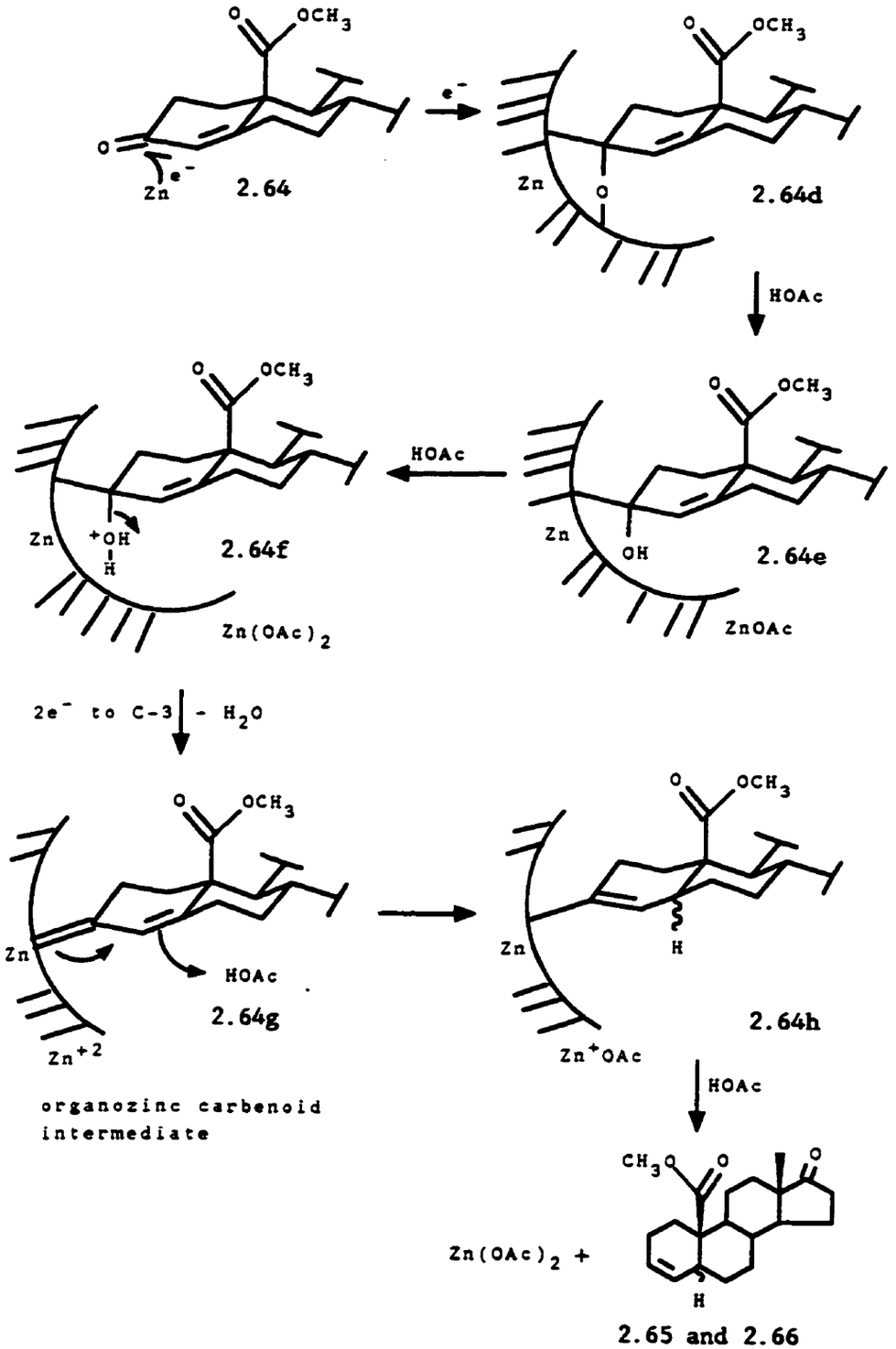
imidazole reagent (Scheme 2.32; path b). However, the trimethylsiloxyderivatives proved again to be difficult to separate. Possibly, better separation could be achieved by employing higher siloxy derivatives. On chromatographic separation of the trimethylsiloxy derivatives the following fractions were identified: (*i*) methyl 5β-androst-3-en-17-on-19-oate **2.65** (7%); (*ii*) a mixture of compounds, **2.65** and **2.66** (52%); (*iii*) methyl 5α-androst-3-en-17-on-19-oate **2.66** (4%); (*iv*) the symmetrical dimer, bis-(methyl 3ξ-siloxy-androst-4-en-17-on-19-oate) **2.68** (5%); and (*v*) the unsymmetrical dimer, bis-(methyl 3ξ-siloxyandrost-4-en-17-on-19-oate) **2.69** (2.5%).

It was observed that a removal of the 3ξ-TMSi group by treatment of **2.68** with nBu_4NF or CsF in THF to obtain **2.67** was unsuccessful. Attempts to hydrogenate the 4,5-double bond of **2.68** with 5% Pd/C in ethyl acetate under normal pressure at room temperature also failed. Compound **2.67** was stable to 0.5M KOH-MeOH and acetic acid conditions at ambient temperature. However, it was unstable, on reflux, in the 0.5M KOH methanolic solution. Treatment of the 3ξ-diol dimer **2.67** with sodium metaperiodate in aqueous methanol gave, as anticipated, the 19-methyl ester **2.64**, which was confirmed by its ^1H and ^{13}C NMR spectrum. Reduction of **2.68** with $LiAlH_4$ at room temperature gave the 17-hydroxy dimer **2.70**. The 19-ester function was not affected, as determined by the presence of the 19-methoxy group in the ^1H NMR spectrum.

Reduction of the 19-methyl ester **2.64** with lithium metal in liquid ammonia, after chromatographic separation, also afforded the dimeric steroid bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) **2.67** (25%) as the major product (Scheme 2.32). The C-3 unsaturated isomers, **2.65** and **2.66**, were not produced.



Scheme 2.33 Proposed mechanism of reductive dimerization of the 19-methyl ester **2.64** to bis-(methyl 3ξ-hydroxyandrost-4-en-17-on--19-oate) **2.67** or **2.71**.



Scheme 2.34 Proposed mechanism of the formation of the methyl 5ξ-androst-3-en-19-oates, **2.65** and **2.66**.

A proposed mechanism for reductive dimerization is presented in Scheme 2.33. A direct electron transfer from a metal to a π system of the ketone group converts the 3-ketone **2.64** to the 3-ketyl **2.64a** (a radical anion). The 3-ketyl **2.64a** abstracts proton or reacts with a cation metal to form the hydroxy radical or the organometallic species, **2.64b** or **2.64c**. Pinacol coupling of the two radicals **2.64b** or **2.64c** gives the bis-(methyl 3-ξ-hydroxyandrost-4-en-17-on-19-oate) **2.67**. A second alternative would be a reaction of the C-3 radical, **2.64b** or **2.64c**, with a π system of the ester group to yield the 19-hemiacetal **2.71**. Although these two compounds, **2.67** and **2.71**, were expected, only the dimer has been produced. The fact that the dimeric compound was formed suggests a stability of the C-3 radical, which presumably results from the stabilizing effect of the 19-ester group *via* its π system.

The proposed mechanism of formation of the unsaturated products, methyl androst-5β- and androst-5α-3-en-17-on-19-oate, **2.65** and **2.66**, is outlined in Scheme 2.34. The reaction commences with transfer of the first electron to a π system of the 3-ketone. The second electron is further transferred more rapidly than the compound diffuses from the metal surface. Protonation of the intermediate **2.64d** to cleave the oxygen-metal bond, followed by water elimination and further successive transfer of two more electrons to C-3 leads to the organozinc carbenoid intermediate **2.64g**. Reaction of the carbenoid **2.64g** with two protons produces the final 5ξ-androst-3-en-17-on-19-oates, **2.65** and **2.66**. Reduction of intermediates **2.64d** → **2.64h** occurs, presumably, when all intermediates are bonded to zinc atoms at the metal surface and leave the surface only after at least one proton has been added to carbon. However, more experimental data are required to resolve the reaction mechanism.

The ^{1}H and ^{13}C NMR spectra were determined for the four compounds, **2.65-2.68**. Structures of compounds **2.67** and **2.68** have been determined by COSY and HSQC, and for compound **2.67** also by NOE and HMBQ.

Elemental analyses of compounds **2.65**, **2.67**, and **2.68** were consistent with their compositions, $C_{20}H_{28}O_3$, $C_{40}H_{54}O_8$, and $C_{46}H_{70}O_8Si_2$, respectively.

The symmetrical structure of the 3,3'-dimers, **2.67** and **2.68**, was revealed in their ^{1}H and ^{13}C NMR spectra. These compounds behaved rather as monomers. The number of the characteristic proton and ^{13}C carbon signals was in agreement either with the hemiacetal structure or with a symmetrical dimer. However, the dimer structure was confirmed by both EIMS and FABMS. EIMS (70 eV) in the high mass spectra of the dimers, **2.67** and **2.68**, did not show their molecular ions at m/z 662 and m/z 806, respectively. In the high mass spectra the following fragment ions were observed: compound **2.67** (Figure 2.12) $M^{+\cdot}$ 662 (0%), m/z 644 $[M - H_2O]^{+\cdot}$ (3%), m/z 626 $[M - 2H_2O]^{+\cdot}$ (18%), m/z 567 $[M - 95]^{+}$ (12%), m/z 508 (8%), m/z 330 $(M - 2H)^{2+\cdot}$ (100%), m/z 270 (78%) and compound **2.68** (Figure 2.13) $M^{+\cdot}$ 806 (0%), m/z 626 $[M - 2TMSiOH]^{+\cdot}$ (0.6%), m/z 567 (0.5%), m/z 403 $[M]^{2+}$ (8%), m/z 147 (100%), m/z 75 $[SiMe_3]^{+}$ (67%).

The structures of **2.67** and **2.68** were determined by FABMS. The FAB mass spectrum of the 3,3'-dihydroxy derivative **2.67** in the glycerol matrix is presented in Figure 2.14. The m/z 663 represents the $[M + H]^{+}$ ion and at m/z 685 the $[M + Na]^{+}$ ion. The molecular weight of the molecule can be obtained by subtracting 1 from 663 and 23 from 685. This indicates that the intact molecular weight is 662 consistent with the dimeric structure of **2.67**.

The FAB mass spectrum of the 3,3'-disilyl derivative **2.68** in the glycerol matrix is shown in Figure 2.15. Again, the peak at m/z 807

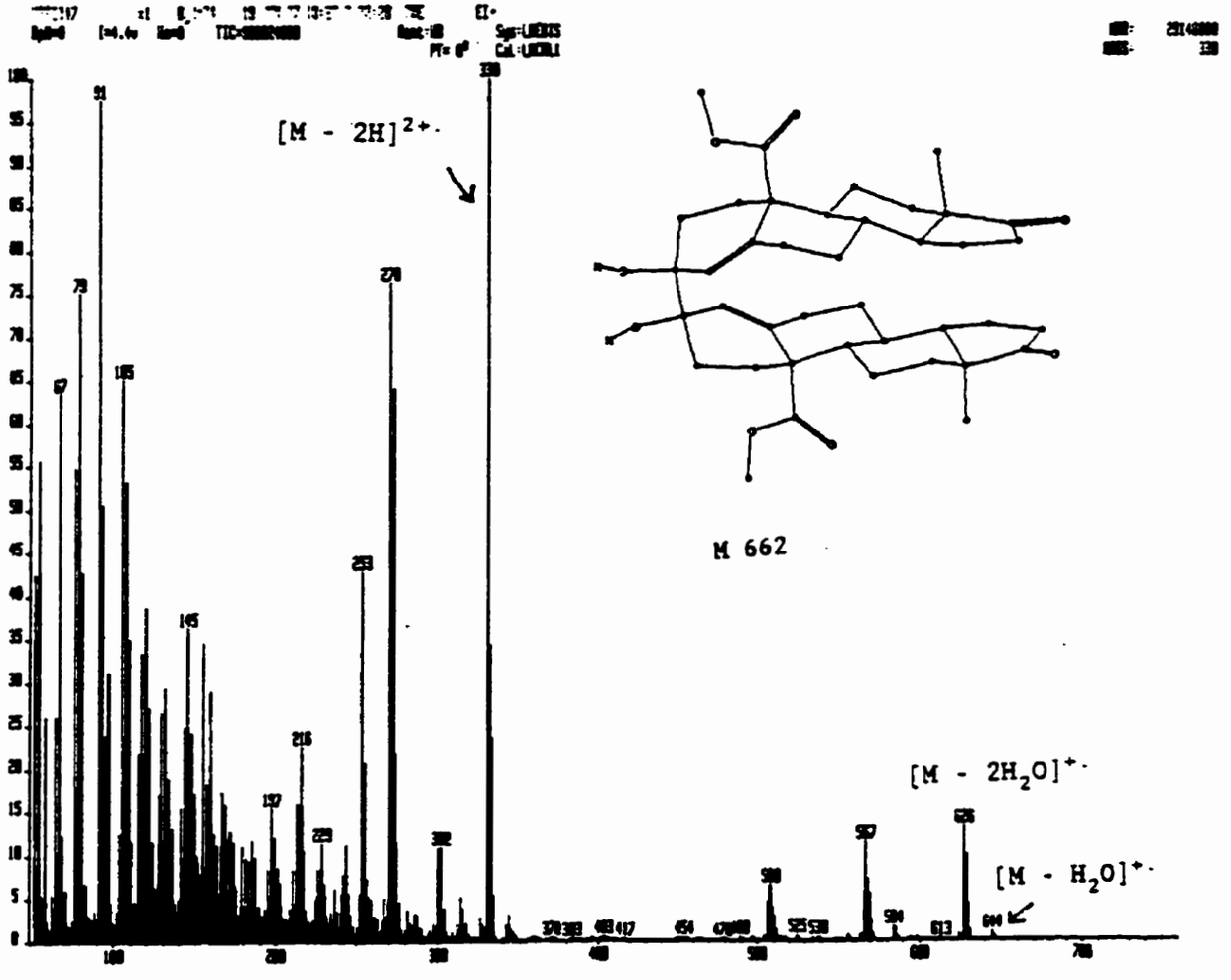


Figure 2.12 EIMS (70 eV) of a symmetrical dimer, bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) **2.67**.

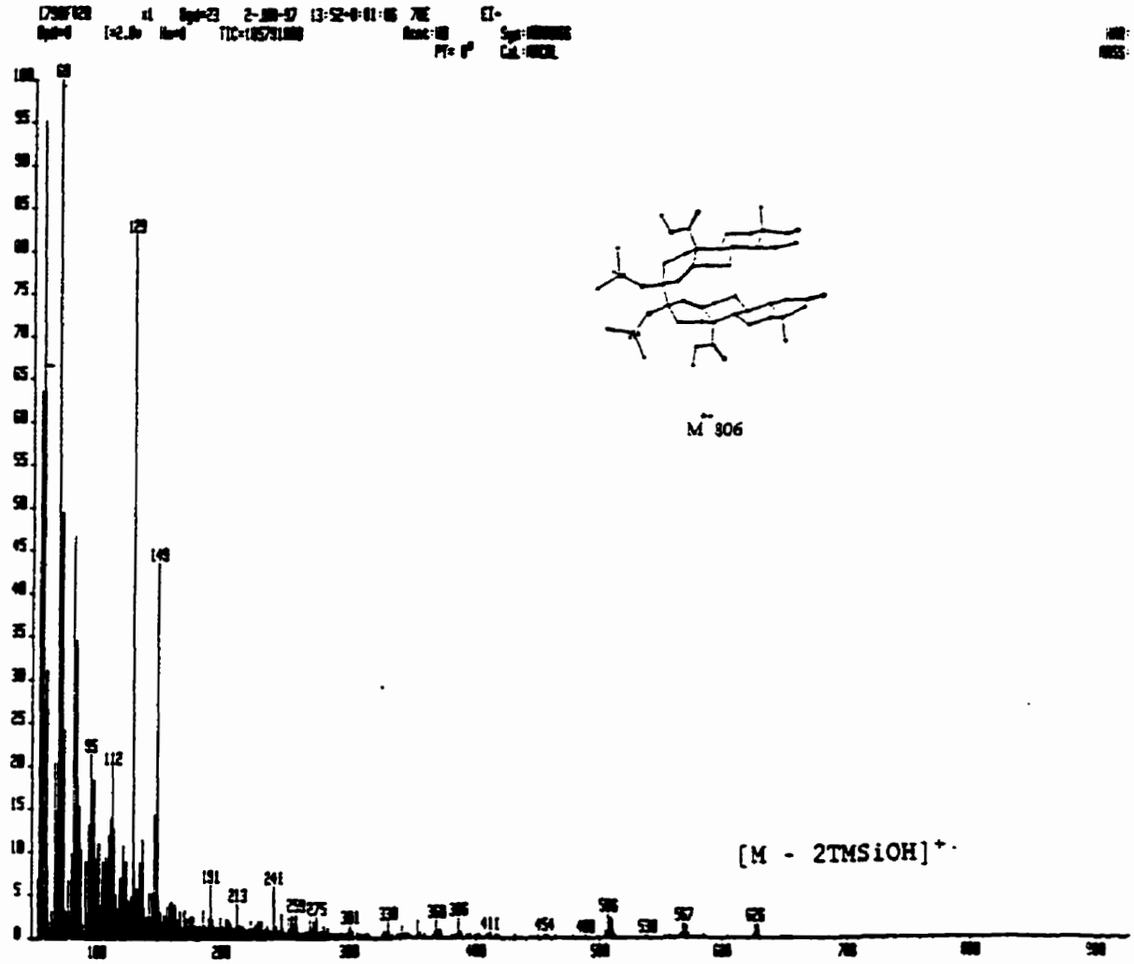


Figure 2.13 EIMS (70 eV) of a symmetrical dimer, bis-(methyl 3ξ-trimethylsilyloxyandrost-4-en-17-on-19-oate) 2.68.

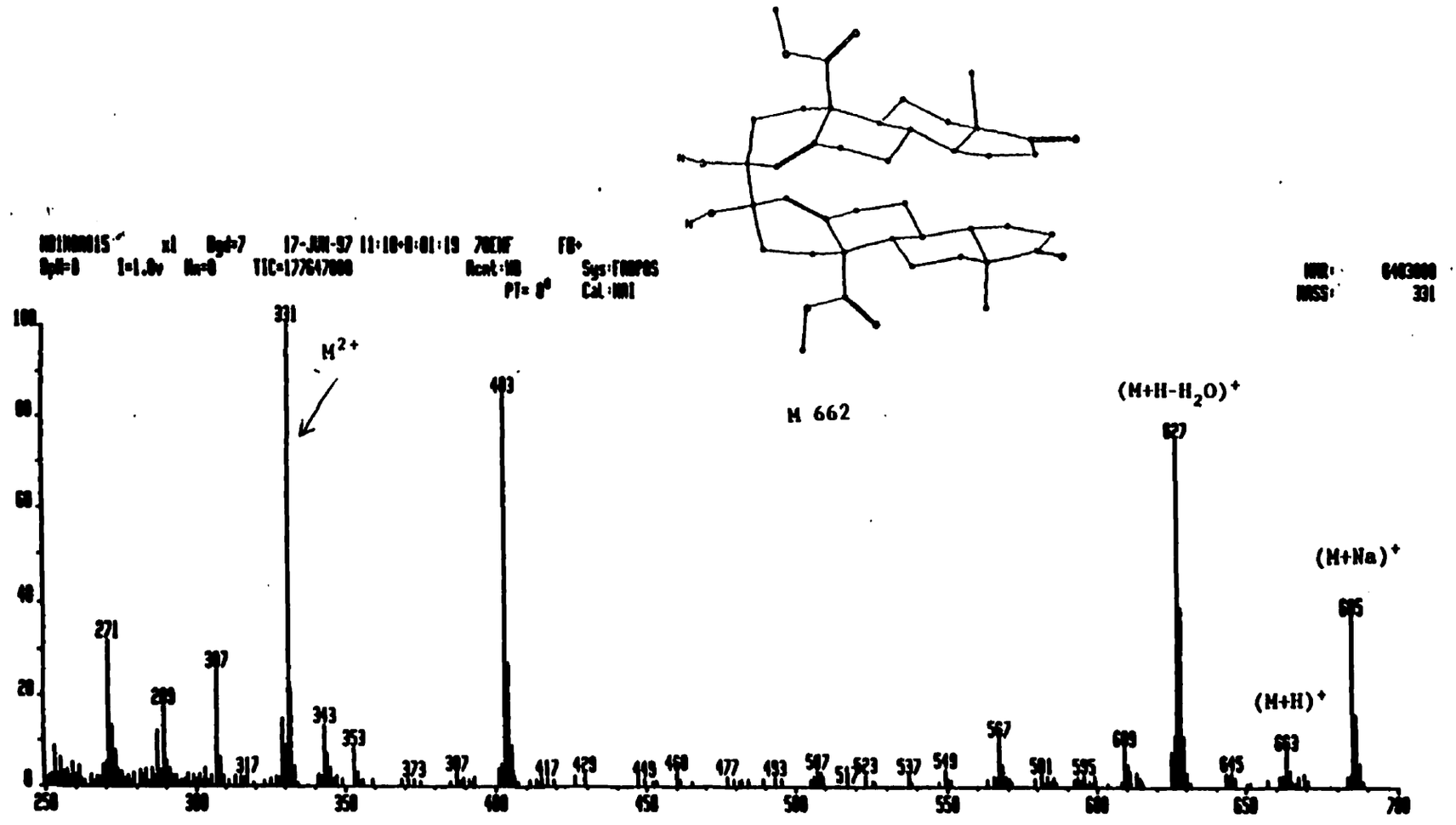


Figure 2.14 FAB mass spectrum of bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) 2.67 in glycerol.

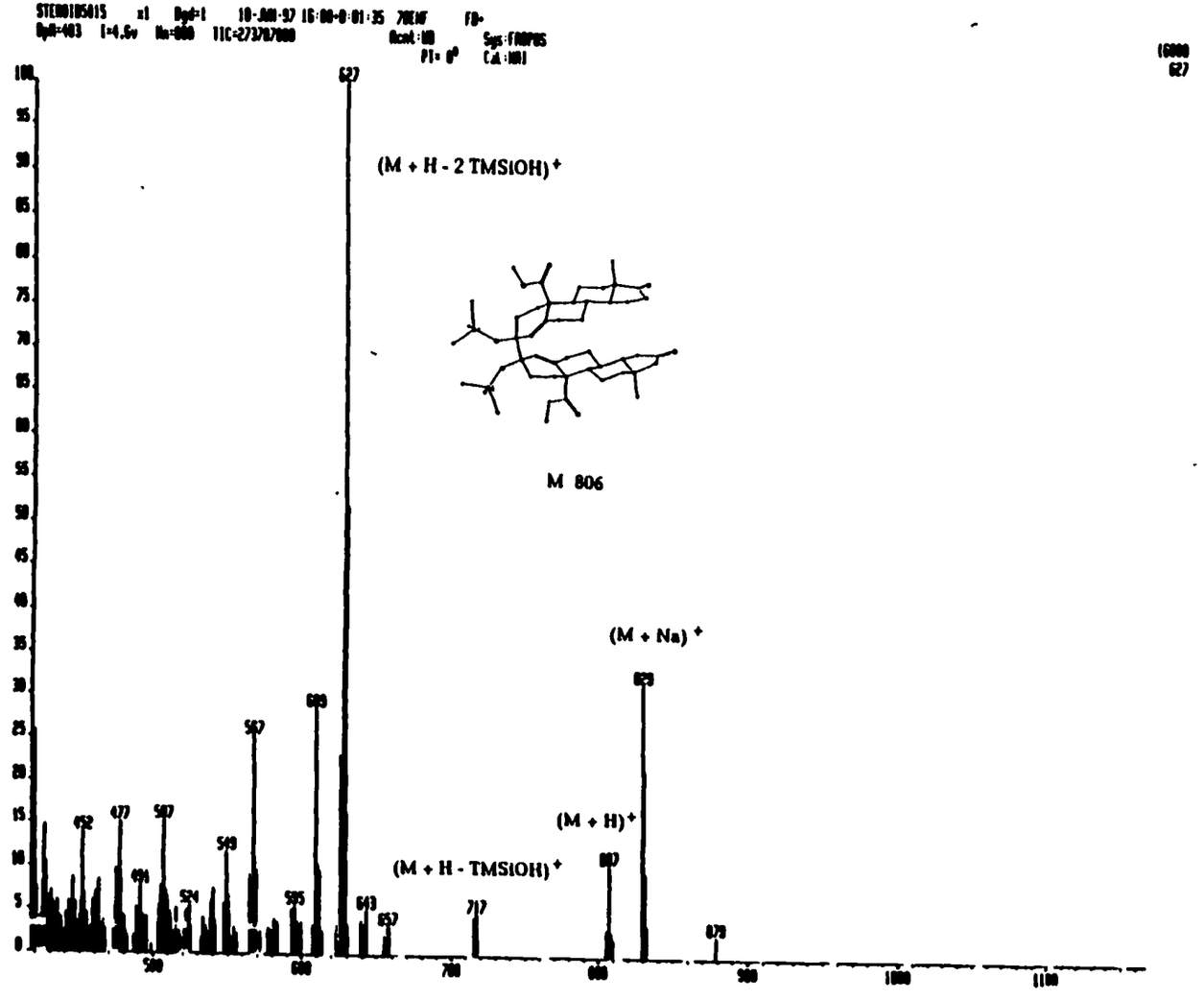


Figure 2.15 FAB mass spectrum of bis-(methyl 3ξ-trimethylsiloxyandrost-4-en-17-on-19-oate) 2.68 in glycerol.

represents the $[M + H]^+$ ion and at m/z 829 the $[M + Na]^+$ ion, i.e. the intact molecule and Na^+. The molecular weight of the molecule can be obtained by subtracting 1 from 807 or 23 from 829. Thus the molecular weight is 806 consistent with the dimeric structure of **2.68**. The base peak, m/z 627, in the spectrum is formed by a fragment ion $[M+H-2TMSiOH]^{+\cdot}$ supporting the presence of two trimethysiloxy groups in the molecule.

An attempt to determine the structure of compound **2.68** by X-ray crystallographic analysis was unsuccessful because of difficulties in obtaining a crystal of good quality (Dr. Bridson, personal communication, 1996).

The structure of the bis-(methyl 3ξ-hydroxy androst-4-en-17-on-19-oate) **2.67** was determined by the following NMR data. The ^1H and ^{13}C NMR data were consistent with the presence of unsaturation at C-4: a singlet at δ_H 5.69 and methine carbon at δ 124.63 as well as a quaternary carbon at δ_c 143.06 were in agreement with the 4,5 double bond. The lack of a quaternary carbon at $\delta_c \sim 198$ (sp^2), was assigned to the 3-carbonyl group, but the presence of a newly formed quaternary carbon at δ_c 73.60 (sp^3). suggested the existence of the hydroxy group at C-3. This conclusion was also supported by ^{13}C NMR spectra of bis-(methyl 3ξ-trimethylsiloxyandrost-4-en-17-on-19-oate) **2.68**. After the silylation of the allylic tertiary hydroxy groups of **2.67**, a quaternary carbon at δ_C 73.60 ppm was shifted downfield to δ_c 77.90 ppm supporting the presence of the hydroxy group at C-3. Additionally, the ^1H NMR spectrum of **2.68** showed a chemical shift corresponding to the trimethylsilyl groups at δ_H 0.004 ppm. The attachment of the methoxy groups to C-19 was determined by the long-range couplings. The resulting spectrum showed cross peaks for two-, three- and four-bonds

connections. Protons of the methoxy group showed three-bond couplings to the quaternary C-19. Similarly, a proton at C-4 showed couplings to C-1, C-2, C-3 and C-10. The signal of protons of the 19-OCH_3 of compound **2.68** had undergone an upfield shift (51.41 ppm) consistent with transformation of the C-3 ketone. The ^{13}C chemical shift of C-19 (174.21 ppm) of **2.68** was not significantly changed from that of the starting compound **2.64** (175.54 ppm). This is more consistent with the dimer structure of compound **2.68** than with the 19-hemiacetal structure **2.71**. Expected values for a quaternary carbon, C-19, of the 19-hemiacetal **2.71** should be *ca* 100 ppm as for acetals (Templeton *et al.*, 1994).

Irradiation of protons of the 19-OCH_3 group of **2.67** showed a NOE enhancement of protons of the angular methyl group, 18-CH_3 (0.85%), 6β-H (0.19%), and 8β-H (0.07%). A ^{13}C T_1 measurement in $CDCl_3$ gave an average T_1 value of 231 ms for the CH_2 carbon atoms compared with 600 ms for CH_2 of other steroid molecules with a faster tumbling (smaller molecule). The T_1 of **2.68** suggests a dimeric product. In general, when rotation of molecules in solution is slow, the effective correlation time, τ_c, is longer and T_1 becomes short according to the equation $T_1 \sim 1/nh(\gamma_c)^2(\gamma_H)^2(r_{CH})^{-6}\tau_c$, where n is the number of attached or nearest neighbour hydrogens, h is Plank's constant divided by 2π, r is the distance from carbon to hydrogen, and τ_c is the effective correlation time for rotations (equivalent to the time for a molecule to rotate one radian, 57°32') (Lambert *et al.*, 1987).

The IR spectrum of the 3ξ-siloxy dimer **2.68** shows the ν_{C-O} vibration of the 19-OCH_3 group of **2.68** is in its normal region, i.e. 1074 cm^{-1}. Interpretation of the spectra of the bis-(methyl 3ξ-hydroxy androst-4-en-17-on-19-oate) **2.67** is complicated by the fact that more

hydrogen bonding can occur. The IR spectrum of **2.67** shows bands at 3595 cm^{-1} ($\Delta\nu$ 33 cm^{-1}) and at 3553 cm^{-1} ($\Delta\nu$ 75 cm^{-1}), which are attributed to the 3-OH and 3'-OH, respectively, due to intramolecular hydrogen bonding. Similar hydrogen bonding and 5-membered ring systems were observable in 5α-androstane-16,17-diols where the hydroxy substituents were *cis* (Brutcher *et al.*, 1962; Combe *et al.*, 1971). Surprisingly, the ν_{C-O} vibration of the 19-methoxy group is not observable at its normal region (1074 cm^{-1}).

Melting point of the 3,3'-trimethylsiloxy dimer **2.68** was higher (258 °C) than the 3,3'-diol dimer **2.67** (218 °C). This suggests an increase in molecular weight rather than the usual protection of the hydroxy groups. In general, protections of the hydroxy groups lead to elimination of hydrogen bonds and subsequently, it gives the lower melting point. It was observed that the 3,3'-disilyl derivative **2.68** was more stable than compound **2.67** both in organic solutions and in the crystalline form.

In the ^1H NMR spectrum of the methyl 5β-androst-3-en-17-on-19-oate **2.65** a multiplet peak at 5.72 ppm and a doublet of doublet of doublets at 5.39 ppm (J 1.75, 3.6, 10 Hz) were assigned to 3-H and 4-H, respectively, corresponding to the introduction of the 3,4-double bond. A methoxy signal at δ 3.69 ppm was assigned to the 19-OCH_3. A broad signal at 2.75 ppm was assigned to the 5β-H based on the absence of an axial coupling to the 6β-H. The broad signal of the 5β-H results from the structural flexibility.

The ^{13}C NMR spectrum of **2.65** showed, instead of the carbonyl group at C-3, two new methine signals at δ_{C-3} 126.80 ppm and δ_{C-4} 130.99 ppm assigned to the 3,4-double bond. A newly formed sp^3 carbon at C-5, confirmed by the ^1H-^1H (2D COSY) and ^1H-^{13}C (HSQC) correlation spectra,

was consistent with structure **2.65**. The ^{13}C chemical shift of a methine carbon at C-9 of structure 5β-3-ene **2.65** was observed at 40.26 ppm.

In the ^{1}H NMR spectrum of 5α-androst-3-en-17-one **2.66** two new doublets of doublets of doublets at 5.55 ppm (J 2.0, 6.5, 9.8 Hz) and 5.46 ppm (J 1.8, 6.4, 9.8 Hz) were assigned to 3-H and 4-H, respectively, corresponding to the introduction of the 3,4-double bond. The doublet of doublets at 2.5 ppm (J 8.2, 12.7 Hz), which overlaps the 16β-H signal, was assigned to the 5α-H: there is an axial coupling to the 6β-H with J = 12.7 Hz. The ^{13}C NMR spectrum of **2.66** showed two new methine signals at δ_{C-3} 125.99 ppm and δ_{C-4} 131.39 ppm assigned to the 3,4-double bond. The tertiary carbon (sp^3) at C-5 was consistent with structure **2.66**.

The structures, 5β-3-ene **2.65** and 5α-3-ene **2.66**, can be distinguished by the chemical shifts of their methine carbons at C-9. The ^{13}C chemical shifts of a methine carbon at C-9 of structure 5α-3-ene **2.66** and structure 5β-3-ene **2.65** were observed at 50.05 ppm and 40.26 ppm, respectively. The difference of the chemical shifts, $\Delta\delta_{C-9}$= -9.77 ppm, going from the 5α-H to 5β-H structure is consistent with the assignemnt.

The structure of the unsaturated dimer, bis-(methyl 3ξ-trimethylsiloxyandrost-4-en-17-on-19-oate) **2.69** has been determined by its ^{1}H and ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed the four pairs of singlet signals. Each pair of the singlet signals had equal intensity. The two singlets at δ 6.69 and 5.35 ppm, δ 3.64 and 3.62 ppm, δ 0.86 and 0.84 ppm, and δ 0.06 and 0.02 ppm were assigned to the two vinylic protons at C-4, the two 19-OCH_3 groups, the two 18-CH_3, and the two $Si(CH_3)_3$, respectively. The ^{1}H and ^{13}C NMR spectra were

consistent with the presence of unsaturation at C-4. Two singlet signals of equal intensity at δ_H 5.69 and δ_H 5.35 ppm and two methine carbons δ_{C-4} 127.30 and $\delta_{C-4'}$ 127.55 as well as two quaternary carbons at δ_{C-5} 140.09 and $\delta_{C-5'}$ 140.82 were in agreement with the presence of two 4,5-double bonds. The lack of a quaternary carbons at *ca* δ 198 (sp^2) were assigned to the 3-carbonyl group, but the presence of two newly formed quaternary carbons at δ_{C-3} 77.89 (sp^3) and $\delta_{C-3'}$ 78.06 (sp^3) suggested the existance of the two siloxy gropups at C-3.

2.3.1 Summary

1. A steroid dimer, with a symmetrical structure, bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) **2.67**, has been synthesized by reduction with Li-NH_3 as well as Zn, 50% H_2O-CH_3COOH conditions.
2. Some chemical properties of compound **2.67** were determined: (*i*) the 4,5-double bond was resistant to catalytic hydrogenation; (*ii*) the two tertiary 3- and 3'-hydroxy groups were protectable with N-(TMSi)imidazole); (*iii*) the two neighbouring 3- and 3'-hydroxyl groups were oxidatively cleaved with $NaIO_4$ to give the 19-methyl ester **2.64**; and (*iv*) alkaline or acetic acid conditions, at ambient temperature, did not affect **2.67**.
3. A structure of the 3,3'-TMSi dimer **2.68** has been supported by the following data: (*i*) its mp 258 °C was higher than the non-derivitized 3,3'-diol dimer **2.67** (mp 218 °C); (*ii*) once protected with TMSi reagent, it did not reacted further with TMSiOTf; (*iii*) its T_1 (231 ms) was consistent with the T_1 (250 ms) of a reported steroid dimer (Templeton *et al.*, 1990); (*iv*) the ^{13}C chemical shift of C-19 (174.21 ppm) of **2.68** was not significantly changed compared with the starting compound **2.64** (175.54 ppm); (*v*) both the molecular ions of **2.67** nor **2.68** were not detected upon electron

ionization (70 eV) in MS, but $[M/2]^+$ was abundant showing half of the molecule; (*vi*) the molecular weight of the intact molecule, **2.67** and **2.68**, was determined by FAB MS.

2.3.2 Experimental

Androst-4-ene-3,17-dion-19-oic acid 2.63

A standard solution of a Jones reagent: 27 g of chromic trioxide (CrO_3) was dissolved in 23 mL of concentrated sulfuric acid diluted with water to a volume of 100 mL.

To a stirred solution of 19-hydroxyandrost-4-ene-3,17-dione **2.1** (10 g, 0.033 mol) in acetone (250 mL), maintained at 10-15 °C in an ice-water bath, was added cold Jones reagent (30 mL, 0.081 mol, 2.5 eq.) dropwise over 40 min. The mixture was stirred until no starting material (2 h) was detected by TLC at which time isopropanol (20 mL) was added to destroy excess reagent. The mixture was extracted with CH_2Cl_2 (350 mL), and the organic layer washed with 43% aqueous $(NH_4)_2SO_4$, water, dried over Na_2SO_4, filtered, and evaporated to dryness to give a crude product. This product was stirred with saturated aqueous $NaHCO_3$ (100 mL) for 30 min. The aqueous layer was extracted with EtOAc, and the EtOAc extract again extracted with aqueous $NaHCO_3$ (20 mL), and then the combined water layers acidified with 10% HCl to give, on filtration, androst-4-ene-3,17-dion-19-oic acid **2.63** (5.0 g, 0.017 mol, 52%), mp 146-148 °C (decomp.), (from CH_2Cl_2-LP), (lit., mp 146 °C; Ueberwasser *et al.*, 1963)

^{1}H NMR of **2.63** ($CDCl_3$): δ 5.95 (d, J 1.22, 1H, 4-H), 0.91 (s, 3H, 18-CH_3).

^{13}C NMR of **2.63** ($CDCl_3$): δ 33.68 (1), 34.78 (2), 198.73 (3), 127.19 (4), 161.55 (5), 32.57 (6), 31.37 (7), 35.56 (8), 53.68 (9), 50.52 (10), 21.64 (11), 29.97 (12), 47.58 (13), 50.93 (14), 21.98 (15), 35.70

(16), 220.0 (17), 13.90 (18), 175.54 (19).

Androst-4-ene-3,17-dion-19-oic acid methyl ester 2.64

From androst-4-ene-3,17-dione-19-oic acid **2.64**:

The crude product **2.63** (2.26 g, 6.84 mmol) was treated with freshly prepared diazomethane (CH_2N_2) in diethyl ether (150 mL) for 5 min. Evaporation of the organic solvent gave a crude product (2.27 g), which after crystallization yielded compound **2.64** (2.10 g, 6.01 mmol, 88%), mp 142-145 °C (from EtOAc-LP), (lit., mp 142-142.5 °C, from diethyl ether, Dyer and Harrow, 1979a).

From bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) **2.67**:

To a solution of bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) **2.67** (13.4 mg, 0.04 mmol) in warm methanol (3 mL) was added, in one portion, $NaIO_4$ (150 mg, 0.70 mmol, 17 eq.) dissolved in hot water (1 mL). The mixture was refluxed for 4 h until no starting material was detected by TLC, and then diluted with CH_2Cl_2 (20 mL). The organic layer was washed with water, dried, filtered, and evaporated to give a residue of **2.64** (8 mg). The product was confirmed by its ^1H and ^{13}C NMR spectra.

^1H NMR of 2.64 ($CDCl_3$): δ 5.90 (d, J 1.51, 1H, **4-H**), 3.76 (s, 3H, 19-OCH_3), 0.90 (s, 3H, 18-CH_3).

^{13}C NMR of 2.64 ($CDCl_3$): δ 33.82 (1), 34.91 (2), 198.57 (3), 126.76 (4), 161.96 (5), 32.56 (6), 31.35 (7), 35.59 (8), 53.75 (9), 50.85 (10), 21.64 (11), 30.06 (12), 47.52 (13), 50.88 (14), 21.92 (15), 35.69 (16), 219.97 (17), 13.77 (18), 171.63 (10-$OCOCH_3$), 50.85 (10-$OCOCH_3$).

Bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) 2.67 and **Bis-(methyl 3ξ-trimethylsiloxyandrost-4-en-17-on-19-oate) 2.68.**

Reductive dimerization of **2.64** with Zn in 50% H_2O-CH_3COOH

(i) *Dimerization and FCC separation*: compounds **2.65** and **2.67**

Methyl androst-4-ene-3,17-dion-19-oate **2.64** (526 mg, 1.6 mmol) was

dissolved in 50% aqueous HOAc (20 mL) and Zn powder (10 g) added in one portion. The heterogenous mixture was stirred at room temperature for 6 h and then filtered to remove the excess zinc, which was washed with 50% aqueous CH_3COOH, water, Et_2O, and CH_2Cl_2. The filtrate was extracted with CH_2Cl_2 and the organic layer washed with water, saturated aqueous $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated to give a glassy residue (500 mg), which on FCC, on elution with 3-25% acetone-LP, yielded the following fractions given in order of their elution: methyl 5β-androst-3-en-17-on-19-oate **2.65** (75 mg, 0.24 mmol, 15%), mp 143-145.5 °C, (from Et_2O-LP), (Found: C, 75.81; H, 9.15. $C_{20}H_{28}O_3$ requires C, 75.91; H, 8.92%), a mixture of **2.65** and methyl 5α-androst-3-en-17-on-19-oate **2.66** (150 mg), bis-(methyl 3ξ-hydroxyandrost-4-en-17-on-19-oate) **2.67** (67 mg, 1.7 mmol, 0.20 mmol, 13%), mp 217-220 °C (from CH_2Cl_2-EtOAc-LP), (Found: C, 72.38, H, 8.62. $C_{40}H_{54}H_8$, MW 662.3819, requires C, 72.26, H, 8.49%), and a mixture of unidentified compounds (120 mg).

^{1}H NMR of **2.65** ($CDCl_3$) 5β-H: 5.72 (m, 1H, 3-H), 5.38 (dd, J 1.75, 3.6 10.0, 1H, 4-H), 3.67 (s, 3H, 19-OCH_3), 2.74 (s, 1H, 5β-H), 2.44 (dd, J 9.3, 19.1, 1H, 16β-H), 0.96 (s, 3H, 18-CH_3).

^{13}C NMR of **2.65** ($CDCl_3$) 5β-H: δ 28.77 (1), 32.03 (2), 130.98 (3), 126.80 (4), 39.18 (5), 28.52 (6), 25.70 (7), 35.58 (8), 40.26 (9), 48.08 (10), 21.45 (11), 21.86 (12), 47.29 (13), 51.56 (14), 21.62 (15), 35.98 (16), 221.51 (17), 14.00 (18), 176.81 (19), 51.42 (19-OCH_3).

^{1}H NMR of **2.67** ($CDCl_3$): δ δ 5.69 (s, 1H, 4-H), 3.69 (s, 3H, 19-OCH_3), 0.93 (s, 3H, 18-CH_3).

^{13}C NMR of **2.67** ($CDCl_3$): δ 29.71 (1), 28.61 (2), 73.60 (3), 124.63 (4), 143.06 (5), 33.94 (6), 32.10 (7), 36.19 (8), 52.74 (9), 50.22 (10), 21.71 (11), 31.70 (12), 47.83 (13), 51.54 (14), 21.71 (15), 35.72 (16),

221.0 (17), 13.84 (18), 174.32 (19) 51.81 (19-OCH_3).

IR of the 3,3'-dihydroxy-dimer **2.67** (CH_2Cl_2): 3595.0 (3ξ-OH), 3553.0 (3'ξ-OH), 2939.0, 1737 (17-C=O), 1250 (C-OH), 1137, 1009 (C-O).

EIMS of **2.67** (MW 662): m/z 644 $[M - H_2O]^{+\cdot}$ (3%), m/z 626 $[M - 2H_2O]^{+\cdot}$ (18%), m/z 567 $[M - 95]^{+}$ (12%), m/z 508 (8%), m/z 330 $(M - 2H)^{2+\cdot}$ (100%), m/z 270 (78%).

FABMS of **2.67** (MW 662) (glycerol): m/z 685 $[M+Na]^{+}$ (48%), m/z 663 $[M+H]^{+}$ (8%), m/z 627 $[M+H-2H_2O]^{+}$ (75%), m/z 403 (85%), m/z 331 $[M]^{2+}$ (100%).

(ii) *Dimerization, TMSi derivatization, and FCC separation* : compounds **2.65, 2.66, 2.68, and 2.69.**

Methyl androst-4-ene-3,17-dion-19-oate **2.64** (2.22 g, 6.45 mmol) was dissolved in 50% aqueous HOAc (40 mL) and Zn powder (40 g) added. The heterogenous mixture was stirred at room temperature for 2.5 h and then filtered to remove zinc, which was washed with 50% aqueous CH_3COOH, water, acetone and CH_2Cl_2. The filtrate was extracted with CH_2Cl_2 and the organic layer washed with water, saturated aqueous $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated to give a glassy residue (2.11 g). The residue was treated with 1-(trimethylsilyl)imidazole reagent (2.24 mL, 15 mmol) in CH_2Cl_2 (5 mL) for 1h, when no starting material was detected by TLC. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated to give a residue, which on FCC, on elution with 30% Et_2O-LP, gave fractions given in order of their elution: (i) methyl 5β-androst-3-en-17-on-19-oate **2.65** (133 mg, 0.42 mmol, 6.5%), mp 143.4-145.5 °C (from Et_2O-LP); (ii) a mixture of 5β-androst-3-en-17-on-19-oate **2.65** and methyl 5α-androst-3-en-17-on-19-oate **2.66** (1.06 g, 3.35 mmol, 52%); (iii) methyl 5α-androst-3-en-17-on-19-oate **2.66** (70 mg, 0.22 mmol, 3.5%) not pure enough to

crystallize; (iv) bis-(methyl 3ξ-trimethylsiloxyandrost-4-en-17-on-19-oate) **2.68** (270 mg, 0.335 mmol, 5%), mp 258-260°C (from CH_2Cl_2-EtOAc), (Found: C, 67.97; H, 8.91. $C_{46}H_{70}O_8Si_2$, MW 806.4609, require C, 68.27; H, 8.97%), and a noncrystalline compound **2.69** (130 mg, 0.160 mml, 2.5%) identified by its ^1H NMR spectrum as the unsymmetrical dimer, bis-(methyl 3ξ-trimethylsiloxyandrost-4-en-17-one-19-oate).

^1H and ^{13}C NMR of **2.65** ($CDCl_3$): see above.

^1H NMR of **2.66** ($CDCl_3$) 5α-H: δ 5.55 (ddd, J 2.05, 6.52, 9.78, 1H, 3-H), 5.46 (dd, J 1.77, 9.82, 1H, 4-H), 3.66 (s, 3H,19-OCH_3), 2.5 (d, J 12.73, 5α-H overlaps with 16β-H), 2.51 (dd, J 8.79, 10.13, 16β-H).

^{13}C NMR of **2.66** ($CDCl_3$) 5α-H: δ 31.54 (1), 24.41 (2), 131.39 (3), 125.99 (4), 35.81 (5), 30.41 (6), 27.50 (7), 44.50 (8), 51.30 (9), 50.03 (10), 21.74 (11), 32.02 (12), 47.23 (13), 51.54 (14), 21.81 (15), 35.81 (16), 220.91 (17), 13.86 (18), 174.65 (19) 51.11 (19-OCH_3).

^1H NMR of **2.68** ($CDCl_3$): δ 5.62 (s, 1H, 4-H), 3.67 (s, 3H, 19-OCH_3), 2.60 (ddd, J 4.5, 13.8, 1H, 6β-H), 2.22 (dd, J 9.05, 19.17, 1H, 16β-H), 0.90 (s, 3H, 18-CH_3), 0.015-(-0.07) [s, 9H, 3α-$OSi(CH_3)_3$].

^{13}C NMR of **2.68** ($CDCl_3$): δ 31.07 (1), 30.50 (2), 77.90 (3), 127.27 (4), 140.85 (5), 33.95 (6), 31.57 (7), 36.03 (8), 53.09 (9), 50.02 (10), 21.64 (11), 31.35 (12), 47.83 (13), 51.26 (14), 21.74 (15), 35.80 (16), 220.88 (17), 13.88 (18), 174.21 (19), 51.41 (19-OCH_3), 2.55 3α-$OSi(CH_3)_3$.

T_1 (sec) of ^{13}C of **2.68** ($CDCl_3$): 0.206 (1;CH_2), 0.220 (2;CH_2), 0.414 (4;CH), 3.737 (5;C), 0.229 (6;CH_2), 0235 (7;CH_2), 0.416 (8;CH), 0.431 (9;CH), 0.235 (11;CH_2), 0.250 (12;CH_2), 1.210 (14;CH), 0.231 (15;CH_2), 0.239 (16;CH_2), 1.093 (18;CH_3), 0.441 (19;OCH_3), 4.286 [$Si(CH_3)_3$].

IR (cm^{-1}) of **2.68** (CH_2Cl_2): 2955, 1735 (17-C=O), 1252 (C-OH), 1074 (19-OCH_3).

EIMS of **2.68**: $M^{+\cdot}$ 806 (0%), m/z 626 $[M - 2TMSiOH]^{+\cdot}$ (0.6%), m/z 567 (0.5%), m/z 403 $[M]^{2+}$ (8%), m/z 147 (100%), m/z 75 $[SiMe_3]^+$ (67%).

FABMS of **2.68** (MW 806) (glycerol): m/z 829 $[M+Na]^+$ (35%), m/z 807 $[M+H]^+$ (15%), m/z 717 $[M+H-TMSiOH]^+$ (8%), m/z 627 $[M+H-2TMSiOH]^+$ (100%).

^{1}**H NMR** of **2.69** (unsymmetrical dimer) ($CDCl_3$): δ 5.69 (s, 1H, 4-H), 5.35 (s, 1H, 4-H), 3.64 (s, 3H, 19-OMe), 3.62 (s, 3-H, 19-OMe), 0.86 (s, 3H, 18-CH_3), 0.84 (s, 3H, 18-CH_3), 0.06 (s, 9H, 3-$OSiMe_3$), 0.02 (s, 9H, 3-$OSiMe_3$).

^{13}**H NMR** of **2.69** (unsymmetrical dimer) ($CDCl_3$): 29.72/30.34 (1/1'), 28.95/29.72 (2/2'), 77.89/78.06 (3/3'), 127.30/127.55 (4/4'), 140.09/140.82 (5/5'), 33.80/33.96 (6/6'), 31.42./31.52 (7/7'), 35.89/35.96 (8/8'), 52.87/52.27 (9/9'), 49.98/49.86 (10/10'), 21.63/21.79 (11/11'), 30.73/30.89 (12/12'), 47.58/47.71 (13/13'), 51.10/51.14 (14/14'), 22.13/22.55 (15/15'), 35.71/35.71 (16/16'), 220.54/220.59 (17/17'), 13.76/13.81 (18/18'), 173.53/173.83 (19/19'), 51.22/51.26 (19-OCH_3/19'-OCH_3), 2.37/3.19 (3-$OSiMe_3$/3'-$OSiMe_3$).

<u>Reductive cyclization of **2.64** with Li-NH_3: compound **2.67**</u>

To a stirred mixture of liquid ammonia (150 mL) and THF (10 mL) containing dissolved lithium metal (700 mg, 2.0 mmol) was added a solution of the methyl androst-4-ene-3,17-dion-19-oate **2.64** (709 mg, 2.06 mmol) in THF (20 mL) over 20 min. Stirring was continued for a further 1 h at which time solid NH_4Cl (6 g, 11 mmol) was added. Addition of the CH_2Cl_2 (150 mL), followed by evaporation of the ammonia, under a gentle stream of argon, left an organic layer, which was washed with water, dried over Na_2SO_4, filtered, and evaporated to give a glassy residue (620 mg), which on FCC, on elution with 18% EtOAc-LP, gave 3,3'-dihydroxy dimer **2.67** (170 mg, 0.51 mmol, 25%), mp

217-220 °C (from CH_2Cl_2-EtOAc-LP) (Found: C, 72.38, H, 8.62. $C_{40}H_{54}H_8$, MW 662.3819, requires C, 72.26, H, 8.49%).

^1H and ^{13}C NMR of **2.67** ($CDCl_3$): see above.

Bis-(methyl 3ξ-trimethysiloxy-17,19-dihydroxy-androst-4-ene) 2.70

To a solution of bis-(methyl 3ξ-trimethylsiloxyandrost-4-en-17-on-19-oate) **2.70** (35 mg, 0.434 mmol) in Et_2O-THF (1.5 mL-0.5 mL) was added solid $LiAlH_4$ (3.3 mg, 0.087 mmoL, 2 eq.) in one portion. After 5 min. of stirring, TLC showed no starting material. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water, dried, filtered, and evaporated to give a solid residue of **2.70** (24 mg, 0.031 mmol, 71%), mp (TC) 235-238 °C (Elemental analysis was not done).

^1H NMR of **2.70** ($CDCl_3$): δ 5.60 (s, 1H, 4-H), 3.66 (s, 3H, 19-OCH_3), (ddd, J 4.5, 13.8, 1H, 6β-H), 0.76 (s, 3H, 18-CH_3), - 0.00033 [s, 9H, 3α-$OSi(CH_3)_3$].

^{13}C NMR of **2.70** ($CDCl_3$): δ 30.63 (1), 30.53 (2), 77.92 (3), 127.01 (4), 141.60 (5), 34.15 (6), 32.00 (7), 36.54 (8), 53.17 (9), 50.10 (10), 22.11 (11), 31.28 (12), 42.98 (13), 51.04 (14), 23.33 (15), 36.75 (16), 81.73 (17), 11.12 (18), 174.24 (19), 51.17 (19-OCH_3), 2.59 3ξ-$OSi(CH_3)_3$.

2.4.0 ATTEMPTED SYNTHESIS of 19(R/S)-AMINO-5β,19-CYCLOANDROSTANE-3,17-DIONE

Introduction

The goal of the work in this section was to synthesize 19(R/S)-amino-5β,19-cycloandrostane-3,17-dione **2.79b** (Scheme 2.41) *via* a reductive cyclization of androst-4-ene-3,17-dion-19-al 19-oxime **2.73** or its derivatives **2.80** and **2.80a** (Scheme 2.43). Unlike androst-4-ene-3,17-dion-19-al **2.2** and androst-1-ene-3,17-dion-19-al **2.28** treatment of the 19-oxime **2.73** with Zn in glacial acetic acid did not lead to cyclization but instead to its deoximation to the 19-aldehyde **2.2** (Scheme 2.41). The 19-aldehyde **2.2** reacted further to give the 5β,19-cyclopropanol **2.4** prepared earlier (see Scheme 2.3). The observed deoximation is in agreement with reported procedures that effect reductive cleavage of the N-O bond (Ahmed and Siddiqui, 1969; Corey and Richman, 1970). Unprotected ketooximes can be converted to ketones by reductive cleavage using zinc-acetic acid at 100 °C (Ahmed and Siddiqui, 1969) or zinc-aqueous acetic acid at 25 °C (Corey and Richman, 1970). The mechanism of these oxime deoximations is not fully established.

Polarographic investigations of oximes showed that the reductions involved two two-electron polarographic waves in acid solutions at pH < 4 (Lund, 1959, 1964). On the first wave, oximes were reduced to imines. On the second wave, i.e. at the more negative potential, the imines were reduced to amines. The reduction process, at the first wave, involves reduction of the protonated oxime and proceeds *via* initial cleavage of the N-O bond, in preference to the C-N bond, to form the imine. For example, the postulated intermediate, the imine, has been isolated after polarographic reduction of 2,4-dihydro-

xybenzophenone oxime to 2,4-dihydroxybenzophenone imine (Lund, 1964). The electrode reaction corresponding to the first wave of the oxime can be formulated as follows:

$$R_1R_2C{=}N\text{-}OH + H^+ \rightleftharpoons R_1R_2C{=}NH^+\text{-}OH + 2e^- + 2H^+ \rightarrow R_1R_2C{=}NH_2^+ + H_2O$$

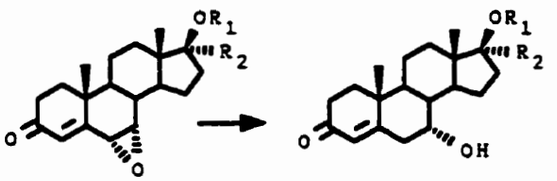
and for the second wave as:

$$R_1R_2C{=}NH_2^+ + 2e^- + 2H^+ \rightarrow R_1R_2CH\text{-}NH_3^+ \qquad \text{(Lund, 1964)}$$

The site of the protonation of the species to which the transfer of electrons occurs is not known. Both N and O may be considered with N the most likely basic centre.

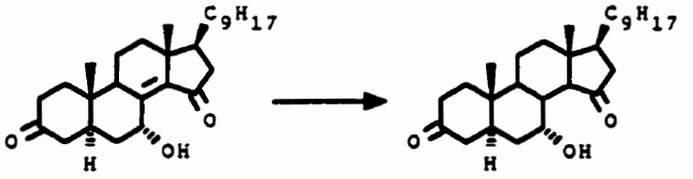
Attempts to obtain the cyclopropylamine **2.79b** (Scheme 2.41) by treatment of androst-4-ene-3,17-dione 19-TBDMSi oxime **2.81** with Li-NH_3 (Scheme 2.43) resulted in the elimination of the 10-CH=N-OTBDMSi substituent yielding the 5(10)-unsaturated derivatives, estr-5(10)-ene-3,17-dione **2.3** and 17β-hydroxy estr-5(10)-en-3-one **2.7**. In the latter, reduction of the 17-ketone function also occurred (Scheme 2.43). Analogous examples of reductive elimination of functional groups have been reported (see below). In general, α,β-unsaturated ketones with a good leaving group at the γ position undergo elimination to give, initially, metal dienolates on reaction with alkali metals in ammonia. Quenching these enolates with ammonium chloride allows the isolation of the β,γ-unsaturated ketone. Isomerization of the β,γ-double bond into conjugation with the ketone may occur by further treatment with base or acid. Reductive eliminations that involved expulsion of hydroxide ions (Amendolla *et*

al., 1954; Anthonsen *et al.*, 1969), alkoxide ions (Masamune *et al.*, 1969), and acetate ions (Spencer *et al.*, 1965), as well as lactone fission (Bruderer *et al.*, 1956; Howe *et al.*, 1959; Kulkarni *et al.*, 1965), and epoxide ring opening (Scheme 2.35), (Irmscher *et al.*, 1964) have been reported. In some cases, it was observed that reduction reactions were faster than elimination reactions. For example, reductions of γ-hydroxy enones with Li-NH_3 at -80 °C gave the saturated ketone (Scheme 2.36), (Dauben *et al.*, 1961; DeClereq *et al.*, 1974; Van Hulle *et al.*, 1974). This phenomenon was explained by temperature dependence of the reaction, i.e. at the lower temperature, elimination was slower than reduction.



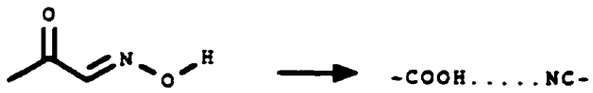
R_1= H
R_2= H or CH_3

Scheme 2.35 Epoxy ring opening by Li-NH_3 (Irmscher *et al.*, 1964).

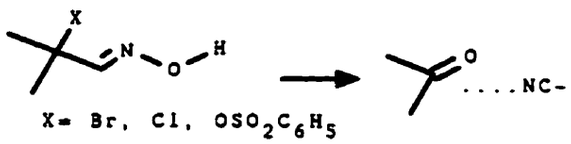


Scheme 2.36 Reduction of the 8,14-double bond by Li-NH_3 instead of 7α-hydroxy elimination (Dauben *et al.*, 1961).

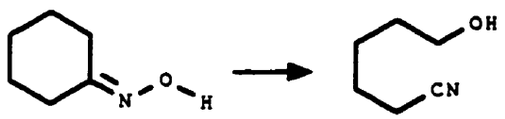
1. Cleavage of a ketooxime (Hendrickson, 1986).



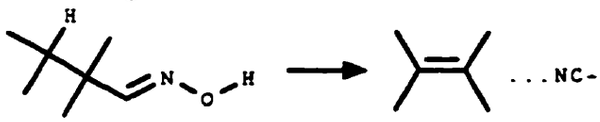
2. Direct cleavage of an oxime (Hendrickson, 1986).



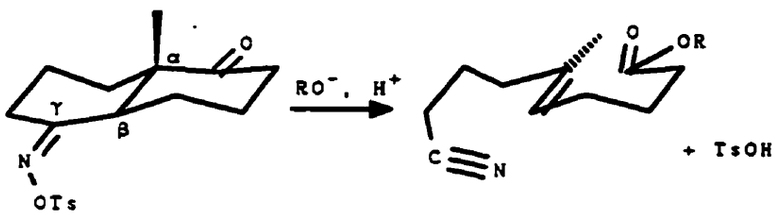
3. Cleavage of an oxime and nucleophilic quenching (Corey and Jorgensen, 1976).



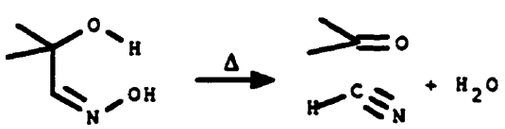
4. Cleavage of an oxime and elimination (Hendrickson, 1986).



5. Cleavage of γ-keto-ketoxime (Eisele *et al.*, 1968).



6. Pericyclic fragmentation (Hendrickson, 1986).

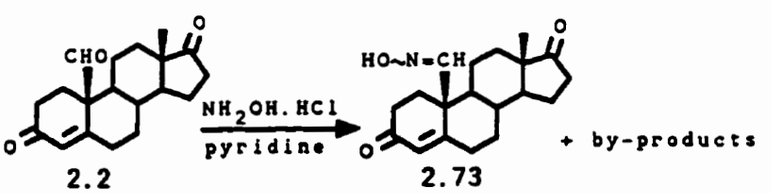


Scheme 2.37 Fragmentations involving the oxime functional group.

Lithium-ammonia reductions of 2,3-dialkyl-4-hydroxy-2-cyclopentanones have been shown to yield products having the 3-alkyl group and the 4-hydroxy group *trans* with a high degree of stereoselectivity (DeClereq *et al.*, 1974; Van Hulle *et al.*, 1973). The occurrence of β protonation *cis* to the oxygen function has been explained by a cation bridging factor. Elimination reactions can also be termed as fragmentation reactions. In general, a fragmentation, is the reverse of a construction, and a skeletal rearrangement is a reaction that combines a fragmentation with a construction. Many fragmentations have demanding stereoelectronic requirements, i.e. anticoplanar elimination orientation. Fragmentations specific to oximes are presented in Scheme 2.37. These fragmentation reactions produce pairs of functional groups (Hendrickson, 1986; Corey and Jorgensen, 1976; Eisele *et al.*, 1968).

Results and Discussion

The most direct method for preparing the androst-4-ene-3,17-dione 19-oxime derivative **2.73** (Scheme 2.38) is reaction of androst-4-ene-3,17-dione-19-al with hydroxylamine hydrochloride. While this reaction gave the desired 19-oxime **2.73** (41%; based on the ^1H NMR spectrum), as the major product, four additional by-products were also

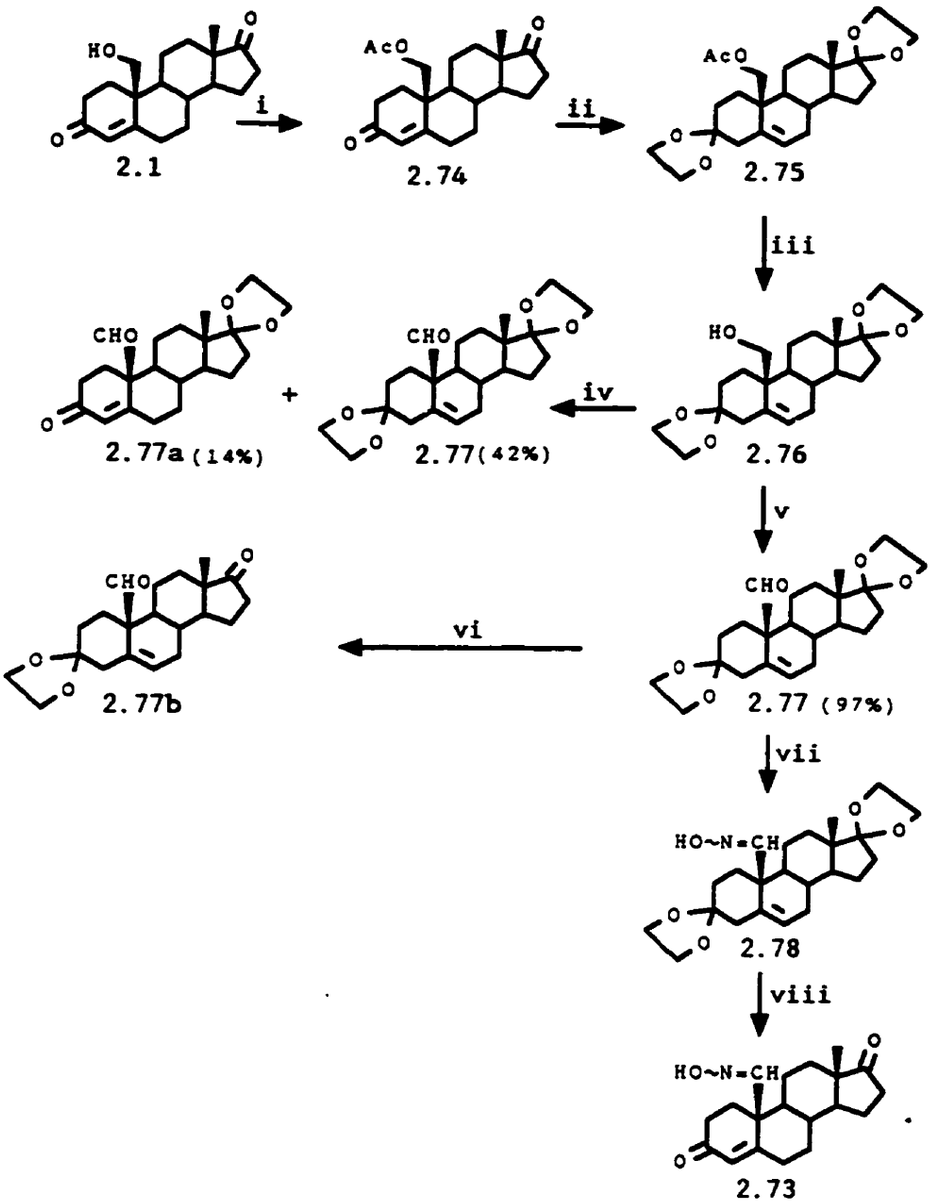


2.2
androst-4-ene-3,17-dion-19-al

2.73

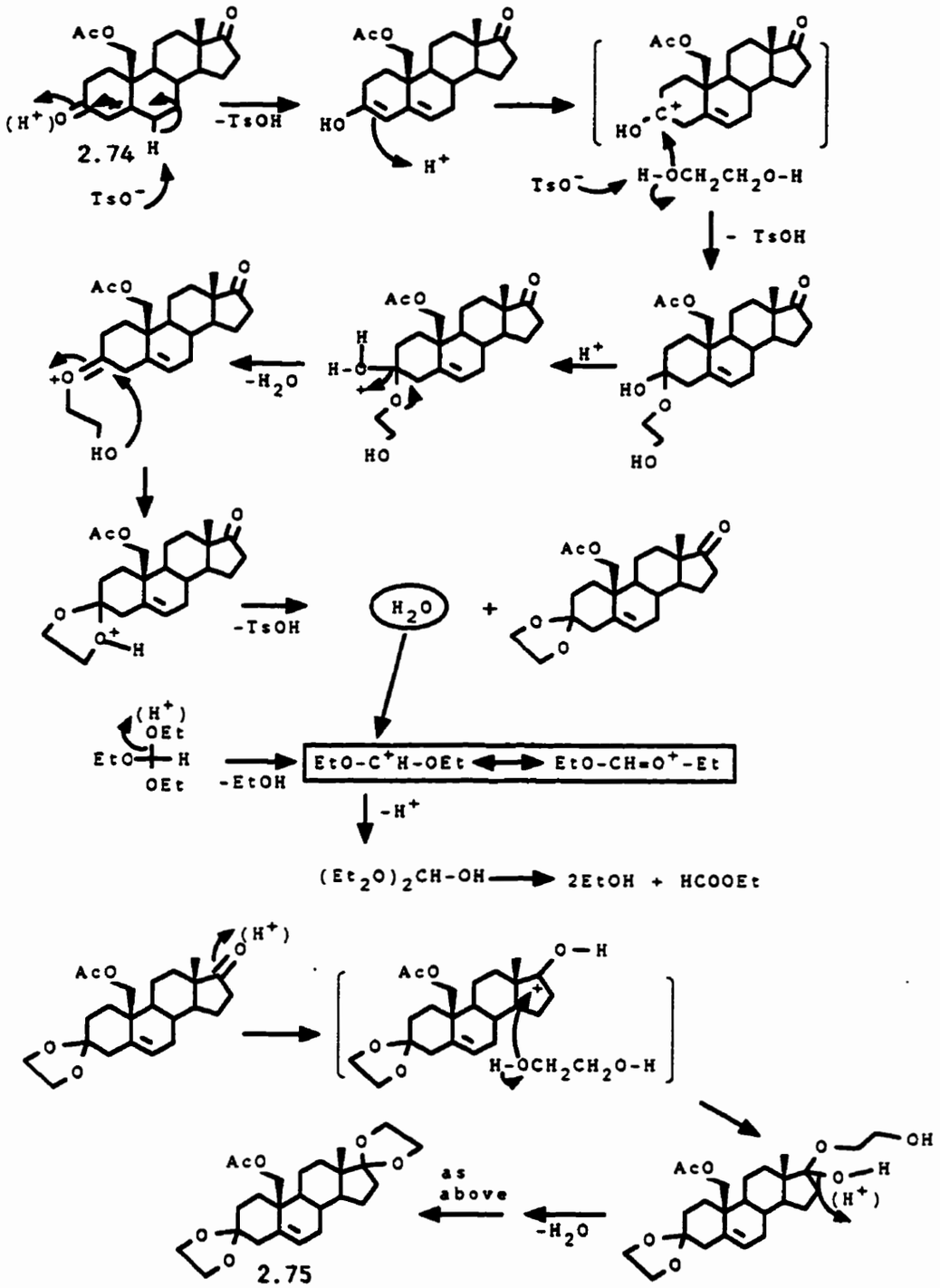
Scheme 2.38 Reaction of androst-4-ene-3,17-dion-19-al with $NH_2OH.HCl$.

observed by TLC. Because of the close proximity of the R_f values to androst-4-ene-3,17-dion-19-al 19-oxime **2.73** on TLC, no product was separated. Therefore, the target compound, the 19-oxime **2.73** was synthesized, in an overall yield of 29%, by a method reported by Lovett *et al.* (Scheme 2.39), (Lovett *et al.*, 1984). As outlined in Scheme 2.39, an intermediate, 3,17-bis(ethylenedioxy)-androst-5-en-19-al **2.77** was prepared in four steps from 19-hydroxyandrost-4-ene-3,17-dione **2.1** by modification of procedures described by Lovett (Lovett *et al.*, 1984). Three synthetic steps, ketalization (**2.74** → **2.75**), oxidation (**2.76** → **2.77**), and deprotection (**2.78** → **2.73**) have been improved (Scheme 2.39). Because direct ketalization of 19-hydroxyandrost-4-ene-3,17-dione **2.1**, was unsuccessful (Lovett *et al.*, 1984), the 19-hydroxyl group was protected as the 19-acetate (Knox *et al.*, 1965). Ketalization of 19-acetoxyandrost-4-ene-3,17-dione **2.74** was accomplished in 81% yield by treatment with 1,2-ethanediol and triethyl orthoformate in tetrahydrofuran at ambient temperature in the presence of a catalytic amount of *p*-toluenesulfonic acid (PTSA) giving solely **2.75**. Under these conditions the desired 3,17-bisketal **2.75** was obtained, without monoketal by-products. In general, ketalization of steroid 4-ene-3,17-dione derivatives by treatment with 1,2-ethanediol and p-toluenesulfonic acid, under reflux with the elimination of water (Dean-Stark apparatus) leads to a mixture of 3,17-diketals and 3-monoketals (*ca* di:mono; 4:1), (Djerassi, 1963). Due to the series of equilibria involved (e.g., TSOH + H_2O ⇌ TsO^- + H_3O^+), ketalization at C-17 is never complete, under the above conditions. As outlined in Scheme 2.40, the reaction with the orthoester can be described as a catalyzed formation of ketals from 1,2-ethanediol and ketone (MacKenzie and Stocker, 1955). The ethyl orthoformate plays the role of



Reagents: i, Ac_2O, DMAP, CH_2Cl_2; ii, $HOCH_2CH_2OH$, $(EtO)_3CH$, p-TsOH, THF; iii, 0.5M KOH, MeOH; iv, PDC; v, TPAP, NMO, CH_3CN/CH_2Cl_2; vi, 50% H_2O-CH_3COOH; vii, $NH_2OH.HCl$, pyridine; viii, p-TsOH, acetone.

Scheme 2.39 Synthesis of androst-4-en-3,17-dion-19-al 19-oxime **2.73** and the related compounds, **2.77**, **2.77a**, and **2.77b** [steps iii, iv, vii according to Lovett *et al.* (Lovett *et al.*, 1984)].



Scheme 2.40 Proposed mechanism of the 3,17-diketal **2.75** formation catalyzed by triethyl orthoformate and PTSA.

a dehydrating agent, as it reacts with acid and water. The proposed mechanism of diketalization is outlined in Scheme 2.40. A series of equilibria are involved, again stemming from the relatively facile formation of the key intermediate carbocations as shown in parentheses in Scheme 2.40. The water molecule is captured by the $EtOCH^{+}$-OEt cation and the equilibrium is driven over by the irreversible formation of ethyl formate. Carbocations of a type $ROCH^{+}$-OR are greatly stabilized by the resonance due to the presence of adjacent oxygen, bearing an unshared pair of electrons, as shown in a box in Scheme 2.40 (March, 1992; Pavlova *et al.*, 1986). Hydrolysis of 3,17-bis(ethylenedioxy)-androst-5-ene-19-yl acetate **2.75** in 0.5 M KOH-methanol gave 3,17-bis-(ethylenedioxy)-androst-5-en-19-ol **2.76** in a yield of 71%. Although the non-acidic reagent, pyridinium dichromate (PDC), as well as the acid-free solvent, CH_2Cl_2, were employed to oxidize 3,17-bis-(ethylenedioxy)-19-hydroxyandrost-5-ene **2.76** to the 19-aldehyde **2.77**, it did not prevent the formation of an undesired by-product, identified as 17-ethylenedioxyandrost-5-en-3-on-19-al **2.77a**. Modification of this step was performed by employing tetrapropylammonium perruthenate (TPAP) and N-methylmorpholine . N-oxide (NMO) as the oxidation reagents (Griffith and Ley, 1990; Oh and Robinson, 1994), giving a higher yield of 3,17-bis(ethylenedioxy)-androst-5-en-19-al **2.77** (97%). Reaction of 3,17-bis(ethylenedioxy)-androst-5-en-19-al **2.77** with hydroxylamine hydrochloride in pyridine at ambient temperature gave 3,17-bis(ethyl-enedioxy)androst-5-en-19-al 19-oxime **2.78**. The formation of the 19-oxime **2.78** is both general acid (HCl) and general base (pyridine) catalyzed. General acid activates the 19-aldehyde function. General base catalysis dehydration of the tetrahedral intermediate by nitrogen deprotonation resulted in elimination of hydroxide ion (Jencks, 1964;

Sayer, 1973). The attempted hydrolysis of **2.78** with sulfuric acid in dioxane (Lovett *et al.*, 1984) or with 5% $FeCl_3$ dispersed on silica in acetone (Nishikimi *et al.*, 1989) was not fully successful, because of the formation of by-products. Diketalization of **2.78** in acetone containing a catalytic amount of *p*-toluenesulfonic acid furnished one isomer exclusively of 19-oxime derivative **2.73** (95%). The stereochemistry of the 19-oxime was not determined. In Figure 2.16 are shown *syn* and *anti* structures of the 19-oximes calculated by the MMX force field.

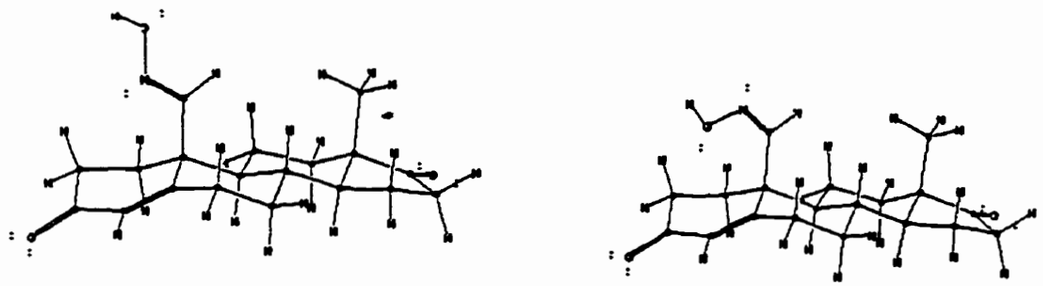
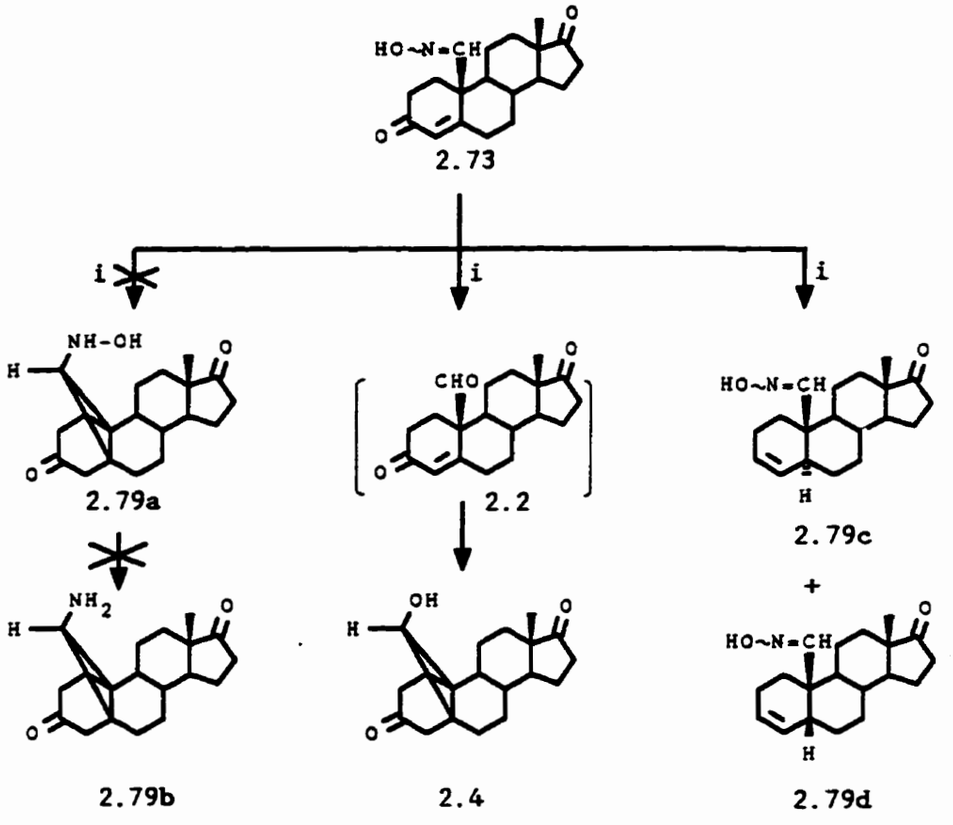


Figure 2.16 Molecular structure of *syn* and anti androst-4-ene-3,17-dion-19-al 19-oxime **2.79** determined by PCmodel (E_{syn} = 39.7 kcal mol^{-1}; E_{anti} = 41.9 kcal mol^{-1}; ΔE= 2.2 kcal mol^{-1}).

The next step in the proposed synthesis of 19(R/S)-amino-cycloandrostanedione **2.79b** (Scheme 2.41) was a reductive cyclization of **2.79** with zinc in 50% aqueous acetic acid. Since the reaction of the 19-oxime **2.79** with zinc in 50% aqueous acetic acid led to recovery of the starting material **2.79**, the reduction process was repeated with zinc in glacial acetic acid, because of different pH and thus the different reduction potentials involved. However, after stirring the

heterogenous mixture, at ambient temperature for one week, the reaction gave a mixture of the following products, **2.79c**, **2.79d**, and **2.4**, as determined by the ^{1}H NMR spectrum. The reaction failed to afford the desired intermediate 19(R/S)-hydroxyamino-5β,19-cycloandrostane-3,17-dione **2.79a**. The 19(R)-cyclopropanol **2.4** can be formed *via* the aldehyde intermediate **2.2** as shown in Scheme 2.41. No attempt was made to separate the products.

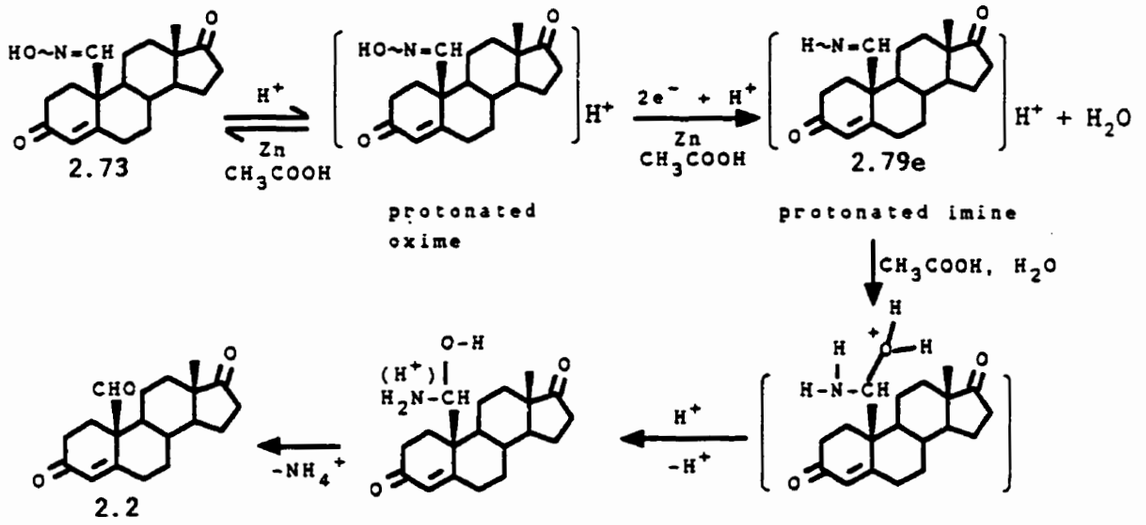


Reagents: *i*, Zn, CH_3COOH

Scheme 2.41 Products of reaction of androst-3-ene-3,19-dion-19-al 19-oxime **2.73** with zinc in glacial acetic acid.

A proposed mechanism of formation of the 19-aldehyde **2.2** *via* reductive deoximation of the 19-oxime **2.73** with zinc in acetic acid is

illustrated in Scheme 2.42. Compound 2.2 arises from reduction of the 19-oxime 2.73 to the 19-imine intermediate 2.79e, followed by its hydrolysis to the aldehyde 2.2. The C-N double bond of the imine is less stable to aqueous hydrolysis than the oxime. The stability of oximes can be attributed to the participation of the atom adjacent to the nitrogen in delocalized bonding according to the equation, RHC=N-OH ⇌ RHC^{-}-$N{=}OH^{+}$, therefore hydrolysis of oximes is more difficult and requires acid or basic catalysis (March, 1992b). A further reaction of the 19-aldehyde 2.2 with zinc in acetic acid furnishes the known 19(R)-cyclopropanol 2.4. The observed stereochemistry of the product in this reaction is the same as that described earlier for reductive cyclization of the 19-aldehyde 2.2 with zinc in 50% aqueous acetic acid (Scheme 2.3).

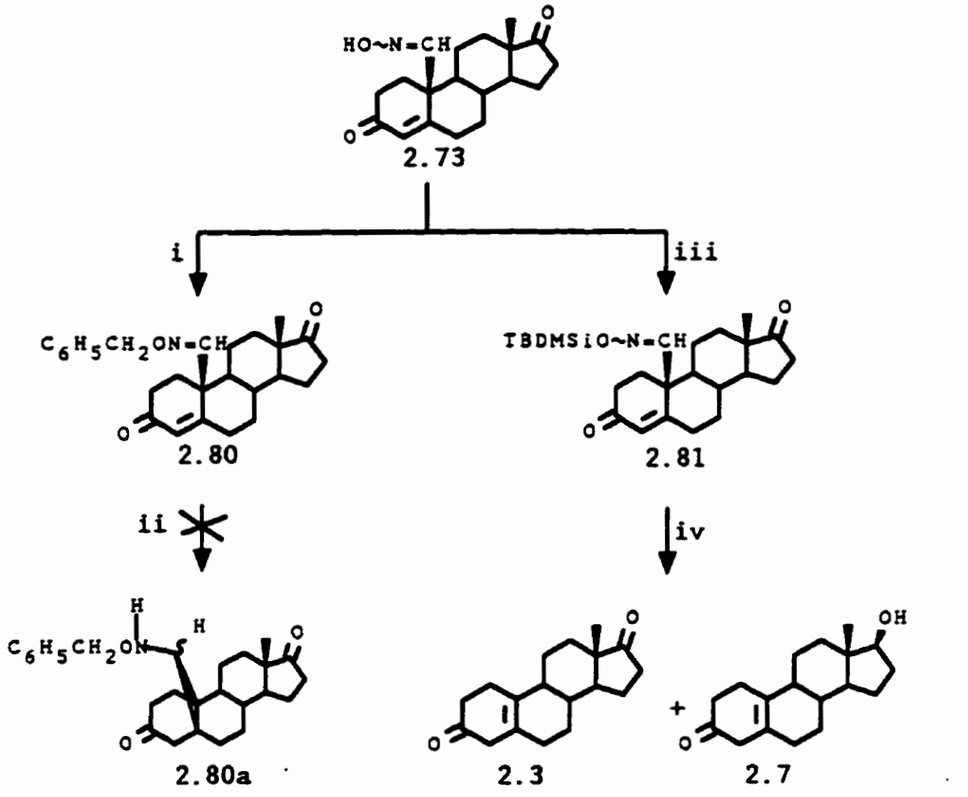


Scheme 2.42 Proposed mechanism of formation of the aldehyde 2.2 *via* reductive deoximation of the 19-oxime 2.73 with zinc in acetic acid.

Based on the products formed, a reduction process can be initiated either at the conjugated double bond or at the oxime function. When the reduction process is initiated at the conjugated double bond, it leads to the 3,4-unsaturated-5ξ 19-oxime derivatives, **2.79c** and **2.79d**, with removal of the 3-keto function (Scheme 2.41). On the other hand, when the reduction process is initiated at the 19-oxime function, it yields the 19-aldehyde intermediate. A similar transformation of testosterone 17β-propionate 3-oxime *via* a hydrolized ketimine to testosterone 17β-propionate was observed by polarographic reduction (Lundi, 1959).

More understanding about the mechanism of the reductive cyclization might be gained from studing the 19-oxime ethers based on their known ability to act as radical acceptors (Corey and Pyne, 1983). Oxime ethers have been utilized with ketyl, alkyl, and vinyl radicals (Corey and Pyne, 1983; Bartlett *et al.*, 1988; Enholm *et al.*, 1990; Pattenden and Schulz, 1993). For example, Bartlett *et al.* (Bartlett *et al.*, 1988) described successful conversion of carbohydrates to carbocyclic derivatives *via* an alkyl radical cyclization of *o*-benzyloximes ethers (aldoximes), when a radical was generated by tin hydride treatment of phenyl thionocarbonate. Provided that the cyclization reaction occurs *via* a radical intermediate, it was expected that the radical generated at C-5 would react with the 19-C-N double bond. The reactions investigated are outlined in Scheme 2.43. Two 19-oxime ether derivatives have been synthesized, androst-4-ene-3,17-dion-19-al 19-*O*-benzyloxime **2.80** and 19-TBDMSi-oxime **2.43**. The 19-oxime androstane-3,17-dion-19-al 19-oxime **2.73** was directly converted into the *o*-benzyloxime **2.80** by reaction with potassium hydride and benzyl bromide in dimethoxyethane. However, the 19-*o*-benzyloxime **2.80** upon

treatment with Zn in 50% aqueous acetic acid led to recovery of the starting material **2.73**. Therefore, a reduction process was planned by employing Li-NH_3. However, *o*-benzyloxime protecting groups, similarly to benzyl ethers, are cleaved upon treatment with Li-NH_3. Therefore,

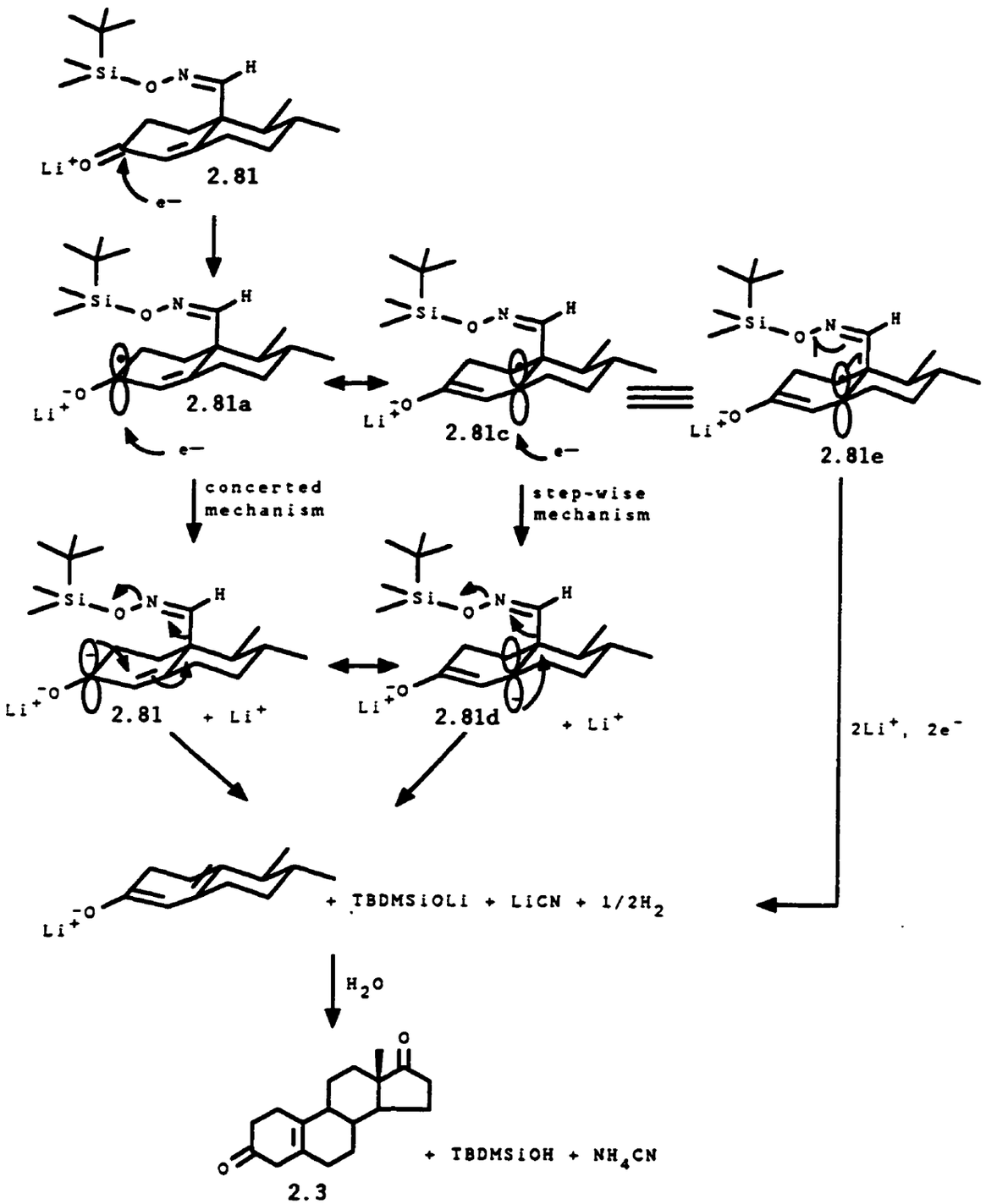


Reagents: **i**, benzyl bromide, KH, glyme; **ii**, zn, 50% aqueous CH_3COOH; **iii**, TBDMSiCl, iPr_2EtN, DMF; **iv**, Li-NH_3-THF, NH_4Cl.

Scheme 2.43 Reactions of the 19-benzyl-oxime **2.80** with Zn in 50% aqueous CH_3COOH and the 19-TBDMSi-oxime **2.81** with Li-NH_3.

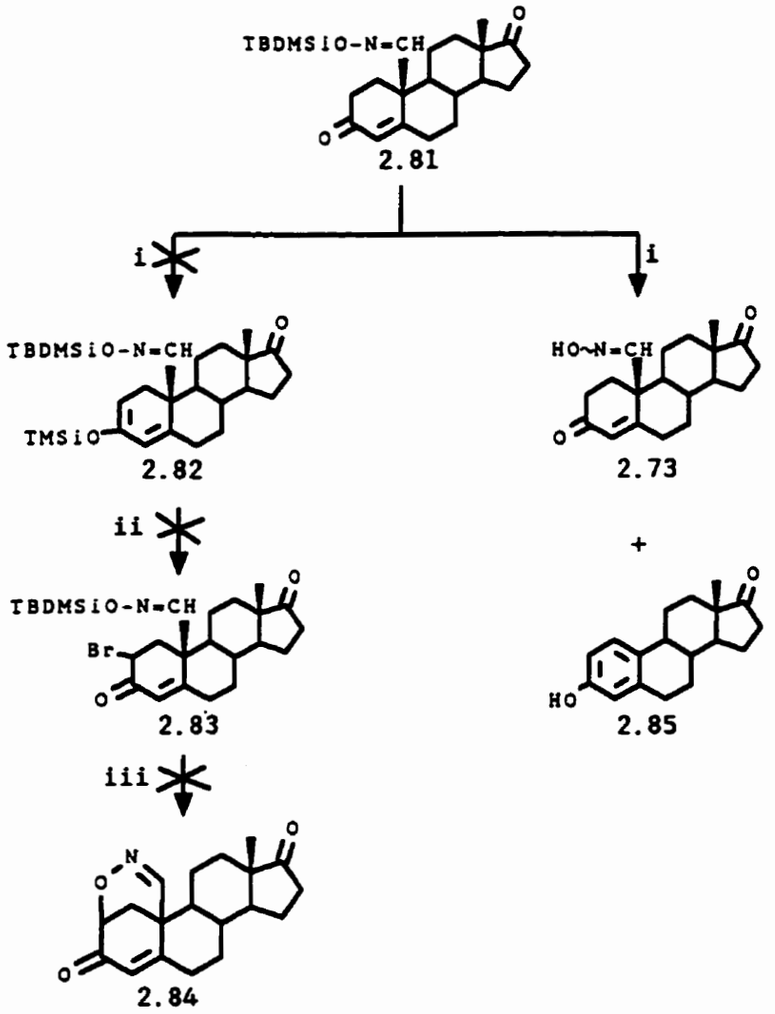
a different 19-oxime ether derivative was required to perform the reaction and accordingly the 19-TBDMSi-oxime **2.81** was prepared by

treatment of the 19-oxime **2.73** with *tert*-butyldimethylsilyl chloride and di-isopropyl ethyl amine in DMF at ambient temperature. Interestingly, attempt to derivatize the 19-oxime **2.73** by treatment with neat TMSi-imidazole, or KH and Me_3SiCl in glyme was not successful. Even with a more powerful reagent than TMSiOTf, trimethylsilyl nonafluoro-1-butanesulfonate, $CF_3CF_2CF_2CF_2SO_2SiMe_3$ (Vorbrüggen and Krolikiewicz, 1979) and *i*-Pr_2EtN in CH_2Cl_2, the reaction was sluggish and not clean, showing two spots on TLC. No attempt was made to identify the compounds. Exposure of the 19-TBDMSi-oxime **2.81** to lithium metal in liquid ammonia at -78 °C led to fission of the C-10-C-19 bond and elimination of the C-10 substituent, to yield two β,γ-unsaturated products, estr-5(10)-ene-3,17-dione **2.3** and 17β-hydroxy estr-5(10)-en-3-one **2.7**. A proposed mechanism of elimination and fragmentation of the 10-CH=N-OTBDMSi substituent is outlined in Scheme 2.44. Although a nucleophilic attack on the C-10 substituent is shown (**2.81a-2.81d**), the reaction may also proceed *via* a radical mechanism (**2.81e**). The intramolecular elimination of the 10-oxime substituent might take place at the dianion (**2.81a** or **2.81d**) or the radical **2.81e** stage of the reaction. There are some factors that can be taken under consideration to explain why cyclization did not take place. The β-position of a radical anion such as **2.81e**, although possibly sufficiently nucleophilic to take part in an intramolecular elimination might not be nucleophilic enough to form a cyclic compound. Cyclization either from **2.81b** or **2.81e**, presumably, requires stereochemical preferences such as approach of the attacking species (radical or nucleophile) at 109° angle to the C-N double bond. However, such an approach can be disfavored because of hindered rotation by the bulky substituent at C-19.



Scheme 2.44 Proposed mechanisms of reduction of androst-4-ene-3,17-dion-19-al 17-TBDMSi-oxime **2.81** with Li in NH_3 via either nucleophiles (**2.81a-2.82d**) or a radical **2.81e**.

On the other hand, the rate of cyclization or elimination might be different. There seems to be no strong evidence to allow one to decide which of two possible eliminations, the anion **2.81e** or the dianion **2.81d**, might be involved in this reaction, but **2.81d** prevails based on the reported fragmentations discussed in the introduction on page 198.



Reagents: i, TMSiOTf, iPr_2EtN; and proposed reagents ii, NBS, THF; iii, $n\text{-}Bu_4NF$.

Scheme 2.45 Attempted synthesis of compound **2.84**.

Another attempt to synthesize a cyclic compound, **2.84**, considered to be a potential aromatase inhibitor, is shown in Scheme 2.45. The reaction was expected to proceed *via* bromination of the TMSi-enol ether **2.82**, followed by a nucleophilic displacement of bromine. However, treatment of the TBDMSi-oxime **2.81**, with TMSiOTf and *i*-Pr_2EtN in CH_2Cl_2 at -78 °C, did not give the expected silyl enol ether **2.82** but instead yielded, on FCC, an unseparable mixture which was composed of the 19-oxime **2.73** and estrone **2.85** (12:15; 20:80) as determined by the ^1H NMR spectrum. As outlined in Scheme **2.45**, the fact that estrone was formed indicates that the TMSiOTf reagent, considered also as a Lewis acid, reacted with oxygen, presumably, leading to the elimination of $(TMSi)_2O$, a molecule of HCN, and a proton from C-1. A similar mechanism could operate at the aromatase active site. This mechanism would explain the lack of inhibitory properties of androst-4-ene-3,17-dione 19-oxime (Lovett *et al.*, 1984). On the other hand, production of compound **2.41** implies that the interaction of the silyl reagent with nitrogen is at the expense of the Si-O bond.

2.4.1 Summary

1. 3,17-Diketal **2.75** (81%) has been synthesized by a modified procedure using ethyl orthoformate, 1,2-ethanediol and PTSA at ambient temperature.
2. 3,17-Bis(ethylenedioxy)-androst-5-en-19-al **2.77** (97%) has been synthesized with highly improved yield by employing *tetra*-propyl-ammonium perruthenate (TPAP) and N-methylmorpholine N-oxide (NMO) as oxidants.
3. Selective deprotection of 3,17-bis(ethylenedioxy)-androst-5-en-19-al **2.77b**, at C-17, with 50% aqueous acetic acid has been

developed.

4. Zinc-acetic acid reduction of compound **2.73** (Scheme 2.41), having the 4,5-conjugated double bond *versus* the 19-oxime function led to: (i) known 19(R)-hydroxy-5β,19-androstane-3,17-dione **2.4** (see above); and (ii) the isomeric 3,4-unsaturated 19-oxime derivatives **2.79c** and **2.79d**.

5. Li-NH_3 reduction of androst-4-ene-3,17-dione 19-TBDMSi-oxime **2.81** led to a heterolytic fission of the C-10-C-19 bond and elimination of the γ-substituent to give the β,γ-unsaturated products **2.3** and **2.7**. A new reaction, i.e. a reductive fragmentation of the conjugated keto δ-ether aldoxime **2.81**, has been developed which can be added to the list of oxime fragmentations. Furthermore, based on the experimental results, it is proposed that analogous steroid compounds, i.e. with the 19-oxime function (protected or not) and a 1,2-double bond and 5α, 5β, or 5-ene could be used as precursors for the preparation of steroid derivatives with C-1-C-10 unsaturation. Compounds with 1,2-unsaturation have not been synthesized and subjected to biological evaluation. Furthermore, steroid derivatives with C-1-C-10 unsaturation could be employed as precursors of the halogen substituted cyclopropyl derivatives (Scheme 1.33, **C** and **D**).

2.4.2 Experimental

For Schemes 2.38 and 2.39

Androst-4-ene-3,17-dion-19-al 19-oxime 2.73

(a) From androst-4-en-3,17-dion-19-al **2.2**

$NH_2OH.HCl$ (685 mg, 9.85, 1.5 eqv.) was dissolved in pyridine (25 mL)

and the 19-aldehyde **2.2** (2.031 g, 6.76 mmol) added. The mixture was stirred for 5 days. Although TLC showed the presence of a small amount of non-reacted starting material, the reaction was terminated. The mixture was cooled in ice-water bath and cold water (500 mL) added. The precipitated products were collected on a filter paper, washed with water, dried in a desiccator over P_2O_5, under reduced pressure to give a crude product (1.40 g). The ^{1}NMR spectrum showed the presence of at least four compounds. The desired 19-oxime **2.73** was produced in 41% (estimated based on the ^{1}H NMR spectrum). No attempt was made to separate the products.

(b) From 3,17-bis(ethylenedioxy)-androst-5-en-19-al **2.73**:

p-Toluenesulfonic acid monohydrate (0.508 g, 2.67 mmol) was added to a solution of 3,17-bis(ethylenedioxy)-androst-5-en-19-al 19-oxime **2.78** (0.607 g, 1.5 mmol) in acetone and the mixture stirred at room temperature for 24 hr. Saturated sodium bicarbonate solution was added slowly to neutralize the acid. The product was extracted with CH_2Cl_2, and the organic layer washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to give a white foamy residue (0.486 g), which after crystallization gave white needles of the 19-oxime **2.73** (0.450 g, 1.43 mmol, 95%), mp 207-209°C (from EtOAc-acetone-hexanes), (lit., mp 204-207.5°C, from EtOAc-hexane; Lovett *et al.*, 1984).

^{1}H NMR of **2.73** ($CDCl_3$): δ 7.82 (br s, 1H, 19-CH=N-O<u>H</u>), 7.58 (s, 1H, 19-C<u>H</u>=NOH), 5.91 (s, 1H, 4-H), 0.89 (s, 3H, 13-Me).

^{13}C NMR of **2.73** ($CDCl_3$): δ 31.21 (1), 32.94 (2), 198.98 (3), 126.48 (4), 163.74 (5), 33.89 (6), 31.95 (7), 35.95 (8), 50.82 (9), 44.95 (10), 20.62 (11), 30.69 (12), 47.32 (13), 53.82 (14), 21.69 (15), 35.69 (16), 220.02 (17), 13.69 (18), 150.32 (19).

For Scheme 2.39

19-Acetoxyandrost-4-ene-3,17-dione 2.74

To 19-hydroxyandrost-4-ene-3,17-dione **2.1** (11.2 g, 0.037 mol) in CH_2Cl_2 (250 mL) was added Ac_2O (70 mL, 0.74 mol, 20 eq.) followed by DMAP (0.450 g, 0.0037 mol, 0.1 eq.) and the mixture stirred at room temperature for 1 hr when TLC indicated that the reaction was complete. Next, methanol was added to destroy excess reagent and then, after 1 hr of stirring, the organic layer was washed with water, saturated sodium bicarbonate, water, dried over Na_2SO_4, and evaporated under reduced pressure to give a foamy residue of **2** (13.1 g), which indicated one product on TLC and in the ^1H NMR spectrum. Compound **2.74** was not crystalline, in agreement with the literature (Joska and Fajkos, 1982) and was used directly in the following reaction.

^1H NMR of **2.74** ($CDCl_3$): δ 5.93 (d, J 17.17 Hz, 1H, 4-H), 4.68 (dd, J_{AB} 11.3, 1.2 Hz, 1H, 19-H), 4.18 (d, J_{AB} 11.3 Hz, 1H, 19-H), 2.02 (s, 3H, 19-$COCH_3$), 0.92 (s, 3H, 13-Me).

^{13}C NMR of **2.74** ($CDCl_3$): δ 32.84 (1), 34.51 (2), 198.99 (3), 126.87 (4), 164.82 (5), 33.49 (6), 31.53 (7), 35.67 (8), 54.02 (9), 41.82 (10), 20.78 (11), 30.85 (12), 47.41 (13), 51.09 (14), 21.57 (15), 35.56 (16), 219.64 (17), 13.73 (18), 66.49 (19), 170.67 (19-O<u>C</u>OCH$_3$), 20.65 (19-OCO<u>C</u>H$_3$).

3,17-Bis(ethylenedioxy)-androst-5-en-19-ol 19-acetate 2.75

A mixture of 19-acetoxyandrost-4-ene-3,17-dione (**2.74**; 12.6 g, 0.037 mol), 1,2-ethanediol (40 mL, 0.72 mol), triethyl orthoformate (60 mL, 0.36 mol), and *p*-toluenesulfonic acid (0.6 g) was stirred in tetrahydrofuran (300 mL), in a stoppered flask, at ambient temperature for 23 hr. Pyridine (20 mL) was then added and the solution concentrated at 30-35 °C on a rotatory evaporator until a pasty, cream

coloured crystalline mass remained. After extraction with EtOAc (3 x 100 mL), the organic layer was washed with water. The water layer was extracted with CH_2Cl_2. The combined extracts were washed with water, saturated sodium bicarbonate, dried with Na_2SO_4, and evaporated to give an oily residue, which after FCC, on elution with 7-10% acetone-LP, gave a white foamy residue of **2.75** (13.0 g, 0.030 mol, 81%), mp 94-97 °C (from CH_2Cl_2-LP), (lit., mp 90.5-95 °C, from hexane, Lovett *et al.*, 1984).

^{1}H NMR of **2.75** ($CDCl_3$): δ 5.57 (br, m, 1H, 5-H), 4.45 (d, J_{AB} 11.8 Hz, 1H, 19-H), 3.90 (m, 9H, C_3 and C_{17}-OCH_2CH_2O + 19-H), 2.58 (dd, J_{AB} 14.25, 2.3 Hz, 1H, 4-H), 2.15. (d, J_{AB} 14.33 Hz, 1H, 4-H), 2.026 (s, 3H, 19-CH_3CO), 0.852 (s, 3H, 13-Me).

^{13}C NMR of **2.75** ($CDCl_3$): δ 30.73 (1), 32.76 (2), 108.95 (3), 41.96 (4), 134.93 (5), 125.82 (6), 31.33 (7), 33.23 (8), 49.53 (9), 39.75 (10), 21.06 (11), 30.56 (12), 45.82 (13), 50.99 (14), 22.68 (15), 34.16 (16), 119.28 (17), 14.26 (18), 65.13 (19) 21.06 (19-$\underline{C}H_3COO$), 170.61 (19-$CH_3\underline{C}OO$), 64.24 + 64.41 (3-OCH_2CH_2O-), 64.52 + 64.57 (17-OCH_2CH_2O-).

19-Hydroxy-3,17-bis(ethylenedioxy)-androst-5-ene 2.76

19-Acetoxy-3,17-bis(ethylenedioxy)-androst-5-ene **2.75** (12.76 g, 0.0295 mol) was dissolved in 5% KOH in methanol (120 mL) and stirred at room temperature for 18 hr, when no starting material was detected by TLC. The mixture was diluted with CH_2Cl_2 (500 mL) and the organic layer washed with water, dried over Na_2SO_4, filtered, and evaporated. Recrystallization of the crude product **2.76** from acetone-hexanes afforded pure 3,17-bis(ethylenedioxy)-19-hydroxyandrost-5-ene **2.76** (8.21 g, 71%), mp 206-209 °C (from acetone-hexanes), (lit., mp 202.5-204 °C, from acetone-hexane, Lovett *et al.*, 1984).

^{1}H NMR of **2.76** ($CDCl_3$): δ 5.72 (m, 1H, 5-H), 3.90 (m, 9H, C_3 and C_{17}

OCH_2CH_2O + 19-H), 3.71 (?), 2.52 (ddd, J 14.05, 8.26, 2.53 Hz 1H, 4-H), 2.19 (d, J 14.06 Hz, 1H, 4-H).

^{13}C NMR of **2.76** ($CDCl_3$): δ 31.46 (1), 32.40 (2), 109.06 (3), 41.91 (4), 134.95 (5), 127.32 (6), 30.90 (7), 33.70 (8), 49.72 (9), 41.75 (10), 21.20 (11), 30.48 (12), 45.98 (13), 51.34 (14), 22.62 (15), 34.25 (16), 119.41 (17), 14.62 (18), 65.14 (19), 62.59+ 64.29 (3-OCH_2CH_2O-), 64.49 + 64.55 [17-(-OCH_2)$_2$].

3,17-Bis(ethylenedioxy)-androst-5-en-19-al 2.77 and 17-Ethylenedioxy-androst-4-en-3-on-19-al 2.77a

Oxidation with PDC.

To a stirred solution of the 19-alcohol **2.76** (2.61 g, 6.68 mmol) in CH_2Cl_2 (30 mL), at ambient temperature, was added pyridinium dichromate (PDC), (3.77 g, 10.0 mmol), (Corey and Schmidt, 1979) and stirring continued for 18 h, when no starting material was detected by TLC. The mixture was diluted with diethyl ether (15 mL) and filtered through a pad of silica. The organic filtrate was washed with water, brine, dried over Na_2SO_4, filtered, and evaporated to give a white foamy residue, which on FCC, on elution with 10% acetone-LP, gave fractions of the 19-aldehyde **2.77** (1.1 g, 2.8 mmol, 42%), mp 165-168 °C (from acetone-LP), (lit., 168.2-172 °C, from acetone-hexane, Lovett *et al.*, 1984) and 17-ethylenedioxy-androst-4-en-3-on-19-al **2.77a** (330 mg, 0.96 mmol, 14%), mp 130-131 °C (from CH_2Cl_2-EtOAc-LP), (Found: C, 72.34; H, 8.51. $C_{21}H_{28}O_4$. 1/4 H_2O (MW 348.958) required C, 72.28; H, 8.23%).

Oxidation with TPAP, NMO.

To a solution of the 19-alcohol **2.76** (6.94 g, 0.018 mol) in CH_3CN-CH_2Cl_2 (1:9) were added N-methylmorpholine N-oxide (NMO) (3.14 g, 0.027 mol, 1.5 eq,) and activated molecular sieves 4 A (3.5 g, powdered) to absorb crystallization water from NMO. The mixture was stirred for 20

min. under argon, and then solid *tetra*-propylperruthenium (TPAP, 311 mg, 0.80 mmol, 0.05 eq.) added in one portion. The reaction mixture was stirred for 1 hr at room temperature under a positive pressure of argon. The reaction mixture was then evaporated to dryness under reduced pressure, the residue taken up in a minimum amount of CH_2Cl_2, and the solution filtered through a pad of silica with excess EtOAc. The filtrate was evaporated under reduced pressure to afford a white solid product **2.77** (6.67 g, 0.0171 mol, 97%), mp 165-168 °C (from acetone-LP), (lit., mp 168.5-172 °C, from acetone-hexane, Lovett *et al.*, 1984).

^{1}H NMR of **2.77** ($CDCl_3$): δ 9.68 (d, J = 0.004 Hz, 1H, 19-H), 5.83 (br m, 1H, 5-H), 3.89 (m, 8H, 3-OCH_2CH_2O and 17-OCH_2CH_2O), 0.80 (s, 3H, 18-CH_3).

(lit., Lovett *et al.*, 1984 (360 MHz, $CDCl_3$): δ 0.785 (s, 3H, 18-CH_3), 3.874 (m, 8 H, C_3 and C_{17} OCH_2CH_2O), 5.814 (br d, J = 5.84 Hz, 1 H, vinyl), 9.663 (s, 1 H, CHO).

^{13}C NMR of **2.77** ($CDCl_3$): δ 31.95 (1), 30.54 (2), 108.74 (3), 142.98 (4) 131.96 (5),127.60 (6), 30.54 (7), 33.34 (8), 48.26 (9), 45.62 (10), 21.74 (11), 30.54 (12), 53.66 (13), 50.33 (14), 22.48 (15), 34.10 (16), 119.11 (17), 14.15 (18), 204.80 (19), 64.32 + 64.53 (3-OCH_2CH_2O-), 64.56 + 65.20 (17-OCH_2CH_2O-).

^{1}H NMR of **2.77a** ($CDCl_3$): 9.93 (s, 1H, 19-CHO), 5.95 (d, J 1.74, 1H, 4-H), 3.89-3.83 (m, 4H, 3-OCH_2CH_2O-), 0.85 (s, 3H, 13-Me).

^{13}C NMR of **2.77a** ($CDCl_3$): 34.04 (1), 29.58 (2), 197.99 (3), 127.32 (4) 161.23 (5), 33.97 (6), 30.86 (7), 37.33 (8), 53.62 (9), 55.27 (10), 21.38 (11), 30.49 (12), 45.58 (13), 49.91 (14), 22.47 (15), 34.31 (16), 118.89 (17), 14.26 (18), 201.15 (19), 64.58 + 65.29 (17-OCH_2CH_2O-).

3-ethylenedioxy-androst-5-en-17-on-19-al 2.77b

Selective deprotection at C-17:

A solution of compound **2.77** (220 mg, 0.64 mmol) in THF (1 mL) was treated with a mixture of 50% aqueous acetic acid (20 mL) at 30-40 °C for 3 h, when no starting material was detected by TLC. The mixture was poured into CH_2Cl_2 (50 mL), and the organic layer washed with water, saturated aqueous $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated to give **2.77b** (190 mg, 0.55 mmol, 86%), mp 164-166 °C (from EtOAc-LP), (Found: C, 72.98; H, 8.31. $C_{21}H_{28}O_4$ (MW 344.455) requires C, 73.23; H, 8.19%).

^{1}H NMR of 2.77b ($CDCl_3$): δ 9.71 (d, J 1.26, 1H, 19-CHO), 5.86 (d, J 1H, 6H), 3.95-3.92 (m, 4H, 3-OCH_2CH_2O-), 0.83 (s, 3H, 13-Me).

^{13}C NMR of 2.77b ($CDCl_3$): δ 30.08 (1), 31.08 (2), 108.59 (3), 42.09 (4), 132.38 (5), 127.01 (6), 31.46 (7), 32.54 (8), 48.58 (9), 47.36 (10), 21.50 (11), 29.64 (12), 53.58 (13), 51.50 (14), 21.64 (15), 35.64 (16), 219.97 (17),13.55 (18), 204.49 (19), 64.38, 64.54 (3-OCH_2CH_2O-)

3,17-Bis(ethylenedioxy)-androst-5-en-19-al oxime 2.78

A solution of 3,17-bis(ethylenedioxy)-androst-5-en-19-al **2.77** (0.734 g, 1.88 mmol) and hydroxylamine hydrochloride (0.239 g, 3.44 mmol, 1.8 eq) in pyridine (10 mL) was stirred under argon at ambient temperature for 24 hr. On chilling the mixture in an ice bath followed by slow addition of 75 mL of cold water, the 19-oxime **2.78** precipitated. The resulting white solid was collected by filtration, washed thoroughly with water, and dried in a desiccator at room temperature over P_2O_5 under reduced pressure to give a solid residue **2.78** (0.667 g, 1.65 mmol). Recrystallization from acetone-hexanes and EtOAc gave white needles of **2.78** (0.650 g, 1.61 mmol, 86%), mp 212-215 °C (from acetone-LP), (lit., mp 210.5-214 °C, from acetone-hexane, Lovett *et al.*,

1984)

^{1}H NMR of 2.78 ($CDCl_3$): δ 7.42 (s, 1H, HON=CH), 7.35 (s, HON=CH), 5.62 (m, 1H, 6-H), 3.90 (m, 8H, C_3 and C_{17}-OCH_2CH_2O), 0.81 (s, 3H, 13-Me)

^{13}C NMR of 2.78 ($CDCl_3$): δ 31.78 (1), 32.93 (2), 109.12 (3), 42.29 (4), 135.21 (5) 124.87 (6), 30.77 (7), 32.40 (8), 49.39 (9), 43.66 (10), 21.35 (11), 30.77 (12), 45.60 (13), 49.99 (14), 22.65 (15), 34.15 (16), 119.30 (17), 14.15 (18), 154.93 (19), 64.28 + 64.49 + 64.52 + 65.17 (3- and 17-OCH_2CH_2O-)

For Scheme 2.43

Androst-4-ene-3,17-dion-19-al 19-*o*-Benzyloxime 2.80

(1) Potasium hydride 35% dispersion in mineral oil (200 mg), was washed with light petroleum ether (2 mL) five times and then stored under LP. When required, a small amount of the oil-free potasium hydride (KH) was dried under a stream of argon.

(2) To a suspension of the above KH (19 mg, 0.47 mmol, 1.6 eq.) in dry DME (1.5 mL) in an ice-water bath was added the 19-oxime **2.73** (96 mg, 0.30 mmol). After stirring the reaction mixture for 10 min. under an argon atmosphere, benzyl bromide (60 μL, 0.50 mmol, 1.6 eq.) was added. The ice-water bath was removed and the reaction mixture stirred for 4 h, when no starting material was detected by TLC. DME was removed under reduced pressure, and an oily residue dissolved in dichloromethane (20 mL). The organic layer was washed with water, saturated aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated, to give a glassy yellow residue, which on FCC, on elution with 10% acetone-LP, afforded fractions of **2.80** (70 mg, 0.17 mmol, 57%), mp 140-143 °C (from CH_2Cl_2-EtOAc). (Found: C, 77.18; H, 7.87; N, 3.41. $C_{26}H_{31}NO_3$ (MW 405.544) requires: C, 77.00; H, 7.70; N, 3.45).

^{1}H NMR of 2.80 ($CDCl_3$): 0 7.55 (s, 1H 19-$C_6H_5CH_2ON$=C<u>H</u>), 5.88 (s, 1H, 4-H), 5.07 (s, 2H, 19-$C_6H_5C\underline{H}_2ON$=CH), 7.32-7.26 (m, 5H, 19-$C_6\underline{H}_5CH_2$-ON=CH), 0.79 (s, 3H, 13-Me).

^{13}C NMR of 2.80 ($CDCl_3$): 31.26 (1), 32.85 (2), 199.69 (3), 126.30 (4), 163.49 (5), 33.93 (6), 31.96 (7), 35.71 (8), 50.74 (9), 45.02 (10), 20.54 (11), 30.59 (12), 47.26 (13), 53.76 (14), 21.63 (15), 35.64 (16), 219.89 (17), 13.64 (18), 149.490 (19).

19-*tert*-Butyldimethysilyl androst-4-ene-3,17-dion-19-al oxime 2.81

To a solution of the 19-oxime **2.73** (788 mg, 2.5 mmol) and *i*-Pr_2NEt (500 μmL, 2.87 mmol) in DMF (14 mL) was added solid TBDMSiCl (1.01 g, 6.7 mmol). The mixture was stirred for 3 h under an argon atmosphere when no starting material was detected by TLC. The mixture was poured into cold diethyl ether (30 mL), and the organic layer washed with cold water, brine, dried over Na_2SO_4, filtered, and evaporated to give a brown oily residue, which on FCC, on elution with 50% Et_2O-LP, afforded the 19-TBDMSi-oxime **2.81** (0.630 mg, 1.5 mmol, 34%), mp 144-146 °C (from CH_2Cl_2-methanol). (Found: C, 69.88; H, 9.15; N, 3.26. $C_{25}H_{39}NO_3Si$ (MW 429.681) requires: C, 69.94; H, 9.19; N, 3.09).

^{1}H NMR of 2.81 ($CDCl_3$): 7.65 (s, 1H, 19-TBDMSi-N=C<u>H</u>), 5.87 (s, 1H, 4-H), 0.91 (s, 9H, CMe_3), 0.88 (s, 3H, 13-Me), 0.13 (s, 3H, $SiMe_2$), 0.14 (s, 3H, $SiMe_2$)

^{13}C NMR of 2.81 ($CDCl_3$): 31.25 (1), 32.91 (2), 198.72 (3), 126.30 (4), 165.59 (5), 34.02 (6), 32.08 (7), 35.93 (8), 50.82 (9), 45.14 (10), 20.66 (11), 30.62 (12), 47.29 (13), 53.94 (14), 21.68 (15), 35.65 (16), 219.80 (17), 13.71 (18), 153.42 (19)

Estr-5(10)-ene-3,17-dione 2.3 and 17β-hydroxyestr-5(10)-en-3-one 2.7

A solution of the 19-TBDMSi-oxime **2.81** (149 mg, 0.307 mmol) in tetrahydrofuran (3 mL) was added over a period of 10 min to a stirred

mixture of liquid ammonia (50 mL), dissolved lithium metal (200 mg, 18 mmoL), and THF (7 mL). Stirring was continued for a further 1 h at which time solid NH_4Cl (5 g, 93 mmol) was added, followed by CH_2Cl_2. Evaporation of ammonia yielded an organic solution, which was washed with water, dried over Na_2SO_4, filtered, and evaporated to give an oily residue (140 mg), which on FCC, on elution with 15% acetone-LP, yielded fractions of estr-5(10)-ene-3,17-dione **2.3** (38 mg, 0.14 mmol, 46%), mp 147-150 °C (from acetone-LP), (lit., mp 144-146 °C; Ueberwasser *et al.*, 1963) and 17β-hydroxyestr-5(10)-en-3-one **2.7** (30 mg, 0.11 mmol, 40%), mp 194-196 °C (from acetone-LP), (lit., mp 193-196 °C; Wilds and Nelson, 1953).

For Scheme 2.45

Androst-4-ene-3,17-dione 19-oxime 2.73 and **Estrone 2.85**

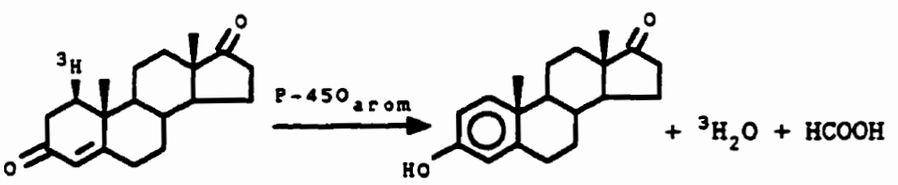
To a cooled (solid CO_2-acetone) solution of TMSiOTf (420 μL, 2.2 mmol, 4 eq.), *i*-Pr_2EtN (130 μL, 0.77 mmol, 1.4 eq), and powdered molecular sieves 4A (66 mg) in CH_2Cl_2 (8 mL) was added, by a syringe, a solution of androst-4-ene-3,17-dione 19-TBDMSioxime **2.81** (250 mg, 0.55 mmol) in CH_2Cl_2 (2.5 mL) over a period of 40 min, under an argon atmosphere. The mixture was stirred for 1.5 h, and the organic layer washed with brine, dried over Na_2SO_4, filtered, and evaporated to give an brown oily residue (400 mg), which on FCC, on elution with 15% acetone-LP, gave a mixture of the 19-oxime **2.73** and estrone **2.85** (30 mg).

^{1}H NMR of 2.85 ($CDCl_3$): δ 7.15 (d, J 8.4, 1-H), (6.64, J 2.7, 8.4, 2-H), 6.58 (d, J 2.62, 4-H), 4.75 (s, 3-OH), 2.85 (m, 6-H), 2.51 (dd, 8.2, 18.3, 16β-H), 0.91 13-Me.

2.5.0 Aromatase Inhibition Activity

Inhibition activity assay for aromatase was carried out in collaboration with Dr. A.H.M. Brodie and Professor Y. Ling in the Department of Pharmacology, School of Medicine, University of Maryland, Baltimore, USA.

Microsomes from human placenta were used as the enzyme source. Aromatase activity was tested by measuring the release of ^3H_2O from [1β-^{3}H]androstenedione (specific activity 15-30 Ci/mmol) according to modified method (Tochigi and Osawa, 1986) of Thompson and Siiteri (Thompson and Siiteri, 1974) (Scheme 2.46). All incubations were performed in a shaking incubator at 37 °C in air in phosphate buffer (10 mM potassium phosphate buffer pH 7.5, containing 100 mM KCl, 1 mM EDTA, and 1 mM dithiothreitol). The assay was carried out in duplicate in 1 mL final incubation volume containing 50 nM [1β-^{3}H]androstenedione, various concentrations (0.1 - 1 μM) of the tested putative inhibitor, 100 μL NADPH, and an aliquot of the microsomal preparation. After 30 minutes incubation the enzymatic reaction was terminated by addition of 2 mL $CHCl_3$. The extraction procedure was repeated twice. The further elimination of traces of labeled substrate in the water phase was performed with dextran-coated charcoal (DCC). Radioactivity in the water phase was determined by a liquid scintillation counting apparatus. The aromatase inhibitor, formestane (4-hydroxyandrost-4-3,17



Scheme 2.46. Enzymatic aromatization of androst-4-ene-3,17-dione.

-dione) at various concentrations (0-600 nM) was used as reference standard.

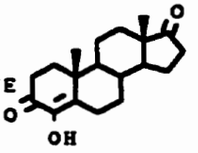
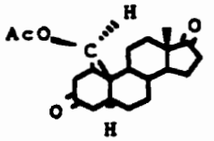
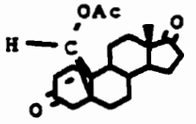
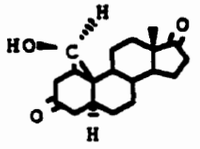
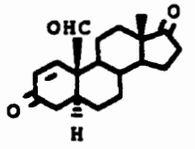
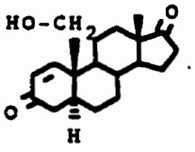
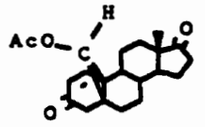
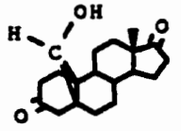
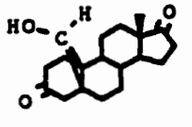
Table 2.4 shows the effect of different synthetic steroid inhibitors on the aromatase activity of placental microsomes. In comparison with Formestane, compounds **2.32**, **2.12**, and **2.29** show aromatase inhibitory potency ranging from 40-50% at a concentration of 1 μM. Two intermediates, **2.28** and **2.27**, and compound **2.15**, show inhibitory potency of *ca* 20% and 9%, respectively, at a concentration of 1 μM. Compounds, **2.4** and **2.5**, show no inhibitory activity at a concentration of 1 μM and only weak activity of 30%, at concentrations of 20 μM.

Among the cyclopropanol steroid derivatives **2.5**, **2.4**, and **2.29**, the following results have been obtained.

Although compounds, **2.4** and **2.5**, showed no inhibitory activity at a concentration of 1 μM, introduction of 1,2-unsaturation in ring A, compounds **2.12** and **2.27**, caused an increase in their inhibitory activity. Of these two, compound **2.12** showed higher activity (44%/1 μM) than its isomer **2.15** (9%/ 1μM) and both higher than its inactive parent compound **2.4** (0%/1 μM). Further modification of compounds **2.4** and **2.5** by shifting the cyclopropanol function from the 5β,19 to 1β,19 position caused a further increase in inhibitory activity. Saturated compounds, **2.32** and **2.29**, were moderately potent inhibitors with their aromatase inhibitory potency varying from 40-50%. Of these two, the 19(S)-acetoxy derivative **2.32** had higher activity than the parent compound **2.29**.

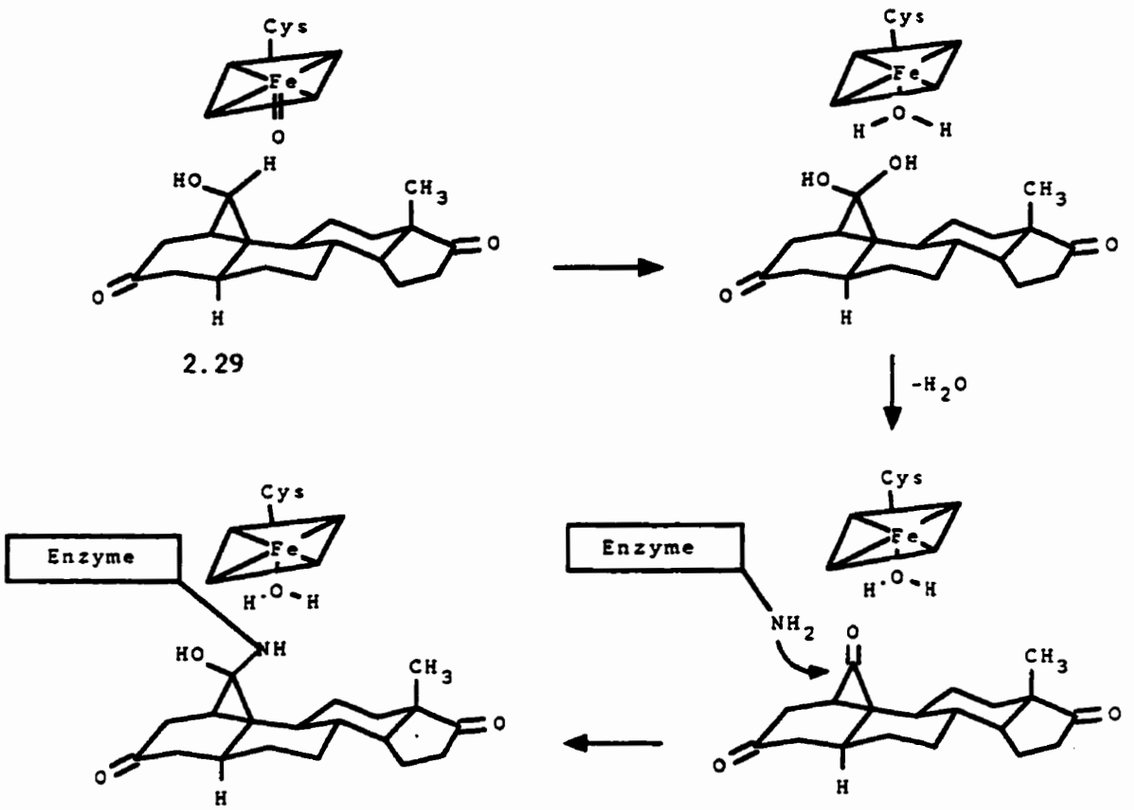
As we can see from Table 2.4, the most potent inhibitor was compound **2.32**, with the 19-acetoxy group above ring A and the 19-hydrogen projected out-of-ring A, which, nevertheless, is more then

Table 2.4 Aromatase inhibition (%) by synthesized compounds versus aromatase inhibitor 4-hydroxyandrost-4-ene-3,17-dione (Formestane).

COMPOUND	INHIBITION [%]	INHIBITOR CONCENTRATION [μM]
FORMESTANE 	99	0.6
2.32 	50	1
2.12 	44	1
2.29 	41.5	1
2.28 	24	1
2.27 	20	1
2.15 	9	1
2.4 	0 30	1 20
2.5 	0 30	1 20

than 2-fold less potent than Formestane.

The proposed mechanism of aromatase inactivation by compound **2.29** is shown in Scheme 2.47. Similar mechanisms may operate for compounds **2.12** and **2.32**, however, after deacetylation of **2.32** by esterase in the blood.



Scheme 2.47 Proposed mechanism of aromatase inactivation by compound **2.29**.

Compound **2.12**, which resulted from modification of its precursor **2.4** by an introduction of the 1,2-unsaturation in ring A, showed significant improvement in its inhibitory potency (44%/1 μM), compared

to the saturated derivative **2.4** (0%/1 μM). Presumably, introduction of the unsaturation and thus flattening of ring A facilitates hydroxylation of the 19-hydrogen, in **2.12**, by a ferroxy (Fe-O·) radical (Akhtar *et al.*, 1994) as shown in Scheme 2.46. Unlike the 19-H in **2.15**, the 19-H in **2.12** is exposed toward the ferroxy radical at the aromatase active site. Presumably, this situation improves the inhibitory properties of compound **2.12**.

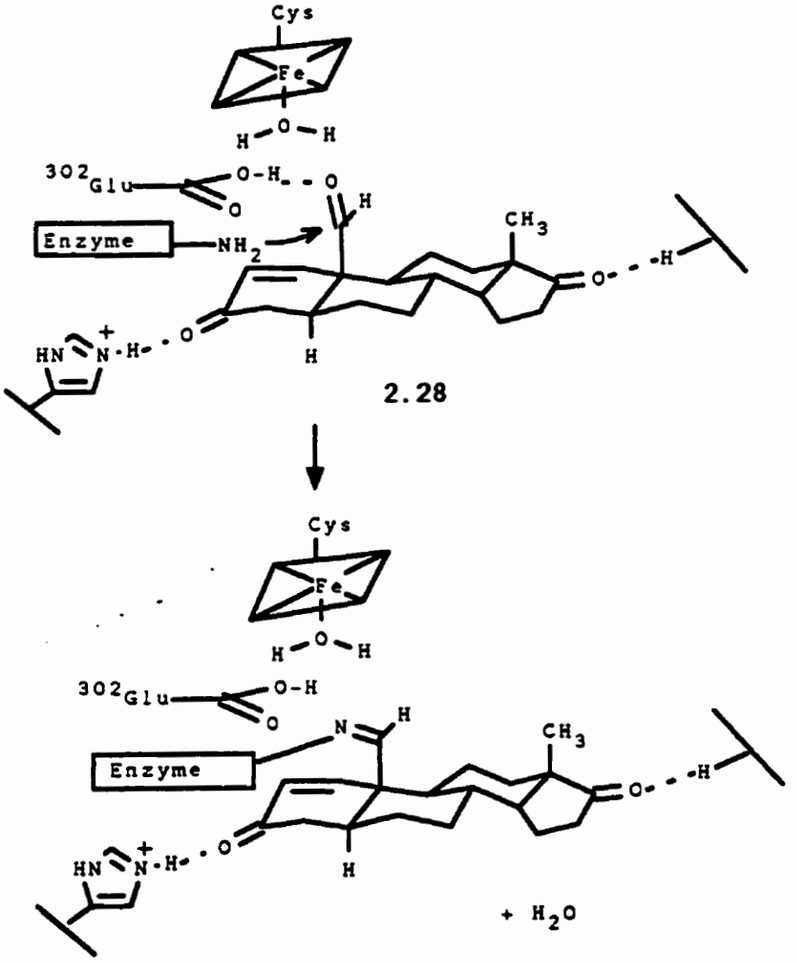
However, the same modification of ring A in compound **2.5** to give compound **2.15** caused only a 9% increase in its inhibitory activity. In both compounds, **2.5** and **2.15**, the 19-hydrogen is projected above ring B. As we can see, the 19(S)-hydrogen is not in a favoured orientation for the facile hydroxylation by aromatase. This could explain their weak inhibition properties.

It is suggested that the difference in activity of compounds **2.32**, **2.12**, and **2.29** in comparison with compounds **2.15** and **2.5** is related to the proximity of the 19-hydrogen to the ferroxy radical at the active site.

The proposed mechanism of aromatase inhibition by 5α-androst-1-ene-3,17-dion-19-al **2.28** is shown in Scheme 2.48. MMX geometrical optimization of compound **2.28** showed that oxygen of the 19-CHO group, in the preferred orientation, is pointed out of rings A and B (Scheme 2.6). The same orientation of the 19-CHO group could be valid at the aromatase active site. Such a situation may lead to a formation of a strong hydrogen bond between the 19-CHO and the aminoacid residue (Glu). Reaction of a nucleophile with the 19-CHO and formation of a covalent bond at the catalytic site may result in the inactivation of the enzyme.

On the basis of the above results, it is important for inhibitory

activity that synthesized steroid inhibitors have both a structural geometry closely related to the aromatase substrate and the 19-cyclopropyl hydrogen, susceptible to hydroxylation, projected toward the heme group of aromatase. These conditions are fulfilled in compounds **2.32**, **2.12**, and **2.29**, which have the highest inhibitory activity. These results are consistent with the assumed mechanism of aromatase inhibition previously discussed in Chapter 1.4.0.



Scheme 2.48 Proposed mechanism of aromatase inhibition by compound **2.28**.

CHAPTER 3

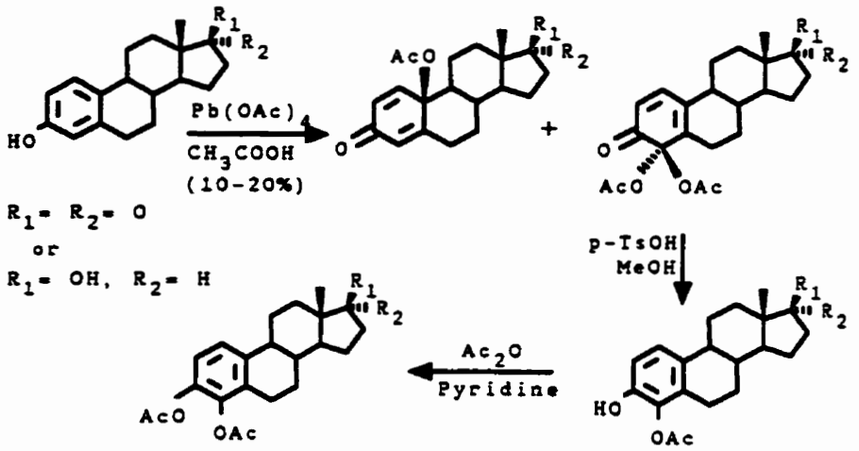
SYNTHESIS OF 4-HYDROXYESTROGENS

3.1.0 Introduction

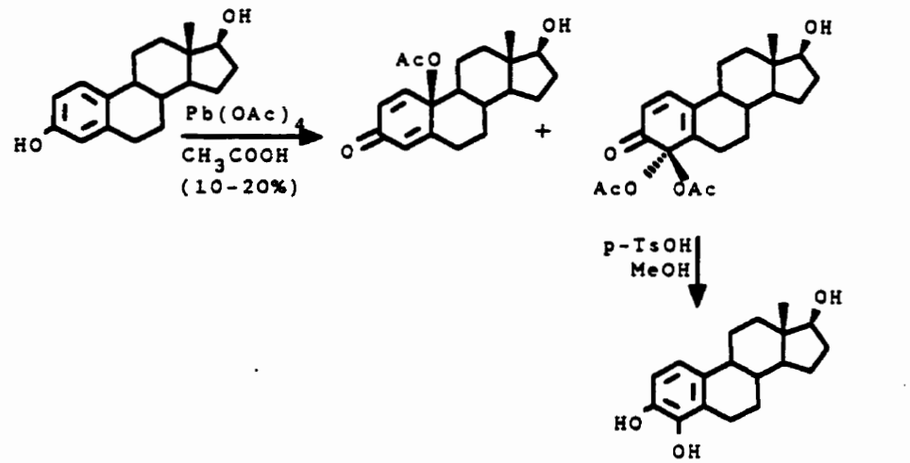
The usual sequence of events in the history of a new class of biological products proceeds from isolation to identification, then synthesis, and evaluation of biological properties. In the case of catechol estrogens, naturally occurring metabolites of estrogens (Bolt, 1979), this order was in part reversed. Their existence was postulated (Westerfeld, 1940), several were synthesized (Niederl and Vogel, 1949), and their pharmacology was studied (Mueller, 1955) some time before they were identified as biological substances. 2-Hydroxyestrone was first isolated from pregnancy urine (Fischman and Gallagher, 1958) and some years later 4-hydroxyestrone (Williams *et al.*, 1974).

The first methods for the preparation of 4-hydroxyestrogens were very laborious and gave only low yields. Preparation of 4-hydroxyestrogen acetates (Scheme 3.1; Gold and Schwenk, 1958) and 4-hydroxyestrogens (Scheme 3.2; Hecker and Walk, 1960) has been described by oxidation of estrone with lead *tetra*-acetate yielding the p-quinol acetate, 10ξ-acetoxy-1,4-estradiene-3,17-dione, together with two *o*-quinone *gem*-diacetates substituted at C- 2 and 4, respectively. The latter compound, 4,4-diacetoxy-1,5(10)-estradiene-3,17-dione, was converted to 4-hydroxyestrone by hydrolysis of the intermediary 4-acetoxyestrone.

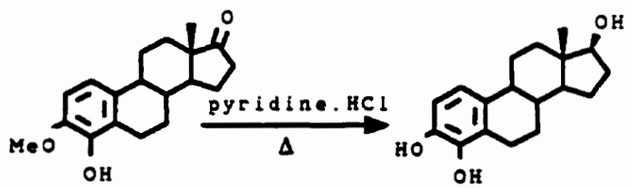
Another synthetic approach to 4-hydroxyestrone (Scheme 3.3; Fischman *et al.*, 1960), involved demethylation of 4-hydroxyestrone monomethyl ether on heating with pyridine hydrochloride. Alternative methods of 4-hydroxyestrogen synthesis were designed based on a reported method for 2-hydroxyestrogens preparation. Procedures involved reduction of 3,4-quinones with KI. These *o*-quinones were synthesized either by direct oxidation of the monophenolic estrogens



Scheme 3.1 Synthesis of 4-hydroxyestrogen derivatives (Gold and Schwenk, 1958).

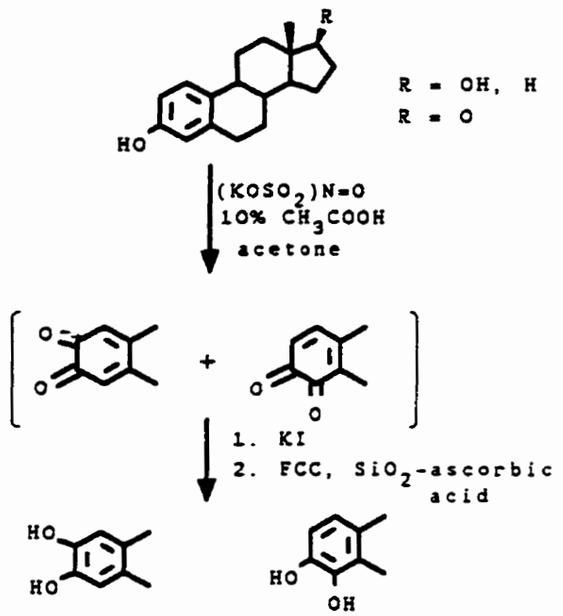


Scheme 3.2 Synthesis of 4-hydroxyestradiol (Hecker and Walk, 1960).



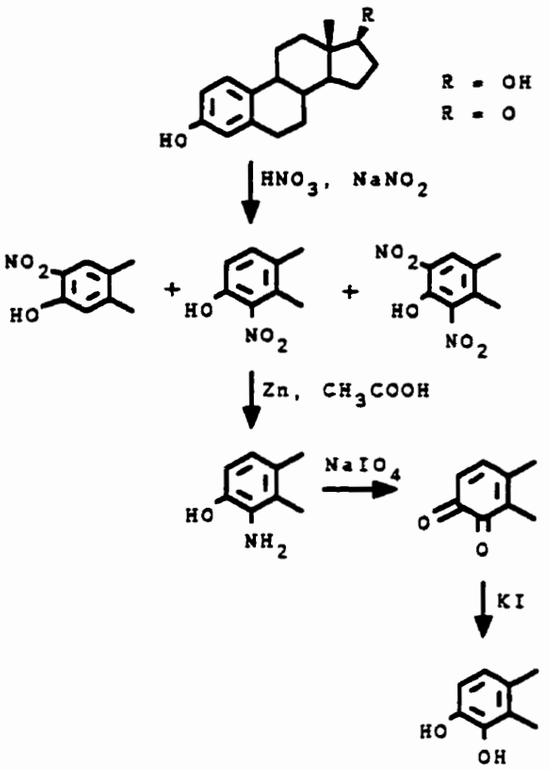
Scheme 3.3 Synthesis of 4-hydroxyestrone (Fischman *et al.*, 1960).

with Fremys's salt (potassium nitrosodisulfonate) (Scheme 3.4; Gelbke *et al.*,1973a,b), or by inverse oxidation of 4-aminoestrogens with $NaIO_4$ (Scheme 3.5; Stubenrauch and Knuppen, 1976).

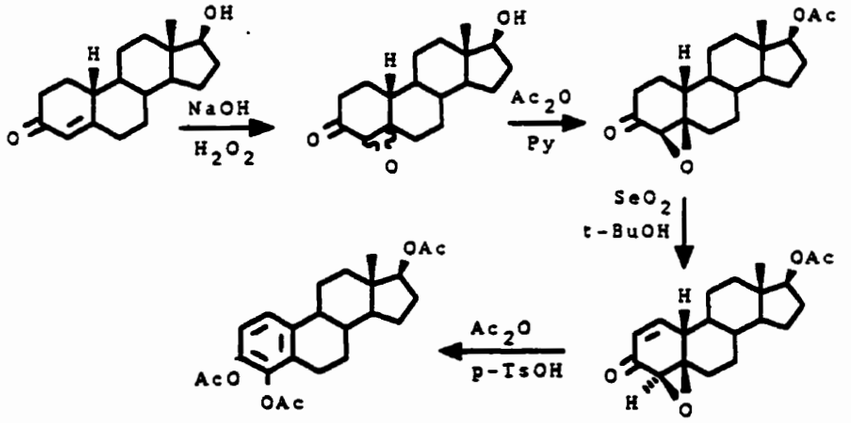


Scheme 3.4 Synthesis of 4-hydroxyestrone or 4-hydroxyestradiol in a one-step procedure (Gelbke *et al.*, 1973a, b).

The first regiospecific preparation of 4-hydroxyestradiol derivatives has been reported (Scheme 3.6; Le Quesne *et al.* 1980). Epoxidation of 19-nor-testosterone by a reported method (Mihailovic *et al.*, 1977), and dehydrogenation with selenium dioxide in anhydrous *tert*-butyl alcohol, followed by aromatization under acidic conditions with *p*-toluenesulfonic acid in acetic anhydride gave 4-hydroxyestradiol 3,4,17β-triacetate. However, no synthetic procedures and no characteristics of the compound were given.

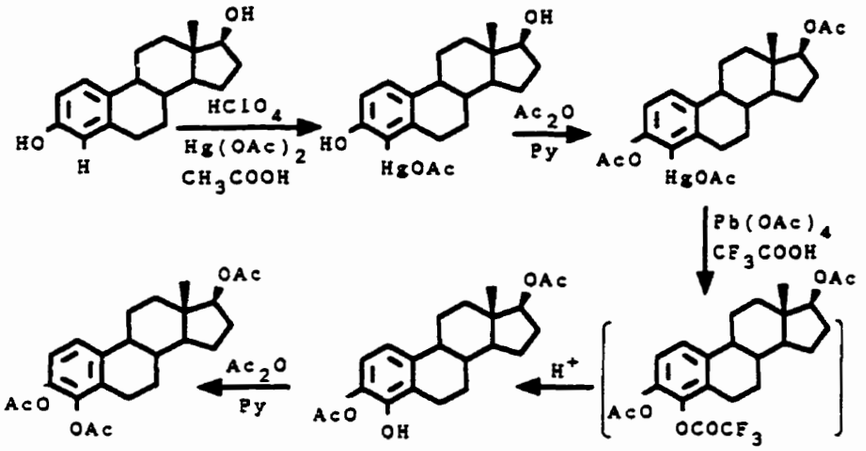


Scheme 3.5 Synthesis of 4-hydroxyestrone or 4-hydroxyestradiol (Stubenrauch and Knuppen, 1976).



Scheme 3.6 Synthesis of 4-hydroxyestradiol triacetate (Le Quesne *et al.*, 1980).

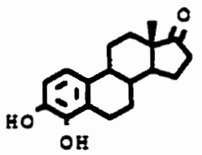
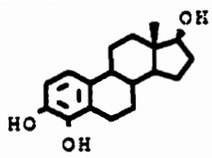
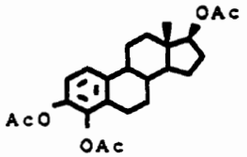
Another route to 4-hydroxyestradiol triacetate involved the 4-acetomercurio derivative of estradiol by using lead tetra-acetate in a metal exchange reaction to introduce the oxygen function at C-4 (Scheme 3.7; Kirk and Slade, 1982).



Scheme 3.7 Synthesis of 4-hydroxyestradiol triacetate (Kirk and Slade, 1982).

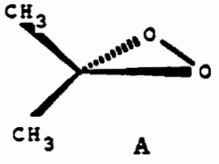
The synthetic methods illustrated in Schemes 3.4 and 3.5 gave mixtures of 2- and 4-functional derivatives, which are difficult to separate and purify from by-products. The methods outlined in Schemes 3.6 and 3.7 led to 4-hydroxyestradiol triacetate. Despite the biological interest in catechol estrogen derivatives, no ^{13}C NMR data were reported for these compounds. There was also inconsistency in ^{1}H NMR data both in assignments of chemical shifts (δ) and in the coupling constant (J), as well (Table 3.1). However, differences in chemical shifts can be explained by performing NMR measurements in different solvents.

Table 3.1 ^1H NMR chemical shifts of 4-hydroxyestrone and 4-hydroxyestradiol, and 4-hydroxyestradiol triacetate.

Catecholestrogen	^1H NMR δ (ppm) J (Hz)			References
	solvent	1-H	2-H	18-CH_3	
	pyridine-d_5	7.1d no J	6.8d no J	-	Gelbke *et al.*, 1973
	pyridine-d_5	7.10d J 11	6.74d J 11	0.80	Abdel-Baky, 1983
	$CDCl_3$:CD_3OD (1:1)	6.77d J 8.3	6.69d J 8.5	0.87	this Thesis
	$CDCl_3$	6.77 J 8.3	6.71 J 8.4	0.87	this Thesis
	acetone-d_6	5.83s		-	Abdel-Baky, 1983
	pyridine-d_5	6.98d J 8	6.74d J 8	0.97	Abdel-Baky, 1983
	$CDCl_3$:CD_3OD (1:1)	6.64d J 8.4	6.61d J 8.4	0.82	this Thesis
	$CDCl_3$	7.18d J 10	6.94d J 10	0.81	Kirk and Slade, 1981
	$CDCl_3$	7.18d J 8.5	6.97d J 8.5	0.82	this Thesis

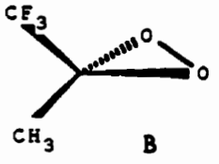
The goal of this project was to synthesize 4-hydroxyestrogens by more efficient and regiospecific routes and make the NMR assignment of protons and carbons. This work describes two synthetic approaches leading to 4-hydroxyestrogens (Schemes 3.12 and 3.16) and three approaches directed to 4-hydroxyestradiols (Schemes 3.11, 3.12, and 3.16). 4-Hydroxyestrogens have been synthesized starting from estr-4-ene-3,17-dione both *via* 1,2-unsaturated 4ξ,5ξ-epoxides (Scheme 3.12) and *via* a thermal rearrangement of 4-chloro-4ξ,5ξ-epoxides (Scheme 3.16). Acetate derivatives of 4-hydroxyestrogens and 4-hydroxyestradiols were also prepared. Synthesis of 4-hydroxyestradiol involved both estr-4-en-17β-ol-3-one and estr-4-en-3,17-one precursors

(Schemes 3.11, 3.12, and 3.16). Syntheses outlined in Schemes, 3.11 and 3.12, are conceptually similar to the synthesis shown in Scheme 3.6. Treatment of 19-nortestosterone **3.1** (Scheme 3.11) and 19-norandrost-4-ene-3,17-dione **3.8** (Scheme 3.14) with a nucleophilic oxidant, alkaline hydrogen peroxide, according to procedures described (Mihailovic *et al.*, 1977; Le Quesne *et al.*, 1986), yielded, as expected, the β-epoxide as the major product. An attempt to introduce an oxygen function into 4ξ-chloroestr-4-ene-3,17-dione **3.13c** having the 4,5-double bond deactivated both by conjugation and by the 4-chlorine substituent, under basic conditions H_2O_2-NaOH, was unsuccessful (Templeton, personal communication). Therefore, attention was paid to epoxidation of compound **3.13c** under neutral conditions. It has been reported that dioxiranes (e.g. **A** and **B**, Scheme 3.8) are powerful electrophilic



A

dimethyldioxirane
(DMD)



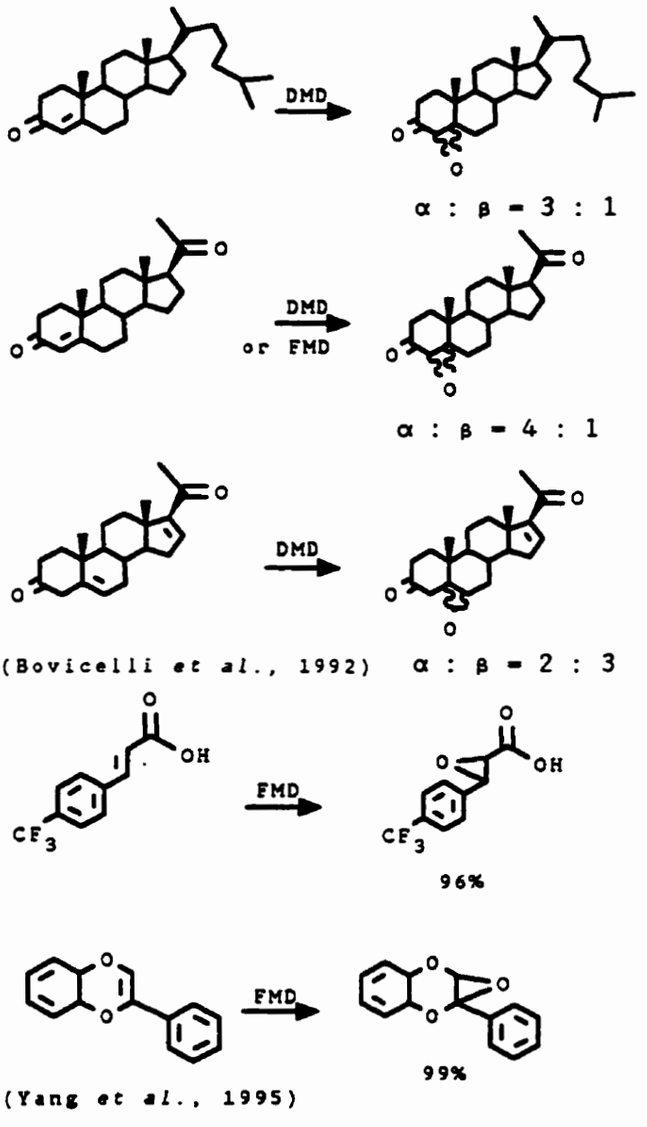
B

1,1,1-trifluoromethyl(methyl)dioxirane
(FMD)

Scheme 3.8 Dioxiranes.

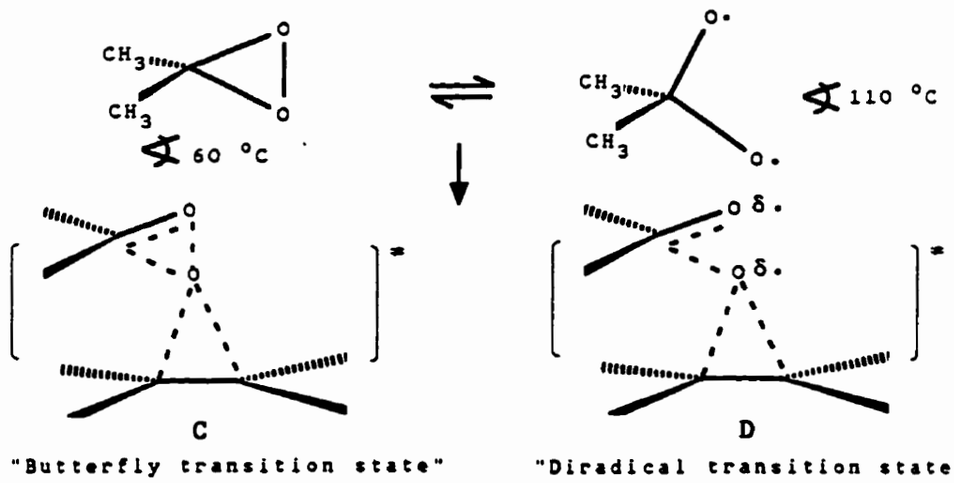
oxidants with high reactivity toward both electron-rich and electron deficient olefins, under neutral conditions (Adam *et al.*, 1989; 1992; Murray, 1989; Curci, 1990; Adam and Hadjiarapoglou, 1993). Dioxiranes, versatile oxidants, can be employed either in their isolated form (Murrey and Jeyaraman, 1985; Adam, 1993) or *in situ* (Yang *et al.*, 1995). Some examples of epoxidations are illustrated in Scheme 3.9.

As shown in Scheme 3.9, the attack of oxiranes, for the first two compounds, proceeds predominantly from the α-face, while the third compound reacts preferentially on the β-face. The "molecular" mechanism of the oxirane reaction with a double bond is, to date inadequately understood. Experimental data such as stereochemistry,



Scheme 3.9 Same examples of epoxidation of olefins using dioxiranes: dimethyldioxirane (DMD) and and 1,1,1-trifluoromethyl(methyl)dioxirane (FMD).

kinetics, activation parameters, isotope effects, reactivity patterns, etc. are consistent with the complex "butterfly" transition state C, initially proposed for peroxy acids as oxygen atom donors, and the novel diradical-like transition state B (Scheme 3.10), (Adam and Hadjiarapoglou and references cited there, 1993). In C, the intact dioxirane delivers the oxygen atom to the alkene, while in D it is the ring-opened dioxirane, i.e. the 1,3-dioxyl diradical. A theoretical estimate of the reaction enthalpy for the ring-opening of the dioxirane into 1,3 diradical was determined to be *ca.* 10 kcal/mol (Harding and Goddard, 1978). Thus, the activation energy for the process was suggested to be less than 15 kcal/mol. However, the direct oxygen transfer by the intact dioxirane, i.e. the "butterfly" transition state C, requires activation energy higher than 15 kcal/mol (Adam and Hadjiarapoglou, 1993). Moreover, it implies that the O-O bond is essentially broken.



Scheme 3.10 The mechanism of oxygen transfer by intact dioxirane (C) and the dioxyl diradical (D) to olefins, (Adam and Hadjiarapoglou, 1993).

In the present work both the treatment of estr-4-ene-3,17-dione **3.12** (Scheme 3.12) and 4-chloroestr-4-ene-3,17-dione **3.13c** (Scheme 3.16) with 1,1,1-trifluoromethyl(methy)dioxirane gave the α-epoxides, **3.13a** or **3.14c**, as a major product.

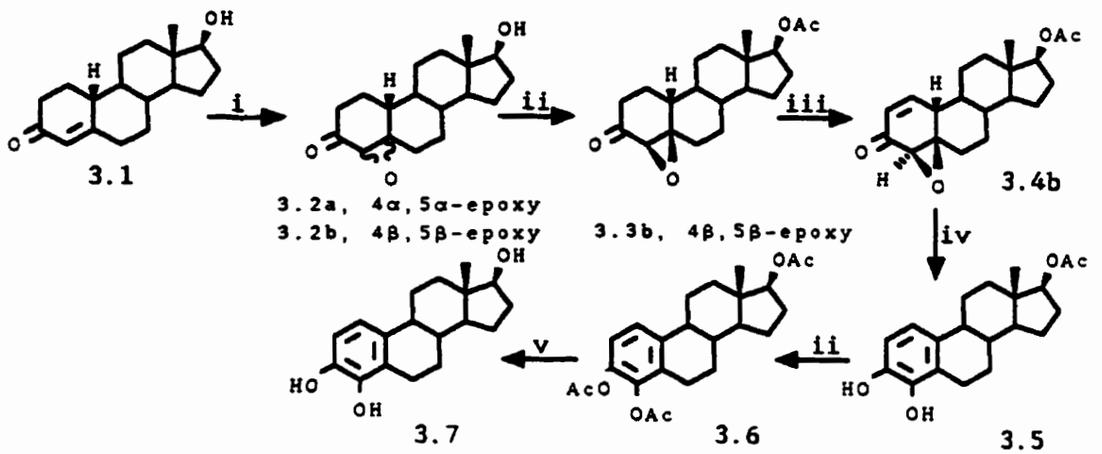
3.2.0 Synthesis of the Three 4-Hydroxy Estrogens: 4-Hydroxyestradiol 17β-acetate, 4-Hydroxyestratriol triacetate, and 4-Hydroxyestradiol from 4β,5β-epoxyestran-17-ol-3-one

Results and Discussion

Scheme 3.11 shows the synthesis of three catechols: 4-hydroxyestradiol 17β-acetate **3.5**, 4-hydroxyestratriol triacetate **3.6** (Kirk and Slade, 1982) and 4-hydroxyestradiol **3.7**. Catechol estrogens **3.5** and **3.7** were synthesized by procedures not reported earlier. Epoxidation of 19-nortestosterone **3.1** by means of 4 M NaOH and 30% H_2O_2 in methanol (Mihalovic *et al.*, 1977) gave a mixture of the 4α,5α- **3.2a** and 4β,5β-epoxides **3.2b** (**3.2a**:**3.2b**;1:9 by comparison of H-4 in the ^1H NMR spectra).

Acetylation of the 4ξ,5ξ-epoxides **3.2a/3.2b** with acetic anhydride in pyridine gave a mixture of 17β-acetoxy-4ξ,5ξ-epoxyestr-3-one, which, after purification on column chromatography, yielded 17β-acetoxy-4β,5β-epoxyestr-3-one **3.3b** as the major product (74%). Dehydrogenation of 17β-acetoxy-4β,5β-epoxyestr-3-one **3.3b**, with selenium dioxide in *t*-butanol and acetic acid (Le Quesene *et al.*, 1986; Menberu *et al.*, 1992), introduced a conjugated double bond at C-1 to give 17β-acetoxy-4β,5β-epoxyestr-1-en-3-one **3.4b** in 53%. Refluxing 17β-acetoxy-4β,5β-epoxyestr-1-en-3-one **3.4b**, in benzene with *p*-toluenesulfonic acid, gave a crude product of 4-hydroxyestradiol 17β-acetate **3.5**, which was directly acetylated with Ac_2O in pyridine and purified on FCC to give

the known 4-hydroxyestradiol triacetate **3.6** (Kirk and Slade, 1982), which was then reduced, with $LiAlH_4$ in ether, to give 4-hydroxyestradiol **3.7**. This is the first time that the catechol estrogen **3.7** was directly synthesized, without contamination by any impurities, *via* acidic aromatization of ring A in compound **3.4b** followed by hydride reduction.



Reagents: **i**, 4N NaOH, 30% H_2O_2, MeOH; **ii**, Ac_2O, pyridine followed by separation on FCC; **iii**, SeO_2, *t*-BuOH, CH_3COOH; **iv**, *p*-TsOH, C_6H_6; **v**, $LiAlH_4$, Et_2O.

Scheme 3.11 Synthesis of 4-hydroxyestradiol 17β-acetate **3.5** and 4-hydroxyestradiol **3.7** from 19-nortestosterone **3.1**.

3.2.1 Summary

1. 4-Hydroxyestradiol 17-β-acetate **3.5** was synthesized by aromatization of ring A of 17β-acetoxy-4β,5β-epoxyestr-1-en-3-one **3.4b** under acidic conditions and identified as 4-hydroxyestradiol triacetate **3.6**.
2. 4-Hydroxyestradiol **3.7** has been synthesized by $LiAlH_4$ reduction of 4-hydroxyestradiol triacetate **3.6**.

3.2.2 Experimental for Scheme 3.11.

4ξ,5ξ-Epoxyestran-17β-ol-3-one 3.2a/3.2b

To a stirred solution of 19-nortestosterone **3.1** (1.0 g, 3.64 mmol) in methanol (100 mL), which was cooled to 5° C in an ice-water bath, was added 30% hydrogen peroxide (5.5 mL), followed by 4 M sodium hydroxide (5.5 mL). After 20 min., TLC showed no starting material and the reaction was quenched with glacial acetic acid (10 mL) and the volume concentrated at reduced pressure to approximately 20 mL. Distilled water was added and the product was extracted with diethyl ether. The organic layer was washed with saturated $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure to produce a mixture of the *4α,5α-epoxide* **3.2a** and *4β,5β-epoxide* **3.2b** (800 mg) in the ratio 1 : 9, respectively, based on the ^1H NMR spectrum. A mixture of the 4ξ,5ξ-epoxides **3.2a/3.2b** was directly subjected to the acetylation.

17β-Acetoxy-4ξ,5ξ-epoxyestran-3-one 3.3a/3.3b

The above residue of 4ξ,5ξ-epoxyestra-17β-ol-3-one **3.2a/3.2b** (800 mg, 2.75 mmol) was treated with acetic anhydride (1.5 ml) in pyridine (5 mL) and allowed to stand at 20°C for 18 h. Excess acetic anhydride was converted into acetic acid by the addition of ice-water and the acetylated product was extracted with diethyl ether. The organic layer was washed with water, 5% HCl, water, saturated $NaHCO_3$, water and then dried over Na_2SO_4, filtered, and evaporated at reduced pressure to form a pale yellow, crystalline residue (1.08 g), which on flash chromatography on elution with 10% acetone-LP, gave fractions of 17β-acetoxy-4β,5β-epoxyestran-3-one **3.3b** (600 mg). Crystallization, yielded of **3.3b** (590 mg, 1.77 mmol, 64%), mp 115-116 °C (from CH_2Cl_2-MeOH), (lit., mp 108 °C, Wehrli *et al.*, 1966; Mihailovic *et al.*, 1977; mp

108-113 °C, Freisen 1991; mp 112 °C, Farmaceutici Italia Soc. Anon., 1975)

17β-Acetoxy-4β,5β-epoxyestr-1-en-3-one 3.4b

To a stirred solution of 17β-acetoxy-4β,5β-epoxyestran-3-one **3.3b** (450 mg, 1.35 mmol) in dry *t*-butanol (70 mL) under argon was added selenium dioxide (solid, 380 mg, 3.42 mmol) and glacial acetic acid (1.5 mL, 0.028 mol). The mixture was gently refluxed, to allow reduced selenium to deposit on the wall of the reaction flask, for 40 hours when TLC showed no starting material. After cooling, reduced selenium was removed by filtration through Celite which was then washed with ethyl acetate and the filtrate concentrated to about 20 mL. The reaction mixture was diluted with ethyl acetate (100 mL) and the organic layer washed with saturated sodium bicarbonate (4x) and water (2x), and dried over Na_2SO_4. Filtration and evaporation to dryness gave a brown residue which on flash chromatography, on elution with 10% EtOAc-LP, yielded a gummy product (245 mg). Crystallization gave 17β-acetoxy-4β,5β-epoxyestr-1-en-3-one **3.4b** (238 mg, 0.72 mmol, 53%), mp 112-116 °C (from EtOAc-CH_2Cl_2), (lit., mp 114-115 °C, Le Quesne *et al.*, 1980; mp 102-110 °C, Friesen, 1991).

4-Hydroxyestradiol triacetate (estra-1,3,5(10)-triene-3,4,17β-triol triacetate) 3.6

To a stirred solution of 17β-acetoxy-4β,5β-epoxyestr-1-en-3-one **3.4b** (200 mg, 0.605 mmol) in benzene (13 mL), was added p-toluenesulfonic acid (26 mg, 0.14 mmol), and the reaction was heated to reflux. After completion of the reaction (2 h) and cooling, the reaction mixture was diluted with ether. The organic layer was washed with saturated $NaHCO_3$, water, saturated NaCl, dried over Na_2SO_4, and evaporated to give a crude sample of 4-hydroxyestradiol 17-acetate **3.5** (170 mg),

which was directly subjected to acetylation.

To a stirred solution of a crude product of 4-hydroxyestradiol 17β-acetate **3.5** (170 mg, 0.515 mmol) in pyridine (3 mL) was added acetic anhydride (0.260 mL). After 2 hr, TLC showed no starting material. The reaction mixture was poured into ice-water and acidified with 5% HCl. After extraction of products with diethyl ether, the organic layer was washed with 5% HCl, water, saturated $NaHCO_3$, water, and dried over Na_2SO_4. Evaporation of the solvent gave a white-brown residue (156 mg) which on flash chromatography, on elution with 10% acetone-LP, yielded fractions of **3.6** (130 mg) which after crystallization gave **3.6** (109 mg, 0.33 mmol, 64%), mp 204-207 °C (from CH_2Cl_2-LP), (lit., mp 192-196 °C, Kirk and Slade, 1982).

4-Hydroxyestradiol [estra-1,3,5(10)-triene-3,4,17β-triol] 3.7

To a stirred solution of 4-hydroxyestradiol triacetate **3.6** (109 mg, 0.262 mmol) in ethyl ether, was added solid $LiAlH_4$ (10 mg, 0.786 mmol). After 4 hours, TLC showed no starting material. The organic layer was washed with 5% HCl, water, saturated sodium chloride, and evaporated to give a white residue which, in air, was oxidized rapidly to a creamy solid (67 mg, 0.232 mmol). Crystallization from benzene-ethyl acetate-2% acetic acid yielded creamy crystals of **3.7** (67 mg, 0.232 mmol, 88%), mp 235-237 °C and s/256-260° C dec., (lit., mp 214-216° C, Gelbke *et al.*, 1973; mp 252-253 °C, Hecker and Walk, 1960)

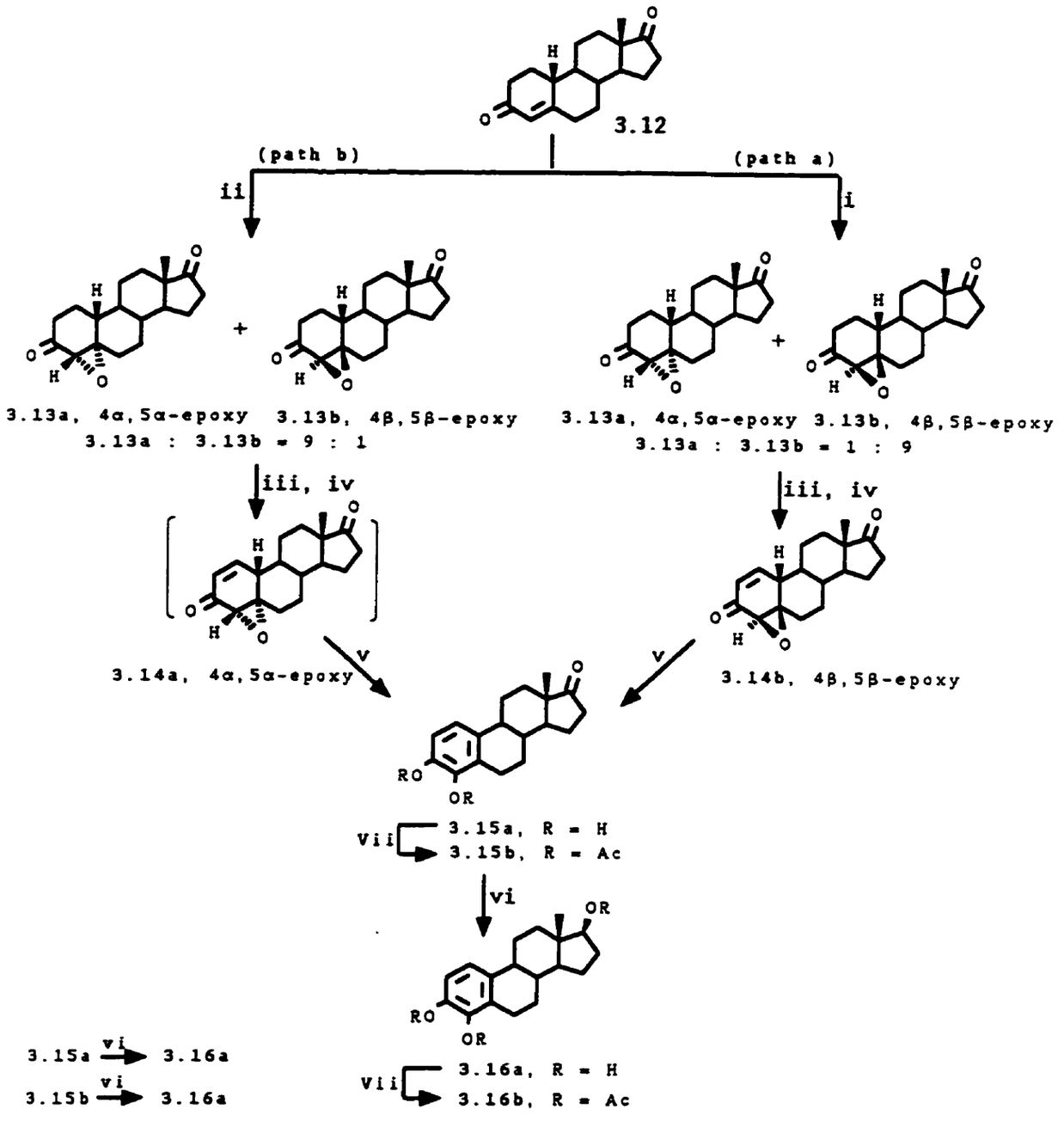
3.3.0 Synthesis of 4-Hydroxyestrone and 4-Hydroxyestradiol from 4ξ,5ξ-epoxyestra-3,17-dione.

Results and Discussion

As outlined in Scheme 3.12 (path a), estr-4-ene-3,17-dione **3.12**, on treatment with alkaline hydrogen peroxide (Le Quesne *et al.*, 1986; Mihailovic *at al.*, 1977), gives a mixture of the α- and β- epoxides, **3.13a** and **3.13b**, (**3.13a:3.13b**; 1:9 by comparison of H-4 in the ^{1}H NMR spectra), which after crystallization yielded 4β,5β-epoxyestra-3,17-dione **3.13b**. On the other hand (path b), estr-4-ene-3,17-dione **3.12**, on treatment with 1,1,1-trifluoromethyl(methyl)dioxirane, Mello reagent, generated *in situ* (Yang *et al.*, 1995), also gave a mixture of the α- and β-epoxides, **3.13a** and **3.13b**, but in the reversed proportion (**3.13a:3.13b**;9:1 by comparison of H-4 in the ^{1}H NMR spectrum). This is a convenient one-step *versus* six-step procedure (Durga, 1979) to introduce an α-epoxy function, into a conjugated double bond of ring A.

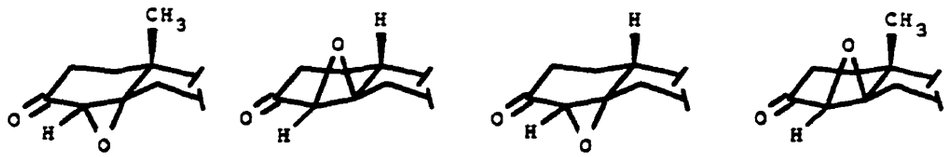
Additionally, an interesting shielding relationship between the orientation of the 4ξ,5ξ-epoxy ring and the proton chemical shift of H-4, in 4α,5α-epoxyandrostane-3,17-dione **3.9a**, 4β,5β-epoxyandrostane-3,17-dione **3.9b**, and its 19-nor analogues, **3.13a** and **3.13b**, has been observed. As outlined in Scheme 3.13, the largest shielding effect of H-4 was observed in compound **3.9b**: upfield shift of the 4-H signal. Compounds **3.9a** and **3.9b** have been synthesized by treatment of both testosterone **3.8** and androst-4-ene-3,17-dione **3.10** with 1,1,1-trifluoromethyl(methyl)dioxirane and H_2O_2-NaOH, respectively (Scheme 3.14).

Treatment of the 4β,5β-epoxide **3.13b** with selenic reagents, $(PhSeO)_2O$ (Barton *et al.*, 1982 a,b) or Ph_2Se_2 (Barton *et al.*, 1979) led to regioselective dehydrogenation of ring A without affecting ring D



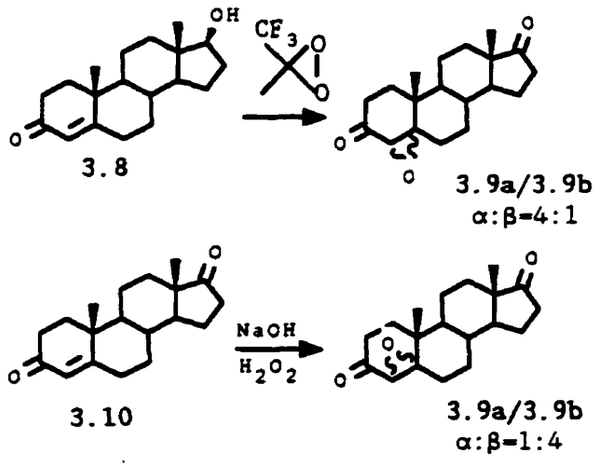
Reagents: **i**, 30% H_2O_2, 4 M NaOH, MeOH; **ii**, 1,1,1-trifluoro(methyl)-dioxirane, CF_3COCH_3, CH_3CN *in situ*; **iii**, separation by crystallization; **iv**, Ph_2Se_2, CSA, 3-$IO_2C_6H_4COOH$ in THF; **v**, p-TsOH, C_6H_6; **vi**, $LiAlH_4$, THF; **vii**, Ac_2O, DMAP, CH_2Cl_2.

Scheme 3.12 Synthesis of 4-hydroxyestrone **3.15a** and its derivatives **3.15b**, **3.16a**, and **3.16b**.



3.9a 3.04 ppm | **3.13b** 3.03 ppm | **3.13a** 3.01 ppm | **3.9b** 2.98 ppm

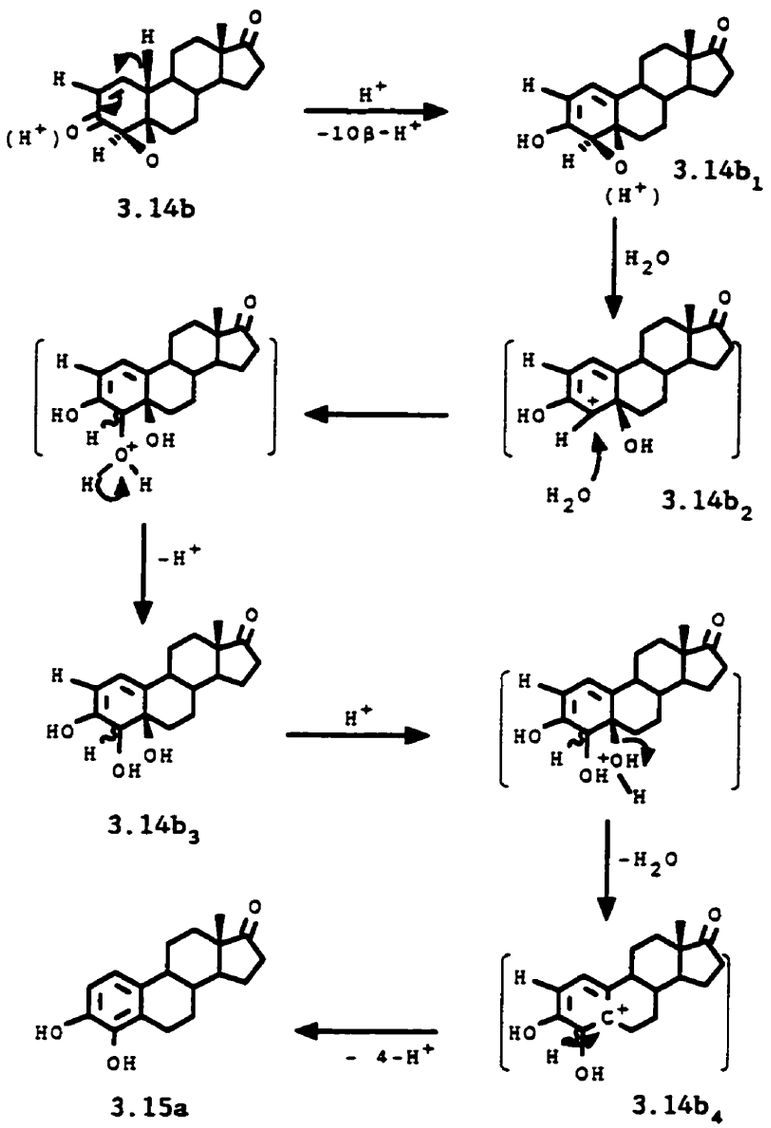
Scheme 3.13 Chemical shifts (δ ppm) of 4-H for 4α,5α- and 4β,5β-epoxy-androstane-3,17-dione, **3.9a** and **3.9b**, and 4α,5α- and 4β,5β-epoxy-estra-3,17-dione, **3.13a** and **3.13b**.



Scheme 3.14 Synthesis of 4α,5α-epoxy- and 4β,5β-epoxyandrostane-3,17-dione, **3.9a** and **3.9b**, respectively.

(Scheme 3.12). When the 4β,5β-epoxide **3.13b** was treated with benzeneselenic anhydride, followed by FCC purification, the 1,2-unsaturated epoxide **3.14b** was obtained in 20-40% yield. On the other hand, treatment of the 4β,5β-epoxide **3.13b** with diphenyldiselenium, camphorsulfonic acid, and 3-iodylbenzoic acid in

tetrahydrofuran (Barton *et al.*, 1989) followed by crystalization gave 4β,5β-epoxyestr-1-ene-3,17-one **3.14b** in a higher yield 50-79%. Similar treatment of the 4α,5α-epoxide **3.13a** with $(PhSeO)_2O$ gave the 1,2-unsaturated epoxide **3.13a** in *ca* 20% yield, contaminated with a by-product, which could not be separated effectively, as shown by the ^1H NMR spectrum. Treatment of **3.13a** with Ph_2Se_2, $3\text{-}IO_2C_6H_4COOH$, and CSA in tetrahydrofuran yielded a mixture of **3.13b** and **3.15a**, as determined from the ^1H NMR spectrum and TLC. On chromatrography of the mixture, the 1,2-unsaturated epoxide **3.14a** was converted to **3.16a** indicating its instability not only to the oxidation conditions but also to the separation conditions. This result also shows that the 4α,5α-epoxy-1-ene **3.14a** is less stable than its isomer **3.14b**, which was stable to silica chromatography. Refluxing 4β,5β-epoxyestr-1-ene-3,17-dione **3.14b** with *p*-toluenesulfonic acid in benzene gave the expected 4-hydroxyestrone **3.15a** (Majgier-Baranowska *et al.*, 1997). Attempts to aromatize ring A by treatment of **3.15b** with p-toluenesulfonic acid in diethyl ether at ambient temperature or in benzene under reflux with water elimination (Dean-Stark) was unsuccessful. This indicates that, for aromatization, not only more vigorous conditions are required, but also the presence of water. Water originates from the crystallized water of p-TsOH. Acetylation of 4-hydroxyestrone **3.15a** furnished 4-hydroxyestrone 3,4-diacetate **3.15b**. Reduction of **3.15a** or **3.16b** with lithium alumnium hydride in THF afforded 4-hydroxyestradiol **3.16a**, which on acetylation with acetic anhydride and DMAP gave 4-hydroxyestradiol 3,4,17β-triacetate **3.16b**. The ^{1}NMR spectra of compounds **3.15a**, **3.15b**, **3.16a**, and **3.16b** showed a pair of downfield doublets consistent with H-1 and H-2 of the aromatic ring (see the experimental part). Their ^{13}C NMR spectra were assigned



Scheme 3.15 Proposed mechanism for formation of 4-hydroxyestrone **3.15a** *via* aromatization of ring A under acidic conditions.

by COSY and HSQC spectra.

The proposed mechanism of ring A aromatization of the 3β,5β-epoxide **3.14b** to yield 4-hydroxyestrone **3.15a** is outlined in Scheme 3.15. Protonation of the 3-carbonyl group of 4β,5β-epoxyestr-1-ene-3,17-one **3.14b** forms the dienol **3.14b**$_1$. Further protonation of the epoxy oxygen results in the ring opening to form an allylic carbocation at C-4 **3.14b**$_2$. The newly formed carbocation is attacked by a molecule of water to yield the 4,5-diol **3.14b**$_3$. Protonation of the tertiary allylic hydroxy group at C-5, followed by its elimination as a molecule of water and the loss of the C-4 proton gives 4-hydroxyestrone **3.15a**.

3.3.1 Summary

1. Introduction of the 1,2-unsaturation in ring A was accomplished in high yield (50-79%), with a catalytic amount of selenium reagent, $(PhSe)_2$, in the presence of camphorsulfonic and 3-iodylbenzoic acid as the oxidant.
2. 4α,5α-Epoxyestra-3,17-dione **3.13a** has been synthesized by epoxidation of estr-4-ene-3,17-dione **3.12** with Mello reagent, 1,1,1-trifluoromethyl(methyl)dioxirane. In this case, introduction of oxygen from the α-face was accomplished in a one-step *versus* a six-step procedure.
3. 4-Hydroxyestrone was obtained upon acidic aromatization of the 4ξ,5ξ-epoxides, **3.14a** and **3.14b**.
4. The methods developed led to the four catechol estrogens, **3.15a**, **3.15b**, **3.16a**, and **3.16b**. Two of them, **3.15a** and **3.15b**, have the 17-keto function while the two remaining, **3.16a** and **3.16b**, have the 17β-hydroxy group.

3.3.2 Experimental for Schemes 3.12 and 3.14.

For Scheme 3.12.

4ξ,5ξ-Epoxyestra-3,17-dione 3.13a/3.13b; (path a)

Epoxidation with H_2O_2-NaOH:

To a stirred solution of estr-4-ene-3,17-dione **3.12** (1.0 g, 3.67 mmol) in MeOH (100 mL), cooled to 5 °C in an ice-water bath, was added 4M NaOH (5 mL) followed by cold 30% hydrogen peroxide (5.5 mL). After 20 min, TLC showed no starting material. The mixture was poured into ice-water and the products extracted with diethyl ether. The organic layer was washed with cold water until neutral, saturated sodium chloride, dried over Na_2SO_4, filtered and the solvent evaporated. The crystalline product (980 mg, 3.75 mmol) mp 148-168 °C consisted of two components (as shown by ^1H NMR and TLC; **3.13a** (4α,5α-epoxy) : **3.13b** (4β:5β-epoxy) = 1:9). Recrystallization gave the 4β,5β-epoxide **3.13b** (830 mg, 2.88 mmol, 78%), mp 166-172 °C (from CH_2Cl_2-methanol). Several recrystallizations gave the 4β,5β-epoxide **3.13b**, mp 170-172 °C (Found C, 74.67; H, 8.84; $C_{18}H_{24}O_3$ requires C, 74.96; H, 8.39%)

4ξ,5ξ-Epoxyestra-3,17-dione 3.13a/3.13b; (path b)

Epoxidation with the Mello reagent *in situ*:

To a stirred solution of estr-4-ene-3,17-dione **3.12** (2.0 g, 7.34 mmol) in acetonitrile (80 mL), cooled in an ice-water bath, was added an aqueous Na_2EDTA solution (30 mL, 4 x 10^{-4} M) followed by the addition of 1,1,1-trifluoroacetone (7 mL) by a precooled graduate cylinder. To this homogenous solution was added in portions a mixture of sodium hydrogen carbonate (4.7 g, 0.057 mmol) and Oxone^R (11.3 g, 0.037 mmol) over a period of 25 minutes (pH = 7). After 1 hr of vigorous stirring, TLC showed no starting material. Water was added to the heterogenous

mixture to dissolve the solid reagents, and the products were extracted with dichloromethane. The organic layer was washed with water, dried over Na_2SO_4, and evaporated to give a solid residue. The crystalline product consisted of the 4α,5α- **3.13a** and 4β,5β-epoxide **3.13b** (as shown by ^1H NMR and TLC; mp 180-195 °C). Recrystallization gave the 4α,5α-epoxide **3.13a** (1.5 g, 5.2 mmol, 71%), mp 215-220 °C (from CH_2Cl_2-methanol). Three recrystallizations gave the 4α,5α-epoxide **3.13a** (1.3 g, 4.5 mmol), mp 220-221 °C, (Found C, 75.07; H, 8.46. $C_{18}H_{24}O_3$ requires C, 74.96; H, 8.39%).

^1H NMR of 3.13a ($CDCl_3$): δ 3.03 (s, 1H, **4β-H**), 0.92 (s, 3H, 13-Me).

^{13}C NMR of 3.13a ($CDCl_3$): δ 20.78 (1), 35.74 (2), 205.62 (3), 61.72 (4), 64.49 (5), 32.98 (6), 31.38 (7), 39.97 (8), 40.54 (9), 46.49 (10), 27.26 (11), 25.63 (12), 47.74 (13), 50.09 (14), 21.68 (15), 36.30 (16), 220.38 (17), 13.79 (18).

4α,5α-Epoxyestr-1-ene-3,17-dione 3.14a and 3.15a

A stirred mixture of diphenylselenium (34.33 mg, 0.11 mmol), 3-iodylbenzoic acid (1.01 g, 3.6 mmol), and camphorsulfonic acid (127.8 mg, 0.55 mmol) in THF (20 mL) was refluxed under argon for *ca* 10 min., until the yellow solution became white, and then a solution of 4β,5β-epoxy **3.13b** (300 mg, mmol) in THF (2 mL) was added. After 4 hr reflux, TLC showed two spots but no starting material. One of the spots, R_f= 0.28 (20% acetone-LP), was seen under UV, the second one, R_f = 0.18, was not detected under UV. The reaction mixture was cooled to RT and the products extracted with CH_2Cl_2. The organic layer was washed with water, saturated $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated to give a yellow residue (120 mg), which on FCC, on elution with 15% acetone-LP, gave non crystalline compound **3.14a** (20 mg) and **3.15a** (30 mg).

^{1}H NMR of 3.14a ($CDCl_3$): 6.74 (dd, J 5.3, 10.3, 1H, 1-H), 5.98 (dt, J 1.6, 1.6, 10.5, 1H, 2-H), 3.28 (t, J 1.6, 1H, 4β-H), 2.71 (dd, J 5.3, 10.9, 1H, 10β-H), 0.88 (s, 3H, 13-Me).

4β,5β-Epoxyestr-1-ene-3,17-dione 3.14b

A stirred mixture of diphenylselenium (34.33 mg, 0.11 mmol), 3-iodylbenzoic acid (1.01 g, 3.6 mmol), and camphorsulfonic acid (127.8 mg, 0.55 mmol) in THF (20 mL) was refluxed under argon for *ca* 10 min., until the yellow solution became white, and then a solution of 4β,5β-epoxy **3.13b** (280 mg, 0.96 mmol) in THF (2 mL) was added. After 4 hr reflux, TLC showed no starting material. The mixture was cooled to RT and CH_2Cl_2, water, and saturated $NaHCO_3$ added. After extraction with CH_2Cl_2, the organic layer was washed with water, dried over Na_2SO_4, filtered, and evaporated to give a yellow solid residue (240 mg), which on crystallization gave **3.14b** (220 mg, 0.76 mmol, 79.5%), mp 213.5-216 °C (from CH_2Cl_2-EtOAc), (Found C, 75.61; H, 7.78; $C_{18}H_{22}O_3$ requires C, 75.50; H, 7.74%).

^{1}H NMR of 3.14b ($CDCl_3$): δ 6.72 (dd, J 10.6, 5.3 Hz, 1H, 1-H), 5.96 (ddd, J 10.8, 1.6, 1.3 Hz, 1H, 2-H), 3.25 (dd, J 1.51, 1.46, 0.95 Hz, 1H, 4-H), 2.63 (dd, J 5.29, 5.28 Hz, 1H, 10-H), 0.95 (s, 3H, 13-Me), 2.48 (dd, J 9.0, 8.33, 1H, 16β-H).

^{13}C NMR of 3.14b ($CDCl_3$): δ 125.25(1), 147.58 (2), 195.66 (3), 61.87 (4), 63.63 (5), 32.25 (6), 31.10 (7), 39.99 (8), 44.82 (9), 49.75 (10), 28.25 (11), 26.22 (12), 47.64 (13), 51.12 (14), 21.65 (15), 35.48 (16), 219.73 (17), 13.81 (18).

4-Hydroxyestrone 3.15a

A solution of 4β,5β-epoxyestr-1-ene-3,17-dione, **3.15b**, (130 mg, 0.454 mmol) and *p*-toluenesulphonic acid (10 mg) in benzene (15 mL) was refluxed under an argon atmosphere for 18 h. The mixture was diluted

with ether (20 mL) and the organic layer washed with cold water until neutral, 4% $NaHCO_3$, water, saturated NaCl, and dried over Na_2SO_4. The solution was concentrated under a stream of nitrogen, and the product was obtained as a white precipitate. Evaporation of the solvent gave a creamy residue (80 mg, 0.28 mmol), which on crystallization yielded **3.15a** (95 mg, 0.332 mmol, 73%), mp 261-263 °C (from benzene/EtOAc/2% CH_3COOH), (lit., (*i*) mp 260-265 °C, Fishman *et al.*, 1960; (*ii*) mp 268-271 °C, Gelbke *et al.*, 1973; (*iii*) mp 260 °C (dec.), Dwivedy *et al.*, 1992).

^{1}H NMR of 3.15a ($CDCl_3$:CD_3OD=1:1): δ 6.70 (d, J 8.77 Hz, 1H, 1-H), 6.66 (d, J 8.34 Hz, 1H, 2-H), 2.95 (dd, J 17.45 Hz, 5.35 Hz, 1H, 6β-H), 2.69 (m, 1H, 6α-H) , 2.49 (dd, J 8.81, 8.83 Hz, 1H, 16β-H), 0.93 (s, 3H, 13-Me).

(lit., **^{1}H NMR of 3.15a** (pyridine-d_5); δ 7.1d (1-H), 6.8d (2-H); no J was given, Gelbke *et al.*, 1973).

(lit., **^{1}H NMR of 3.15a** (pyridine-d_5): δ 7.10 (d, J 11.0, 1H, 1-H), 6.74 (d, J 11.0, 1H, 2-H), 0.80 (s, 3H, 13-Me); Abdel-Baky, 1983).

^{13}C NMR of 3.15a ($CDCl_3$:CD_3OD=1:1): δ 116.67 (1), 112.73 (2), 142.02 (3), 142.39 (4), 132.45 (5), 23.83 (6), 32.04 (7), 38.44 (8), 44.67 (9), 124.37 (10), 26.46 (11), 36.42 (12), 43.49 (13), 51.01 (14), 22.04 (15), 26.69 (16), 219.97 (17), 14.97 (18).

4-Hydroxyestrone diacetate 3.15b

To a stirred solution of 4-hydroxyestrone (30 mg, 0.10 mmol) in CH_2Cl_2 (3 mL) was added DMAP (15 mg) and Ac_2O (0.35 mmol). After 2 hr, methanol (2 mL) was added and the mixture stirred for 0.5 h and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$, water, dried, filtered, and evaporated to give a solid residue of **3.15a** (35 mg, 94%), mp 217-220 °C (from EtOAc-LP) (lit.,

212.5-215.5 °C, Gold and Schwenk, 1958).

^{1}H NMR of 3.15b ($CDCl_3$): 7.20 (d, J 8.6, 1H, 1-H), 6.98 (d, J 8.6, 1H, 2-H), 2.79 (dd, J 5.4, 17.5, 1H, 6β-H), 2.60 (ddd, J 6.8, 11.9, 18.6, 1H, 6α-H), 2.49 (dd, J 8.8, 18.7, 1H, 16β-H), 0.89 (s, 3H, 13-Me.

^{13}C NMR of 3.15b ($CDCl_3$): 120.05 (1), 123.42 (2), 130.67 (3), 138.98 (4), 140.10 (5), 23.50 (6), 25.51 (7), 37.24 (8), 44.09 (9), 140.15 (10), 25.62 (11), 31.41 (12), 47.74 (13), 50.22 (14), 21.46 (15), 35.71 (16), 220.41 (17), 13.70 (18).

4-Hydroxyestradiol 3.16a

(a) From 4-hydroxyestrone **3.15a**:

To a solution of 4-hydroxyestrone **3.15a** (45 mg, 0.157 mmol) in THF (3 mL) was added solid $LiAlH_4$ (8 mg, 0.21 mmol). The mixture was stirred for 1/2 hr, when TLC showed no starting material, and then diluted with CH_2Cl_2 (20 mL). The organic layer was washed with 5% HCl, water, saturated aqueous $NaHCO_3$, water, dried, filterd, and evaporated to give a white residue which, in air, was rapidly oxidized to a creamy solid. Crystallization from benzene-ethyl acetate-2% acetic acid yielded creamy crystals of **3.16a** (35 mg, 0.12 mmol, 77%), mp 235.5-237 °C resolidified and 257-260 °C (dec.), (lit., mp 214-216 °C, Gelbke et al., 1973).

^{1}H NMR of 3.16a ($CDCl_3$:CD_3OD=1:1): δ 6.64 (d, J 8.44 Hz, 1H, 1-H), 6.61 (d, J = 8.36 Hz, 1H, 2-H), 3.68 (dd, J 8.41, 8.63 Hz, 1H, 17α-H), 2.89 (dd, J 5.57, 5.56 Hz, 1H, 6α-H), 2.61 (m, 6β-H), 0.82 (s, 3H, 13-Me) (lit. ^{1}H NMR of **3.16a** (pyridine-d_5): δ 7.1 (d, 1H, 1-H), 6.8 (d, 1H, 2-H), 3.9 (t, 1H, 17α-H); Gelbke et al., 1973).

^{13}C NMR of 3.16a ($CDCl_3$:CD_3OD=1:1): δ 116.64 (1), 112.49 (2), 142.02 (3), 142.39 (4), 133.19 (5), 23.83 (6), 27.28 (7), 38.81 (8), 44.57 (9), 124.37 (10), 26.82 (11), 37.14 (12), 43.49 (13), 50.48 (14), 23.42

(15), 30.12 (16), 81.79 (17), 11.28 (18): based on COSY-45 and HSQC (no reference for ^{13}C).

(b) From 4-hydroxyestrone 3,4-diacetate **3.15b**:

A mixture of 4-hydroxyestrone 3,4-diacetate **3.15b** (59 mg, 0.17 mmol) and $LiAlH_4$ (20 mg, 0.53 mmoL) in THF (3 mL) was stirred for 0.5 h, when no starting material was detected by TLC, and then diluted with CH_2Cl_2 (30 mL). The organic layer was washed with 5% HCl, water, saturated $NaHCO_3$, water, dried over Na_2SO_4, filtered and evaporated to give a residue of 4-hydroxyestradiol **3.16a** (33 mg, 0.11 mmol, 65%), which was directly subjected to acetylation (see procedure for **3.16b**).

4-Hydroxyestradiol 3,4,17β-triacetate 3.16b

(a) From 4-hydroxyestrone **3.15a**:

A solution of 4-hydroxyestradiol **3.16a** (35 mg, 0.12 mmol), from the reduction of 4-hydroxyestrone **3.15a**, in CH_2Cl_2 (3 mL), was treated with Ac_2O (1.5 mL, 16 mmol) and DMAP (15 mg) for 8 h, when no starting material was detected on TLC. Methanol (2 mL) was added, to destroy an excess of the reagent, and stirring continued for 0.5 h. The mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was washed with water, saturated aqueous $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated to give a residue (45 mg), which on crystallization yielded **3.16b** (42 mg, 0.10 mmol, 84%), mp 204-207 °C (from CH_2Cl_2-MeOH), (lit., mp 192-196 °C, no solvent given, Kirk and Slade, 1982).

(b) From 4-hydroxyestradiol 3,4-acetate **3.15b**:

A crude product of 4-hydroxyestradiol **3.16a** (33 mg), from the reduction of 4-hydroxyestradiol 3,4-diacetate **3.15b**, was dissolved in CH_2Cl_2 (3 mL), and then treated with acetic anhydride (1.5 mL, 16 mmol) and DMAP

(15 mg). The mixture was stirred for 8 h, when TLC showed no starting material. Methanol (2 mL) was added, to destroy an excess of the reagent, and stirring continued for 0.5 h, followed by the addition of CH_2Cl_2 (20 mL). The organic layer was washed with water, saturated aqueous Na_2CO_3, water, dried over Na_2SO_4, filtered, and evaporated to give a residue (45 mg), which on crystallization with CH_2Cl_2-methanol yielded **3.16b** (40 mg, 0.096 mmol, 88%), mp 204-207° C (from CH_2Cl_2-MeOH), (lit., mp 192-196° C, no solvent given, Kirk and Slade, 1982).

^{1}H NMR of 3.16b ($CDCl_3$): δ 7.18 (d, 8.6, 1H, 1-H), 6.97 (d, J 8.5, 1H, 2-H), 4.69 (dd, J 7.8, 8.9, 17α-H), 2.75 (dd, J 5.5, 17.3, 1H, 6α-H), 2.57 (m, 1-H, 6β-H), 2.30 (s, 3H, 4-OAc) and 2.27 (s, 3H 3-OAc), 2.06 (s, 17β-OAc), 0.82 (s, 3H, 13-Me_3) (lit., Frisen, 1991; Kirk and Slade, 1982).

^{13}C NMR of 3.16b: δ 123.51 (1), 119.93 (2), 140.03 (3), 140.07 (4), 130.82 (5), 27.55 (6), 26.28 (7), 37.52 (8), 44. 01 (9), 130.82 (10), 25.95 (11), 36.80 (12), 42.79 (13), 49.72 (14), 23.23 (15), 23.68 (16), 82.57 (17), 12.00 (18), 20.32 (3-OCO$\underline{C}H_3$), 168.1 (3-O$\underline{C}OCH_3$), 21.05 (17β-OCO$\underline{C}H_3$), 20.68 (4-OCO$\underline{C}H_3$), 168.6 (4-OCO$\underline{C}H_3$), 21.7 (17-O$\underline{C}OCH_3$), 171.11 (17-OCO$\underline{C}H_3$).

<u>For Scheme 3.14</u>

4ξ,5ξ-epoxyandrostane-3,17-dione 3.9a/3.9b

(a) <u>From testosterone 3.8</u>

To a stirred solution of testosterone 3.8 (100 mg, 0.347 mmol) in acetonitrile (8 mL), cooled in an ice-water bath, was added an aqueous Na_2EDTA solution (3 mL, $4x10^{-4}$M) followed by the addition of 1,1,1-trifluoroacetone (4 mL) by a precooled graduated cylinder. To

this solution was added in portions a mixture of sodium hydrogen carbonate (500 mg, mmol), and Oxone^{R} (1.25g, 4.07 mmol) over 10 min (pH = 7). After 3 hr of vigorous stirring, TLC showed no starting material. Water was added to the heterogenous mixture to dissolve the solid reagents, and the products were extracted with CH_2Cl_2. The organic layer was washed with water, dried, and evaporated to give a mixture of **3.9a/3.9b** (100 mg, 0.331 mmol, 95%), mp (TC) = 158-166 °C. The crystalline product consisted of the 4α,5α- **3.9a** and 4β,5β-epoxide **3.9b** in the ratio, α:β=4:1 based on the ^{1}H NMR spectrum.

(b) <u>From androstenedione **3.10**</u>

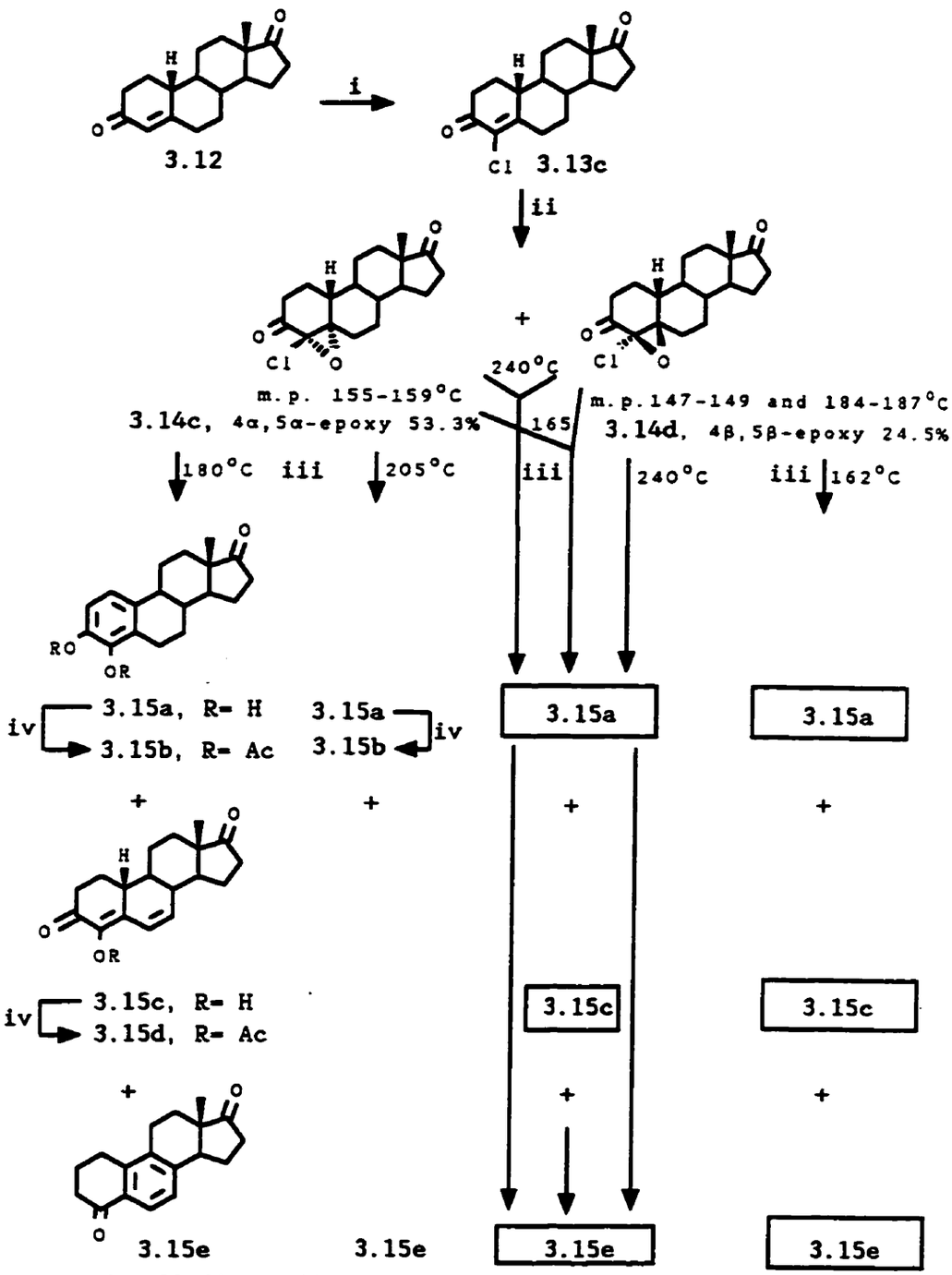
To a stirred solution of androstanedione **3.10** (1g, 3.49 mmol) in MeOH (100 mL), cooled in an ice-water bath was added 4M NaOH (5 mL) followed by cold 30% H_2O_2 (5 mL). After 20 min, TLC showed no starting material. The mixture was poured into cold water and the products extracted with diethyl ether. The organic layer was washed with cold water, brine, dried, filtered, and evaporated to give a residue (900 mg, 2.98 mmol, 85%), which consisted of the 4α,5α- **3.9a** and 4β,5β-epoxide **3.9b** in the ratio, α:β=1:4 based on the ^{1}H NMR spectrum.

3.4.0 Synthesis of 4-hydroxyestrone from 4-chloro-4,5-epoxy-5ξ-estra-3,17-diones *via* thermal rearrangements

Results and Discussion.

4-Chloroestr-4-ene-3,17-dione **3.13c**, in 74% yield, has been produced by chlorination of a solution of the estr-4-ene-3,17-dione **3.12** in pyridine with sulfuryl chloride as described by Mori for the 19-methyl analogue (Mori, 1962). Similar treatment of a solution of **3.12** in pyridine with a commercially available 1M solution of sulfuryl chloride in CH_2Cl_2 (Aldrich Inc.) also yielded 4-chloroestr-4-ene-3,17-dione **3.13c** (81%), (Scheme 3.16). When diluted sulfuryl chloride in methylene chloride was used, the reaction was cleaner than with the neat reagent.

Both dimethyldioxirane (DMD) and 1,1,1-trifluoromethyl(methyl)-dioxirane (FMD; Mello reagent) were employed to introduce oxygen into the 4,5-double bond of **3.13c** (Bovicelli *et al.*, 1992; Yang *et al.*, 1995). For epoxidation of the 4-chloroketone **3.13c**, in which the double bond is deactivated, both by conjugation and by chlorine substitution, longer reaction time and more than a stoichiometric amount of the reagent were required compared with similar compounds without a 4-chloro substituent (Bovicelli *et al.*, 1992). Initially, an attempt to epoxidize **3.13c** with DMD, was not fully successful. Even though excess of the reagent was used, the reaction was very slow, therefore the more reactive Mello reagent was employed (Mello *et al.*, 1989). Treatment of a solution of 4-chloroestr-4-ene-3,17-dione **3.13c** in dichloromethane with 1,1,1-trifluoromethyl(methyl)dioxirane in trifluoroacetone distilled directly into the reaction flask, cooled to -100 °C (Et_2O/solid CO_2), gave a mixture of the 4α,5α- and 4β,5β-chloroepoxides, **3.14c** and **3.14d**, respectively. The chloro-α-



Reagents: i, SO_2Cl_2; ii, FMD, CF_3COCH_3, CH_3CN and then FCC; iii, thermolysis at the indicated temp., 4 min.; iv, Ac_2O, DMAP, CH_2Cl_2.

Scheme 3.16 Synthesis of the chloro-epoxides, **3.14c** and **3.14d**, and their thermal α-halo-epoxide-carbonyl rearrangement products.

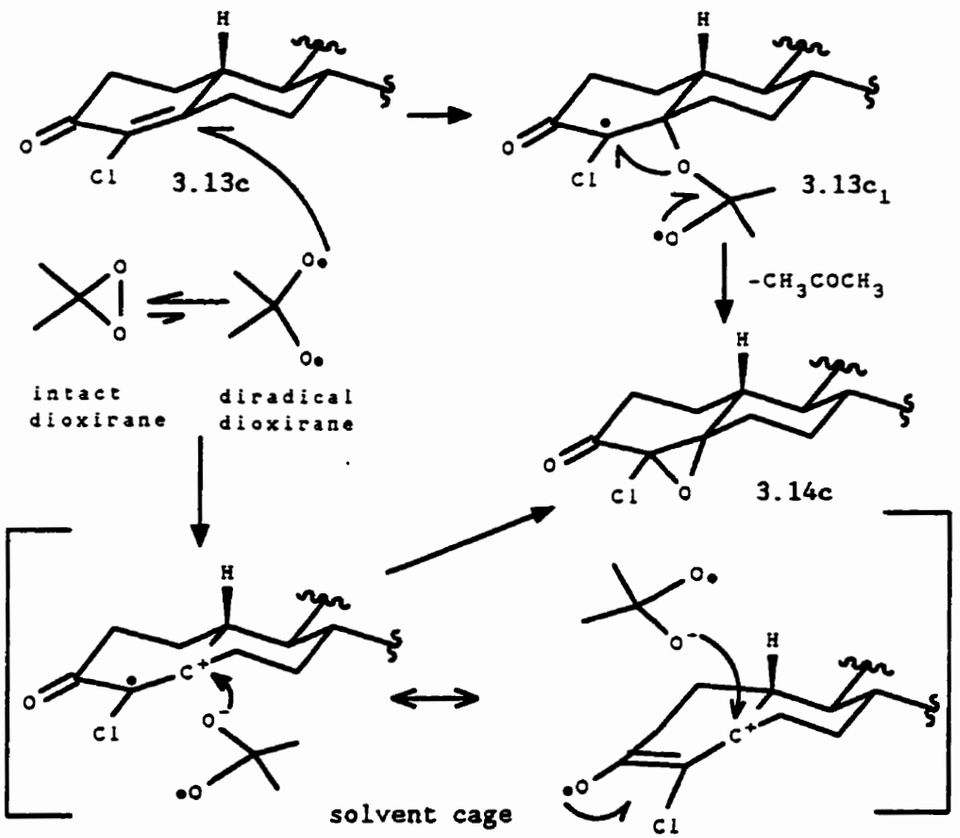
epoxide **3.14c** was the major product [**3.14c** (α):**3.14d**(β) = 7:3] bycomparison of their 6β-H in the ^{1}H NMR spectrum]. A low temperature distillation procedure for the preparation of these reagents was tedious, therefore the more convenient generation of the Mello reagent *in situ*, was employed (Yang *et al.*, 1995). The low temperature distillation procedure gave a mixture of the α- and β-chloroepoxides in a similar ratio; however, when FDM was generated *in situ* the ratio of diastereoisomers showed pronounced dependence on solvent (Table 3.2). Use of a solvent consisting of acetonitrile/CF_3COCH_3 (2:1) gave an epoxide distribution of 1:5.8 in favour of the 4α,5α isomer, while use of CH_2Cl_2/CF_3COCH_3 (50:50) solvent produced not only the 4α,5α-isomer as a major product, but also the 4β,5β epoxide in a significant amount (1:2.4).

Table 3.2 Diastereoselectivity in dioxiranes, DMD and FMD, epoxidations of **3.13c**.

Solvent system/(Dioxirane)	(%)	Epoxides ratio (4β,5β/4α,5α)
acetone/(DMD)	100	1:2.3
CF_3COCH_3-CH_2Cl_2/(FMD)	50:50	1:2.4
CF_3COCH_3-CH_3CN/(FDM)	33:67	1:5.8

Similarly, a solvent dependence on diastereoselectivity in the epoxidation of glycols in the synthesis of 1,2 anhydrosugars (Chow and Danishefsky, 1990) and cyclohex-2-en-ol (Murray *et al.*, 1995) was reported.

Compound **3.14d**, the β-epoxide, appeared to be quite unstable, apparently because of spontaneous rearrangement. During storage either at the ambient temperature or at 5 °C, it changed its appearance from white to pink, red, violet, and then black. The α-epoxide **3.14c** appeared to be stable under the same conditions. Energy calculations for the two isomers by PCmodel are in agreement with these observations. The α-epoxide **3.14c** is about 3.18 kcal mol^{-1} more stable than its β-epoxide **3.14d**. A proposed mechanism of the chloroalkene epoxidation is outlined in Scheme 3.17.



Scheme 3.17 Proposed mechanism of the epoxidation of the 4-chloro-estra-4-ene-3,17-dione.

The mechanism involves a single electron abstraction from the 4,5-double bond on the α-face by the diradical dioxirane to produce either a diradical species or the tight ion pair, a cation radical and an anion radical. A diradical species as well as the pair of anion radicals and cation radicals may react further in the solvent cage, to give the chloroepoxyketone **3.14c**. A similar reaction can occur on the β-face to give the 4β,5β-epoxy isomer. However, when the anion radical of dioxirane escapes from the cage and has a long enough life time, it may approach the cation radical from the α-face. This would explain the remarkable diastereoselectivity observed. Single electron transfer is more rapid than the usual oxygen-transfer oxidation of the intact dioxirane, which is a two-electron transfer process.

Unlike the pair of unsubstituted 4,5-epoxides **3.13a** and **3.13b** differentiation between the structures for the chloro-4ξ,5ξ-epoxides **3.14c** and **3.14d** was not possible from NMR data because of the lack of a proton at C-4. Confirmation of the stereochemistry assigned to the 4α,5α-epoxide **3.14c** was obtained by X-ray crystallography establishing the stereochemistry of both isomeric epoxides **3.14c** and **3.14d** (Figure 3.1). A summary of the crystal data and structure refinement details for the structure determination of **3.14c** is given in Table 3.3.

It was observed that conditions of thermolysis (temperature, a sealed tube) greatly affected formation of products, Scheme 3.16. Dependence of temperature on formation of thermolysis products is shown in Table 3.4. Thermolysis of a mixture of 4-chloro-4ξ,5ξ-epoxides, **3.14c** and **3.14b**, at 165 °C, in a sealed ampoule, yielded three products 4-hydroxyestrone **3.15a**, 4-hydroxyestr-4,6-diene-3,17-one **3.15c**, and estra-5,7,9-triene-4,17-dione **3.15e**, which were separated by flash column chromatography (**3.15a**:**3.15c**:**3.15e**; 1.8:2.6:1).

Table 3.3 Crystallographic data for 4β-chloro-4,5-epoxy-5α-estra-3,17-dione **3.14c**.

compound 3.14c	
Formula	$C_{18}H_{23}O_3Cl$
M_r	322.83
T(°K)	299
Crystal Size (mm)	0.45 x 0.30 x 0.25
Crystal System	Orthorombic
Space Group	$P2_12_12_1$ (no.19)
Cell Dimensions:	
a/Å	11.953(2) [a]
b/Å	18.644(2)
c/Å	7.2521(2)
α, β, γ (°)	90/90/90
Cell Volume/Å^3	1616.1(6)
Z	4
D_{calc}/g cm^{-3}	1.327
F(000)	688
λ/Å (MoKα)	0.71069
$\mu_{(MoK\alpha)}$/cm^{-1}	2.43 (mmm^{-1} or cm^{-1})
Θ min. and max. (°)	9.08-23.39
2Θ max. (°)	50.2
Independent Reflections	1699
Observed Reflections (I.2.00σ(I))	1344
Function Minimized	$\Sigma w(\|F_o\| - \|F_c\|)^2$
Least-square Weights	$4F_o^2/\sigma^2(F_o^2)$
R	0.042
R_w	0.038

[a]Estimated standard deviations in parentheses refer to the last digit.

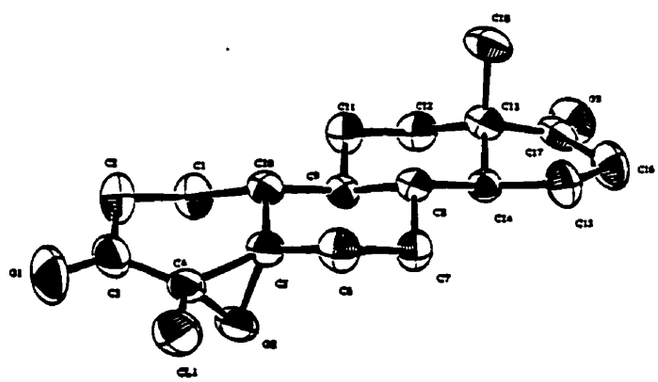


Figure 3.1 X-Ray molecular structure of 4β-chloro-4,5-epoxy-5α-estra-3,17-dione **3.14c**

Table 3.4 Products[a] of thermolysis of compounds **3.14c**, **3.14d**, and their mixture (**3.14c+3.14d**):

Compound	t(°C)	Products (%)[b]		
		3.15a	**3.15c**	**3.15e**
3.14c 4α,5α-epoxide	180	35	33	32
3.14c 4α,5α-epoxide	205	53	--	47
3.14d 4β,5β-epoxide	162	32	41	27
3.14d 4β,5β-epoxide	240	59	--	41
3.14c+3.14d	165	33	33	33
3.14c+3.14d	240	56	--	44

[a] thermolysis conducted in a sealed tube

[b] ratio of products determined based on their ^{1}H NMR spectra

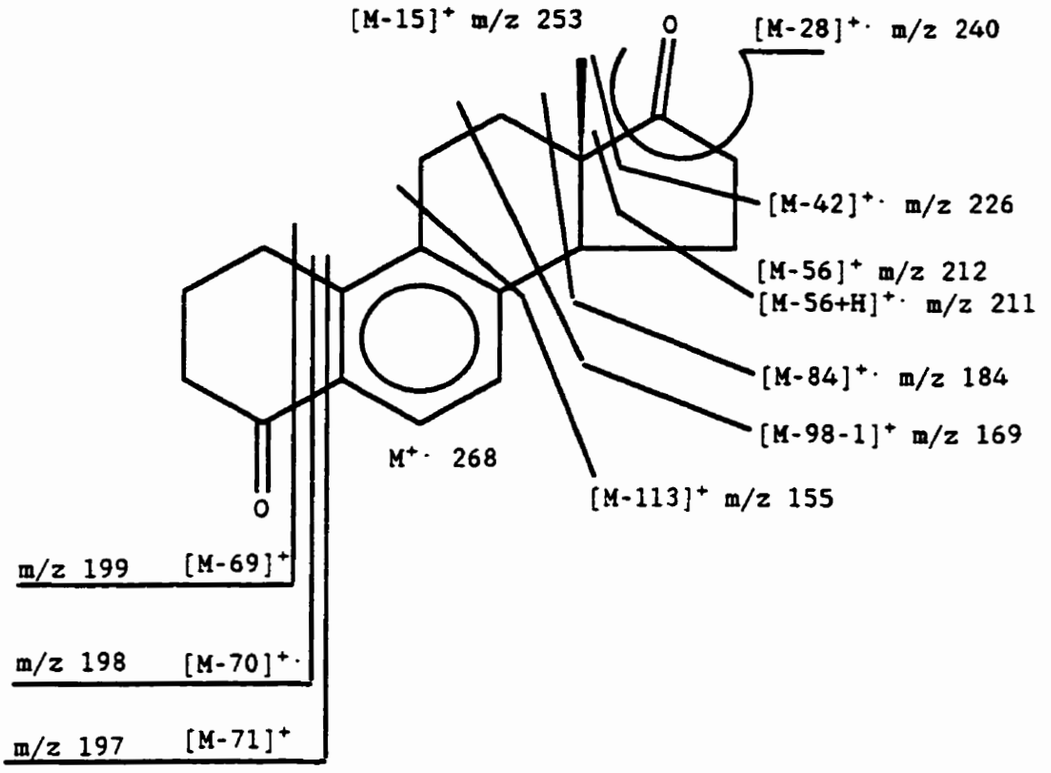
The NMR spectra (^{1}H and ^{13}C) of 4-hydroxyestrone **3.15a** were in agreement with the sample reported above. A separate thermolysis reaction of chloro-α-epoxide **3.14c** at 180 °C, in a sealed ampoule, followed by acetylation of the crude product, again gave three compounds (**3.15b**:**3.15d**:**3.15e**; 2.2:3:1). Similar thermolysis of chloro-α-epoxide **3.14c** at 205 °C, followed by acetylation of the crude product and separation on FCC furnished two products (**3.15b**:**3.15e**; 1.4:1). Thermolysis of chloro-β-epoxide was performed at 162 °C and 240 °C and yielded, respectively, three (**3.15a**:**3.15c**:**3.15e**; 1.8:1.9:1) and two (**3.15a**:**3.15d**; 2.4:1) products. Their ratio was determined by comparison of the appropriate peaks in their ^{1}H NMR spectra (see the experimental part).

In conclusion, thermolysis of the chloro-4α,5α-epoxide **3.14c** or the chloro-4β,5β-epoxide **3.14d** or their mixture, **3.14c** and **3.14d**, conducted in sealed tubes at 50-60 °C above their melting points, produces two aromatic compounds: 4-hydroxyestrone **3.14a** and estra-5,7,9-triene-4,17-dione **3.15e**. On the other hand, thermolysis of **3.14c** or **3.14d** or

a mixture of **3.14c** and **3.14d** in a sealed tube, but conducted just 20 - 30° C above their melting points, gives one product more, 4-hydroxyestra-4,6-diene-3,17-dione **3.15c**, in addition to the two aromatic compounds **3.15a** and **3.15e**.

4-Hydroxyestra-4,6-diene-3,17-dione **3.15c** was identified by comparison of the ^{1}H and ^{13}C NMR spectra with those of the 10-Me analogue previously reported (Templeton *et al.*, 1988). The structure of **3.15e** was established from ^{1}H and ^{13}C NMR, IR, EIMS, and elemental analysis as follows. In the ^{1}H NMR spectrum a pair of doublet signals at δ 7.11 ppm (J_{H6-H7} 8Hz) and δ 7.94 ppm (J_{H6-H7} 8 Hz) were assigned, respectively, to the C-6 and C-7 aromatic hydrogens of ring B. Downfield shift of the signals of 1 ppm from the normal aromatic region can be attributed to the presence of the carbonyl group at C-4. The ^{13}C NMR spectra of **3.15e** showed the presence of seven methylene, three methine, and seven quaternary carbons. Two quaternary carbons at δ 219.21 and δ 198.39 were assigned to the C-17 and C-4 carbonyl groups, respectively. The ^{1}H-^{1}H and ^{1}H-^{13}C correlation was assigned by COSY and HSQC analysis. The IR spectrum showed an unsaturated carbonyl group (1660 cm^{-1}), consistent with a conjugated ketone, and aromatic bands (1658, 1591, 1575 cm^{-1}). The elemental analysis confirmed a structure of $C_{18}H_{20}O_2$. The electron ionization mass spectra (EIMS) showed the molecular ion, $M^{\cdot +}$ 268, as the base peak. The major ions in the high mass spectral region are as follows: m/z 253 (7), 240 (14), 226 (42), 212 (65.5), 211 (83), 197 (26), 198 (21), 199 (35), 184 (23), and 169 (26), 155 (35) (relative abundance is given in parentheses). These ions arise from the competetive fragmentations of the molecular ions by decompositions of rings A, C, and D. Proposed fragmentations of rings A, C, and D are shown in Scheme **3.18**. As outlined in Scheme 3.19, for

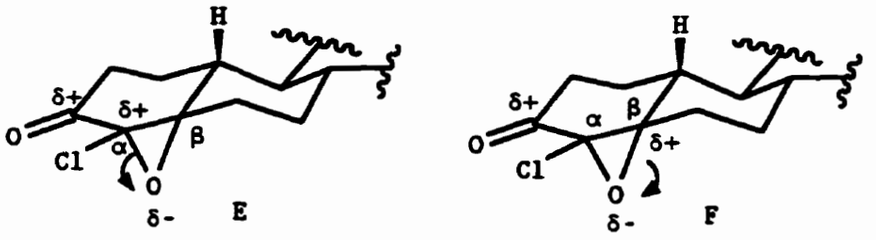
the 1,2 disubstituted conjugated epoxyketone **3.14c**, there are two possible ways for the epoxy ring opening, either at the α-carbon or the β-carbon. However, in **E**, the repulsive interaction between two positive



Scheme 3.18 Proposed fragmentations of rings A, C and D of compound **3.15e**.

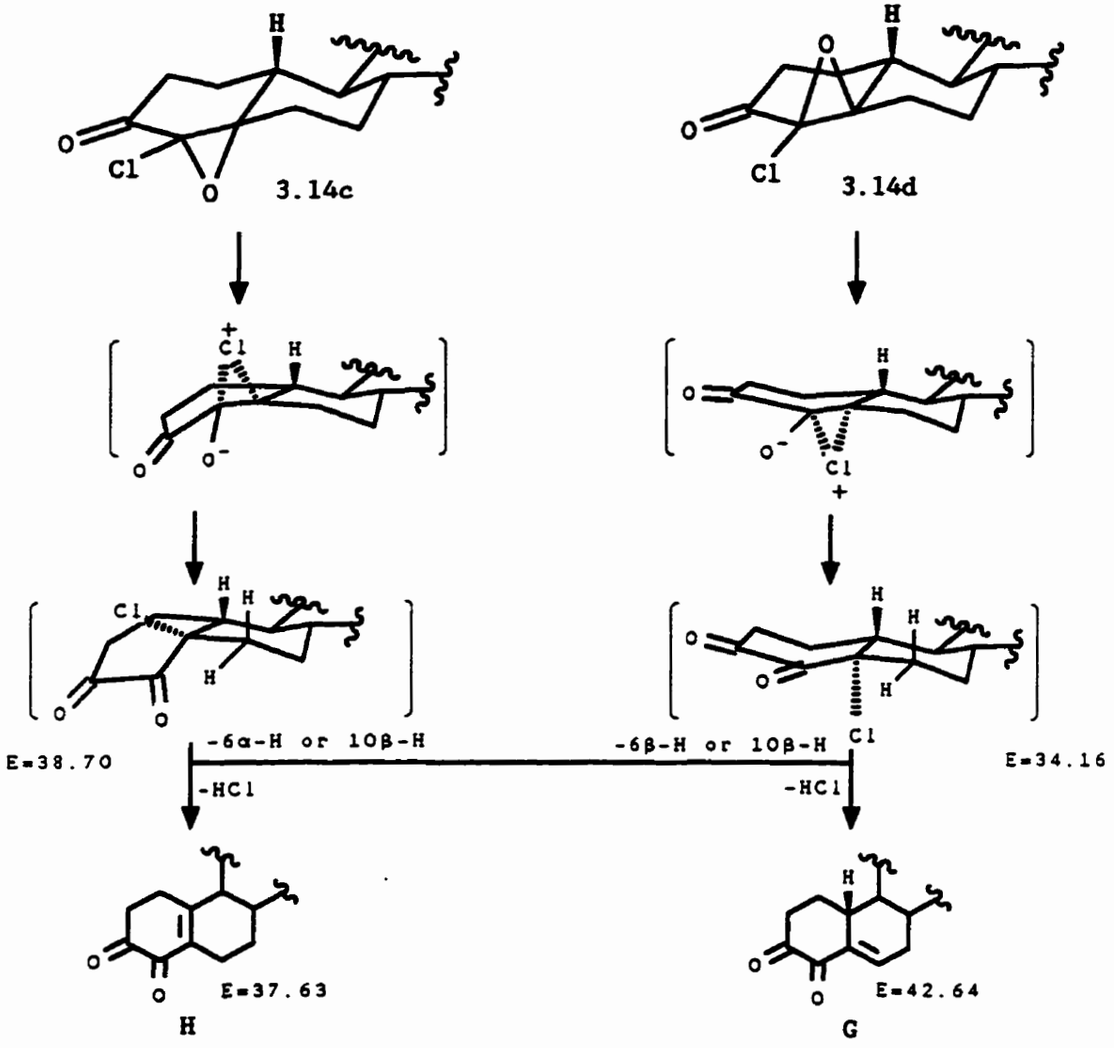
centers will lead to a substantial increase in the energy of activation. On the other hand, in **F**, the repulsion between two of the positive centers is diminished as a function of the distance between them. Therefore, the energy of activation for the path involving opening toward the electron withdrawing groups (**F**) will be less than for the opening of the epoxy ring in the opposite direction (**E**). Both

the 3-carbonyl group and the 4-chloro substituent contribute to the polar effect, which directs the epoxy ring opening. Possible rearrangement mechanisms of the chloroketones **3.14c** and **3.14d** based on the product determination are proposed in Schemes 3.20-3.23.

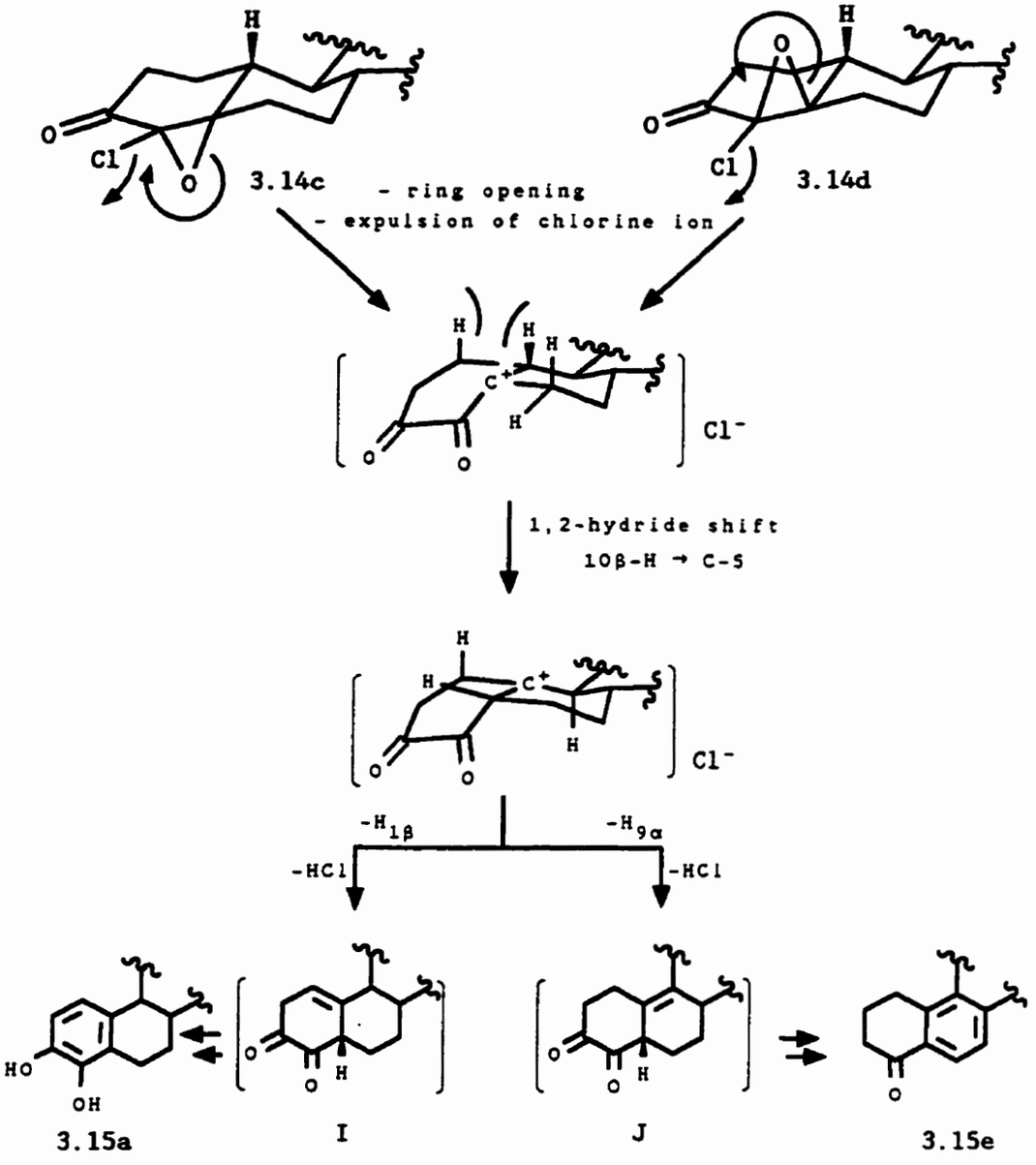


Scheme 3.19 Possible ways of ring opening of 1,2-diconjugated ketone **3.14c**.

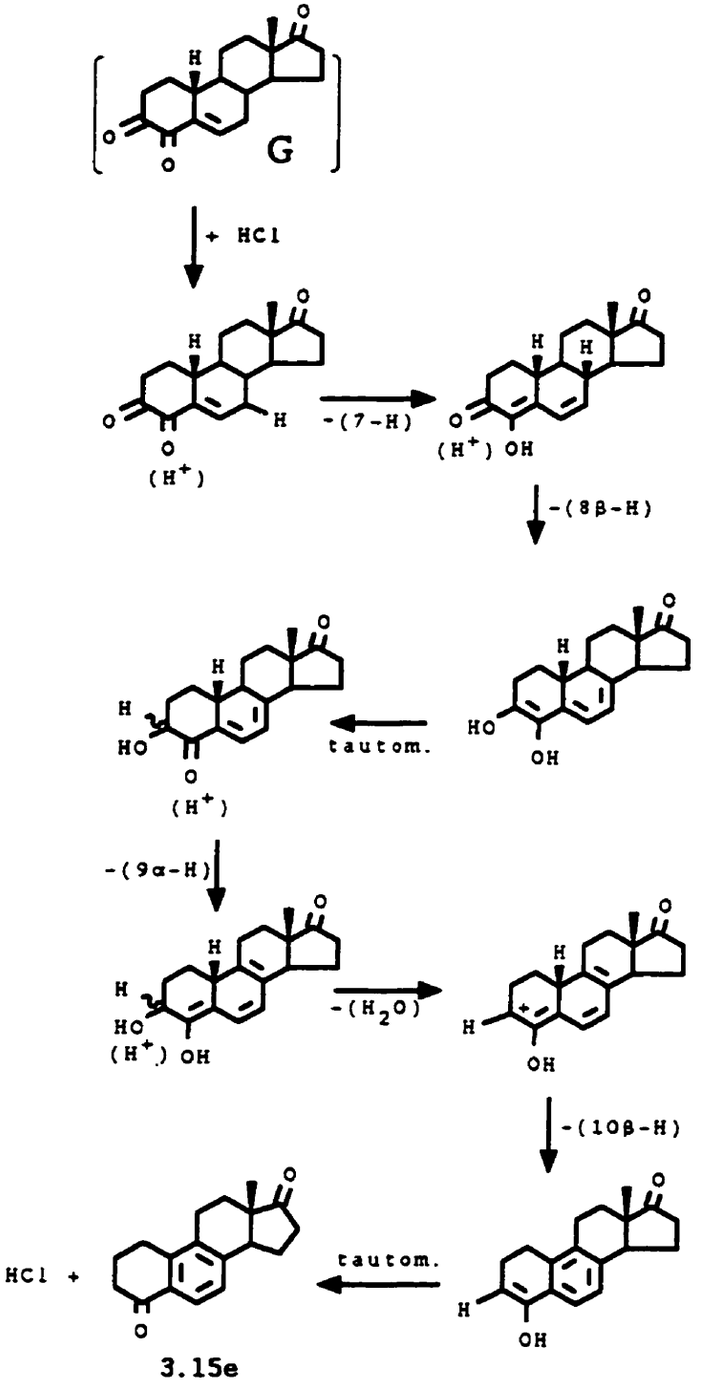
Thermolysis at 248 °C of the diene **3.15c** gave neither the catechol **3.15a** nor the aromatic derivative **3.15e** suggesting that it is not an intermediate in the formation of either **3.15a** and **3.15e**. However, the lack of a HCl molecule which involved an initiation of the rearrangement process should be taken into consideration.



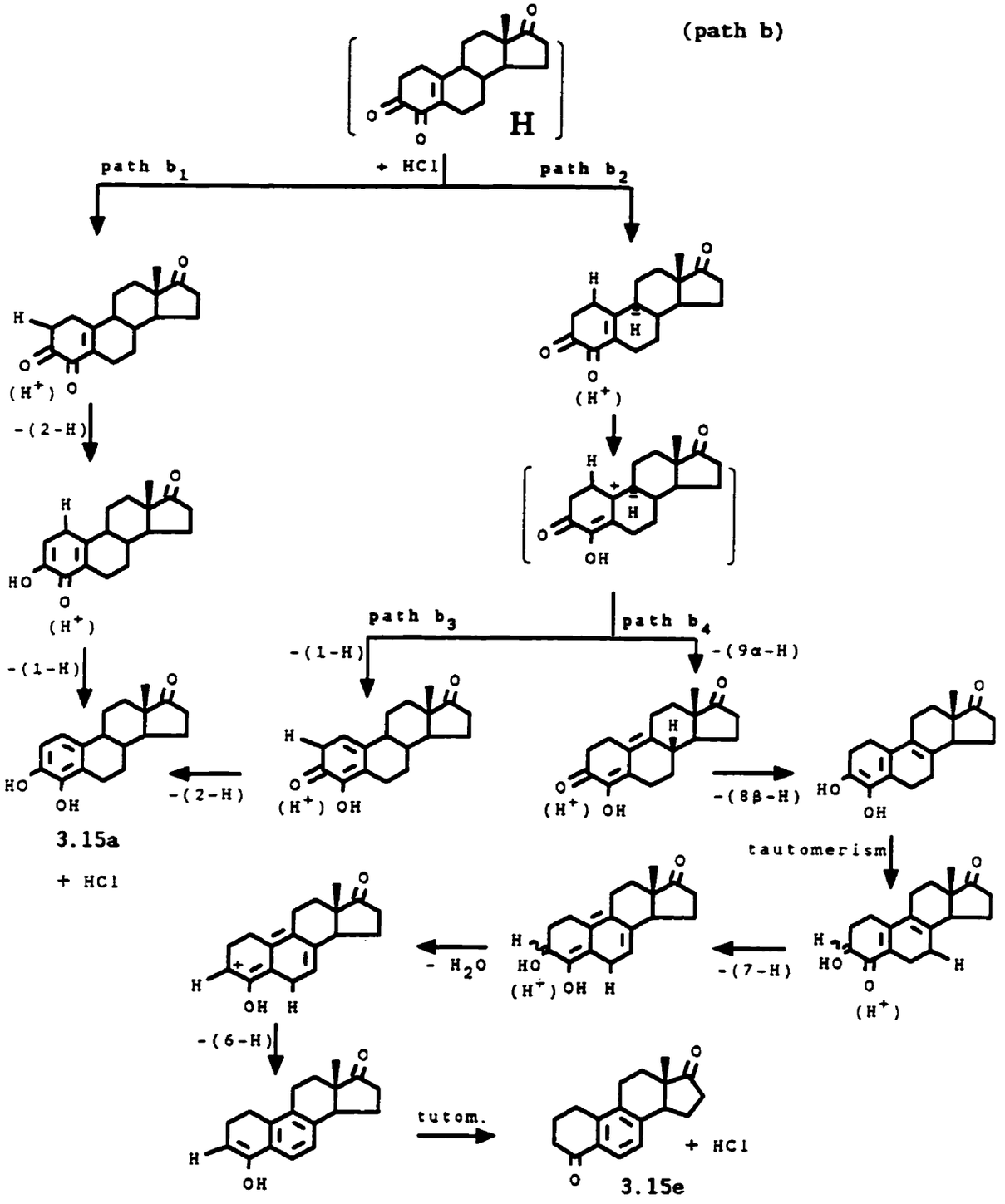
Scheme 3.20 Proposed mechanism of formation of the intermediates G and H *via* chlorine rearrangement from C-4 to C-5 (E in kcal/mol).



Scheme 3.21 Proposed mechanism of formation of intermediates of **I** and **J**, the precursors of **3.15a** and **3.15e**, *via* 1,2 hydride shift of 10β-H to C-5.



Scheme 3.22 Proposed mechanism of the formation of **3.15b** (6-H and 7-H elimination) and **3.15e** (6-H, 7-H, 8β-H, 9α-H, and 10β-H eliminations).



Scheme 3.23 Proposed mechanism of the formation of **3.15a** (10β-H, 2-H, and 1-H eliminations) and **3.15e** (10β-H, 9α-H, 8β-H, 7-H, and 6-H eliminations).

3.4.1 Summary

1. Thermolysis of either 4β-chloro-4,5-epoxy-5α-estra-3,17-dione **3.14c** or 4α-chloro-4,5-epoxy-5β-estra-3,17-dione **3.14d** or their mixture in a sealed tube at 50-60 °C above the melting point of the pure compounds, produces two aromatic compounds: 4-hydroxyestrone **3.15a** and estra-5,7,9-triene-4,17-dione **3.15e**.
2. Thermolysis of either 4β-chloro-4,5-epoxy-5α-estra-3,17-dione **3c** or 4α-chloro-4,5-epoxy-5β-estra-3,17-dione **3.14d** or their mixture in a sealed tube at 20-30 °C above the melting point of the pure compounds, produces a third compound, 4-hydroxyestra-4,6-diene-3,17-dione **3.15c**, in addition to the two aromatic products, 4-hydroxyestrone **3.15a** and estra-5,7,9-triene-4,17-dione **3.15e**. Aromatase inhibition properties of **3.15c** and **3.15d** are suggested to be evaluated.
3. A new compound estra-5,7,9-triene-4,17-dione **3.15e**, with aromatic ring B, has been obtained. Its biological evaluation for estrogenic activity has been suggested.

3.4.2 Experimental for Scheme 3.16

4-Chloroestr-4-ene-3,17-dione 3.13c

Chlorination of 3.12 with neat SO_2Cl_2:

To a stirred solution of estr-4-ene-3,17-dione **3.12** (3.0 g, 11.0 mmol) in dry pyridine (15 mL), cooled in an ice-water bath, was added over 10 min, freshly distilled sulfuryl chloride (1.8 mL, 22 mmol, 2 eq.). The mixture was stirred at ambient temperature for 2 h to allow dichloro products to be converted to the desired compound **3.16**, and then the mixture was poured into cold 5% HCl (400 mL) and stirred for 1/2 hr. The products were extracted with diethyl ether. The organic layer was

washed with 10% HCl (2x), cold water (2x), saturated aqueous $NaHCO_3$ (2x), water until neutral, brine, dried over Na_2SO_4, filtered, and evaporated to yield a creamy residue which on flash chromatography, on elution with 25% EtOAc, gave fractions of 4-chloroketone **3.13c** (2.5 g, 8.14 mmol, 74%), mp 172-180 °C. Three recrystallizations gave **3.13c** (2.4 g, 7.8 mmol, 71%), mp 180-182 °C (from CH_2Cl_2-MeOH).

The ^1H and ^{13}C NMR spectra of **3.13c** are given below.

Chlorination of **3.13c** with 1 M SO_2Cl_2 in CH_2Cl_2:

To a stirred solution of estr-4-ene-3,17-dione **3.12** (2.02 g, 7.4 mmol) in dry pyridine (20 mL), cooled in an ice-water bath, was added, by syringe, 1M SO_2Cl_2 in CH_2Cl_2 (14.88 mL, 2 eq.) over 15 min. The mixture was stirred for 1 hr at 0 °C and then for 2 hr at room temperature, when no starting material was detected on TLC. The mixture was poured into a cold 5% HCl solution (300 mL), and the products were extracted with diethyl ether. The organic layer was washed with water, saturated $NaHCO_3$, water, dried, filtered, and evaporated to give a creamy residue (2.6 g), which on FCC, on elution with 20% EtOAc-LP, gave fractions of **3.13c** (1.74 g, 0.06 mmol, 81%), mp 180-182 °C (from CH_2Cl_2-MeOH), (Found: C, 70.24, H, 7.52, Cl, 11.69. $C_{18}H_{23}ClO_2$ (MW 306.836) requires C, 70.46; H, 7.55; Cl, 11.55%).

^1H NMR of **3.13c** ($CDCl_3$): δ 3.41 (ddd, J 1.8, 2.4, 14.8 Hz,1H, 6α-H), 2.65 (ddd, J 4.30, 4.61, 16.22 Hz, 1H, 7α-H, , 2.49 (dd, J 8.81, 8.83 Hz, 1H, 16β-H). 0.94 (s, 3H, 13-Me).

^{13}C NMR of **3.13c** ($CDCl_3$): δ 25.63 (1), 36.91 (2), 190.89 (3), 127.49 (4), 159.39 (5), 31.81 (6), 29.23 (7), 39.63 (8), 42.31 (9), 44.68 (10), 25.39 (11), 31.26 (12), 47.63 (13), 50.06 (14), 21.60 (15), 35.71 (16), 219.99 (17), 13.79 (18).

4-Chloro-4,5-epoxy-5α-estra-3,17-dione 3.14c and 4-Chloro-4,5-epoxy-5β-estra-3,17-dione 3.14d

(a) Epoxidation of 3.13c with Mello reagent at -78 °C:

1,1,1-Trifluoromethy(methyl)dioxirane solution (0.5-0.8 M 1,1,1-trifluoro-2-propanone in CF_3COCH_3; 12 mL, 6.0 - 9.6 mmol) was directly distilled into a reaction flask (low-temperature distillation, -75 to -100 °C) containing 4-chloroestr-4-ene-3,17-dione **3.13c** (0.5 g, 1.63 mmol) in dichloromethane (10 mL) at *ca* -100 °C (Et_2O/solid CO_2). The organic mixture containing Na_2SO_4 (500 mg), was allowed to stand at -15 °C in the dark, until no further change (75 hr) was observed by TLC. However, because of the presence of the unreacted starting material **3.13c**, after filtration and evaporation of the solvent, the epoxidation process, with freshly prepared reagent (12 mL), was repeated. After a further 75 hr, under the same reaction conditions, TLC showed only a trace of the starting material. Filtration and evaporation of the solvents yielded a yellow residue containing 4α,5α- **3.14c** and 4β,5β-epoxides **3.14d** (α:β = 1:0.72, estimated from the ^1H NMR spectrum or α:β = 1 : 0.47 estimated from FCC fractions) which on FCC on elution with 15 - 25% EtOAc-LP, yielded fractions in order of their elution:

(*i*) *4β,5β-epoxide* **3.14d** (0.131 g), which, after crystallization gave (127 mg, 0.393 mmol, 24.1%), mp 147-149 °C and 184-187 °C (dec.) (from CH_2Cl_2-MeOH), (Found: C, 67.06 ; H, 7.23; Cl, 10.81. $C_{18}H_{23}O_3Cl$ (MW 322.835) requires C, 66.97; H, 7.18; Cl, 10.98%);

^1H NMR of 3.14d ($CDCl_3$): δ 2.49 (ddd, J 1.91, 2.35, 19.03 Hz, 1H, 6β-H), 0.92 (s, 3H, 13-Me), 2.49 (dd, J 8.81, 8.83 Hz, 1H, 16β-H).

^{13}C NMR of 3.14d ($CDCl_3$): δ 16.91 (1), 31.86 (2), 196.38 (3), 82.11 (4), 71.67 (5), 28.31 (6), 30.36 (7), 40.15 (8), 44.17 (9), 41.10 (10),

21.69 (11), 35.65 (12), 47.74 (13), 50.03 (14), 25.62 (15), 31.08 (16), 219.77 (17), 13.90 (18).

(ii) *4ξ,5ξ-epoxides* **3.14c/3.14d** (0.30 mg, 0.093 mmol, 5.7%) and

(iii) *4α,5α-epoxide* **3.14c** (281 mg) which after crystallization gave (275 mg, 0.852 mmol, 52.3%) mp 155-159 °C (from CH_2Cl_2-MeOH). (Found: C, 67.01 ; H, 7.20; Cl, 10.77 $C_{18}H_{23}O_3Cl$ (MW 322.835) requires C, 66.97; H, 7.18; Cl, 10.98%);

^{1}H NMR of **3.14c** ($CDCl_3$): δ 2.65 (ddd, J 2.02, 2.46, 19.24 Hz, 1H, 6β-H), 2.49 (dd, J 8.81, 8.83 Hz, 1H, 16β-H), 0.93 (s, 3H, 13-Me).

^{13}C NMR of **3.14c** ($CDCl_3$): δ 20.61 (1), 35.71 (2), 195.89 (3), 82.56 (4), 69.65 (5), 27.27 (6), 29.56 (7), 39.47 (8), 46.18 (9), 41.27 (10), 21.62 (11), 31.29 (12), 36.25 (13), 49.95 (14), 25.50 (15), 31.29 (16), 220.15 (17), 13.78 (18).

(b) Epoxidation of **3.13c** with Mello reagent *in situ* at ±5 °C

To a stirred solution of the 4-chloroketone **3.13c** (1.0 g, 3.26 mmol) in acetonitrile (50 mL), cooled in an ice-water bath, was added aqueous Na_2EDTA solution (20, mL, 4 x 10^{-4} M) followed by the addition of 1,1,1-trifluoroacetone (25 mL; bp 22 °C) in a precooled graduated cylinder. To this solution was added a mixture of sodium hydrogen carbonate (15 g, 0.183 mol) and Oxone^R (25 g, 0.08 mmol) over a period of 1 h (pH = 7). After 24 h, TLC showed still the presence of the starting material **3.13c**. Therefore, the heterogenous mixture was again treated with the same amount of the above reagents. Although after 24 h, TLC still showed the presence of the chloroketone **3.13c** (starting material), the reaction was terminated. The heterogenous mixture was poured into water (150 mL), and products were extracted with dichloromethane. The organic layer was washed with water, dried over

Na_2SO_4, filtered, and evaporated to give an oily residue which on FCC, on elution with 15-25% EtOAc-LP, yielded fractions given in order of their elution: the chloro-4β,5β-epoxide **3.14d** (60 mg, 0.186 mmol, 6%), the chloro-4α,5α-epoxide **3.14c** (360 mg, 1.12 mmol, 35%), and the starting material, 4-chloroketone **3.13c** (70 mg, 0.23 mmol, 7%), mp 180-182 °C.

A. Thermolysis of chloro-4,5-epoxides in a sealed tube: ratio of products determined based on the ^{1}H NMR spectra.

(i) Thermolysis of 4β-chloro-4,5-epoxy-5α-estra-3,17-dione **3.14c**:

Compounds **3.15a, 3.15c, and 3.15e**. Thermolysis of the 4α,5α-epoxy **3.14c** (220 mg) in a sealed ampoule at 180 °C gave three products: 4-hydroxyestrone **3.15a** (35%), 4-hydroxyestra-4,6-diene-3,17-dione **3.15c** (32%), and estra-5,7,9-triene-3,17-dione **3.15e** (32%) based on their ^{1}H NMR spectra. The mixture was then acetylated (see below).

Compounds **3.15a and 3.15e.** Thermolysis of the same compound **3.14c** (120 mg), under the above conditions, but at a higher temperature, 205 °C, yielded only two products: estra-5,7,9-triene-4,17-dione **3.15e** (40.5%) and 4-hydroxyestrone **3.15a** (50%), based on their ^{1}H NMR spectra. The mixture was further acetylated (see below).

(ii) Thermolysis of 4α-chloro-4,5-epoxy-5β-estra-3,17-dione **3.14d**:

Compounds **3.15a, 3.15c, and 3.15e.** Thermolysis of the 4β,5β-*epoxy* **3.14d** (10 mg), in a sealed melting point tube at 162 °C, gave three products: 4-hydroxyestrone **3.15a** (32%), 4-hydroxyestra-4,6-diene-3,17-dione **3.15c** (41%), and estra-5,7,9-triene-4,17-dione **3.15e** (21%) based on their ^{1}H NMR spectra.

Compounds 3.15a and 3.15e. Thermolysis of 3.14d (10 mg), under the above conditions but at higher temperature, 240 °C, gave only two products: 4-hydroxyestrone 3.15a (36%) and estra-5,7,9-triene-4,17-dione 3.15e (29%) based on their ^{1}H NMR spectra

(iii) Thermolysis of a mixture of 4 ξ-chloro-4,5-epoxy-5ξ-estra-3,17-dione 3.14c and 3.14d:

Compounds 3.15a, 3.15c, and 3.15e. Thermolysis of a mixture of the chloro-4ξ,5ξ-epoxides, 3.14c and 3.14d, (570 mg), in a sealed ampoule at 165 °C, gave three products: 4-hydroxyestrone 3.15a (33%), 4-hydroxyestra-4,6-diene-3,17-dione 3.15c (33%), and estra-5,7,9-triene-4,17-dione 3.15e (33%) based on their ^{1}H NMR spectra.

Compounds 3.15a and 3.15e. Thermolysis of a mixture of the chloro-4ξ,-5ξ-epoxides, 3.14c and 3.14d, (295 mg), in a sealed ampoule at 240 °C, gave two products: 4-hydroxyestrone 3.15a (55%) and estra-5,7,9-triene-4,17-dione 3.15e (44%) based on their ^{1}H NMR spectra.

B. Thermolysis of a mixture of 4ξ-chloro-4,5-epoxy-5ξ-estra-3,17-dione 3.14c and 3.14d: ratio determined after FCC

***(i)* 4-hydroxyestrone 3.15a, 4-hydroxyestra-4,6-diene-3,17-dione 3.15c, and estra-5,7,9-triene-4,17-dione 3.15e**

A mixture of the chloro-4ξ,5ξ-epoxides, 3.14c and 3.14d, (570 mg, 1.77 mmol), in a sealed ampoule, was immersed in an oil bath preheated to 165 °C until effervescence ceased (5 min). The crude product on FCC on elution with 10% EtOAc-LP, gave fractions: 4-hydroxyestrone 3.15a (110 mg, 0.41 mmol, 23.3%), 4-hydroxyestra-4,6-diene-3,17-dione 3.15c (170 mg, 0.594 mmol, 33.5%), mp 234-235 °C (from CH_2Cl_2-EtOAc), (Found: C,

75.16; H, 7.74. $C_{18}H_{22}O_3$ (MW 286.374) require C, 75.49; H, 7.74), and estra-5,7,9-triene-4,17-dione **3.15e** (60 mg, 0.223 mmol, 13%), mp 128-130 °C (from CH_2Cl_2), (Found C, 80.25; H, 7.80. $C_{18}H_{20}O_2$ (MW 268.359) requires C, 80.56; H, 7.51%)

^{1}H NMR of 3.15c ($CDCl_3$): δ 6.78 (dd, J 2.87, 9.88 Hz, 1H, 6-H), 6.23 (s, 1H, 4-OH), 6.15 (dd, J 2.08, 9.84 Hz, 1-H, 7-H), 0.96 (s, 3H, 13-Me).

^{13}C NMR of 3.15c ($CDCl_3$): δ 24.55 (1), 26.51 (2), 194.22 (3), 129.11 (4), 141.17 (5), 136.13 (6), 122.93 (7), 46.42 (8), 40.29 (9), 39.63 (10), 31.29 (11), 35.47 (12), 48.61 (13), 48.36 (14), 21.40 (15), 35.64 (16), 219.70 (17), 13.69 (18).

^{1}H NMR of 3.15e ($CDCl_3$): δ 7.94 (d, J 8.1, 1H, 6-H), 7.10 (d, J 8.02, 1H, 7-H), 3.01 (dd, J 12.44, 5.92, 1H, 14α-H), 0.75 (s, 3H, 13-Me).

^{13}C NMR of 3.15e ($CDCl_3$): 38.44 (1), 22.52 (2), 24.22 (3), 198.39 (4), 134.16 (5), 123.45 (6), 125.07 (7), 142.84 (8), 143.35 (9), 131.36 (10), 26.24 (11), 28.89 (12), 46.86 (13), 47.22 (14), 21.45 (15), 36.42 (16), 219.21 (17), 13.60 (18).

(ii) **4-hydroxyestrone 3.15a and estra-5,7,9-triene-4,17-dione 3.15e**

A mixture of the chloro-4ξ,5ξ-epoxides, **3.14c and 3.14d**, (295 mg, 0.914 mmol), in a sealed ampoule, was immersed in an oil bath preheated to 240 °C until effervescence ceased (5 min). The crude product, on FCC on elution with 10% EtOAc-LP gave fractions: 4-hydroxyestrone **3.15a** (50 mg, 0.173 mmol, 19%), and estra-5,7,9-triene-4,17-dione **3.15e** (70 mg, 0.26 mmol, 28.5%), mp 128-130 °C (from CH_2Cl_2).

C. **Thermolysis of a mixture of 4ξ-chloro-4,5-epoxy-5ξ-estra-3,17-dione 3.14c and 3.14d under reduced pressure; *ratio of products determined after FCC.***

4-hydroxyestrone 3.15a and 4-hydroxyestra-4,6-diene-3,17-dione 3.15c

A flask containing a mixture of **3.14c** and **3.14d** (114 mg, 0.353 mmol), attached to a water pump, was immersed in an oil-bath preheated to 230 °C until effervescence ceased (4 min). The brown residue on flash chromatography, on elution with 25% EtOAc-LP, gave fractions: 4-hydroxyestrone **3.15a** (18.2mg, 0.063 mmol, 18%), mp 263-265 °C (from AcOEt-$CHCl_3$-2% CH_3COOH), [lit., (i) mp 268-271 °C, Gelbke *et al.*, 1973; (ii) mp 266-270 °C dec., from benzene-methanol, Fischman *et al.*, 1960], and 4-hydroxyestra-4,6-diene-3,17-dione **3.15c** (57.4 mg, 0.2 mmol, 56.6%), mp 234-235 °C (from CH_2Cl_2-EtOAc).

D. **Acetylation of thermolysis products from 4β-chloro-4,5-epoxy-5α-estra-3,17-dione 3.14c:**

(i) **4-Hydroxyestrone 3,4-diacetate 3.15b, 4-Hydroxyestra-4,6-diene-3,17-dione 4-acetate 3.15d and Estra-5,7,9-triene-3,17-dione 3.15e**

The chloro-α-epoxide **3.13c** (220 mg, 0.35 mmol), in a sealed ampoule, was immersed in an oil bath preheated to 180 °C until effervescence ceased (ca 4 min). The product in CH_2Cl_2 (20 mL) was treated with Ac_2O (1.8 mL) and DMAP (10 mg) for 2 h, when no starting material was detected by TLC. The reaction mixture was treated with methanol (2 mL), to destroy excess reagent, and then poured into water. The organic layer was washed with water, saturated sodium bicarbonate, dried over Na_2SO_4, filtered, and evaporated to give a glassy residue, which on

flash chromatography, on elution with 30-50% Et_2O-LP, yielded fractions: (*i*) 4-hydroxyestrone 3,4-diacetate **3.15b** (40 mg, 0.11 mmol, 31.4%), mp 217-220 °C (from EtOAc-LP), (Found: C, 71.26; H, 7.06. $C_{22}H_{26}O_5$ (MW 370.449) requires C, 71.33; H, 7.07), (lit., 212.5-215.5 °C, from benzene-cyclohexane, Gold and Schwenk, 1958), (*ii*) 4-hydroxy-estra-4,6-diene-3,17-dione 4-acetate **3.15d** (44 mg, 0.144 mmol, 41.3%), mp 216-218 °C (from CH_2Cl_2-EtOAc), (Found: C, 73.08; H, 7.46; $C_{20}H_{24}O_4$ (MW 304.39) requires C, 73.15; H, 7.37%), and (*iii*) estra-5,7,9-triene-3,17-dione **3.15e** (20 mg, 14%), mp 128-130 °C (from CH_2Cl_2).
^1H and ^{13}C NMR of **3.15b**, **3.15d**, and **3.15e** see above.

(*ii*) **4-Hydroxyestrone 3,4-diacetate 3.15b and Estra-5,7,9-triene-3,17-dione 3.15e**

The chloro-α-epoxide **3.14c** (120 mg, 0.372 mmol), in a sealed ampoule, was immersed in an oil bath preheated to 205 °C until effervescence ceased (*ca* 4 min.). The products dissolved in CH_2Cl_2 (10 mL) were treated with acetic anhydride (1 mL) and DMAP (11 mg). After 2 hr, TLC showed no starting material, and the reaction mixture was quenched with methanol (2 mL) to destroy excess reagent. The mixture was poured into water and the organic layer washed with water, dried over Na_2SO_4, filtered, and evaporated to give a glassy residue which, on flash chromatography, on elution with 45-80% Et_2O-LP, yielded fractions: (*i*) 4-hydroxyestrone 3,4-diacetate **3.15b** (40 mg, 0.11 mmol, 29.6%), mp 217-220 °C (from EtOAc-LP), (lit., 212.5-215.5 °C, from benzene-cyclohexane, Gold and Schwenk, 1958) and (*ii*) estra-5,7,9-triene-3,17-dione **3.15e** (40 mg, 0.15 mmol, 29.6%), mp 128-130 °C (from CH_2Cl_2).
^1H and ^{13}C NMR of **3.15b** and **3.15e** see above.

E. Thermolysis of 4-hydroxyestra-4,6-diene-3,17-dione 3.15c

4-Hydroxyestra-4,6-diene-3,17-dione **3.15c** (10 mg), in a sealed melting tube, was immersed in Wood's metal (50% Bi, 25% Pb, 12.5% Sn, 12.5% Cd) preheated to 248 °C for 4 minutes to yield starting material **3.15c** as identified by the ^{1}H NMR spectrum.

CHAPTER 4

SYNTHESIS OF 17α,21α-CYCLOSTEROIDS AS POTENTIAL MECHANISM-BASED INHIBITORS OF 3α,20β- OR 17β,20α-HYDROXYSTEROID DEHYDROGENASE

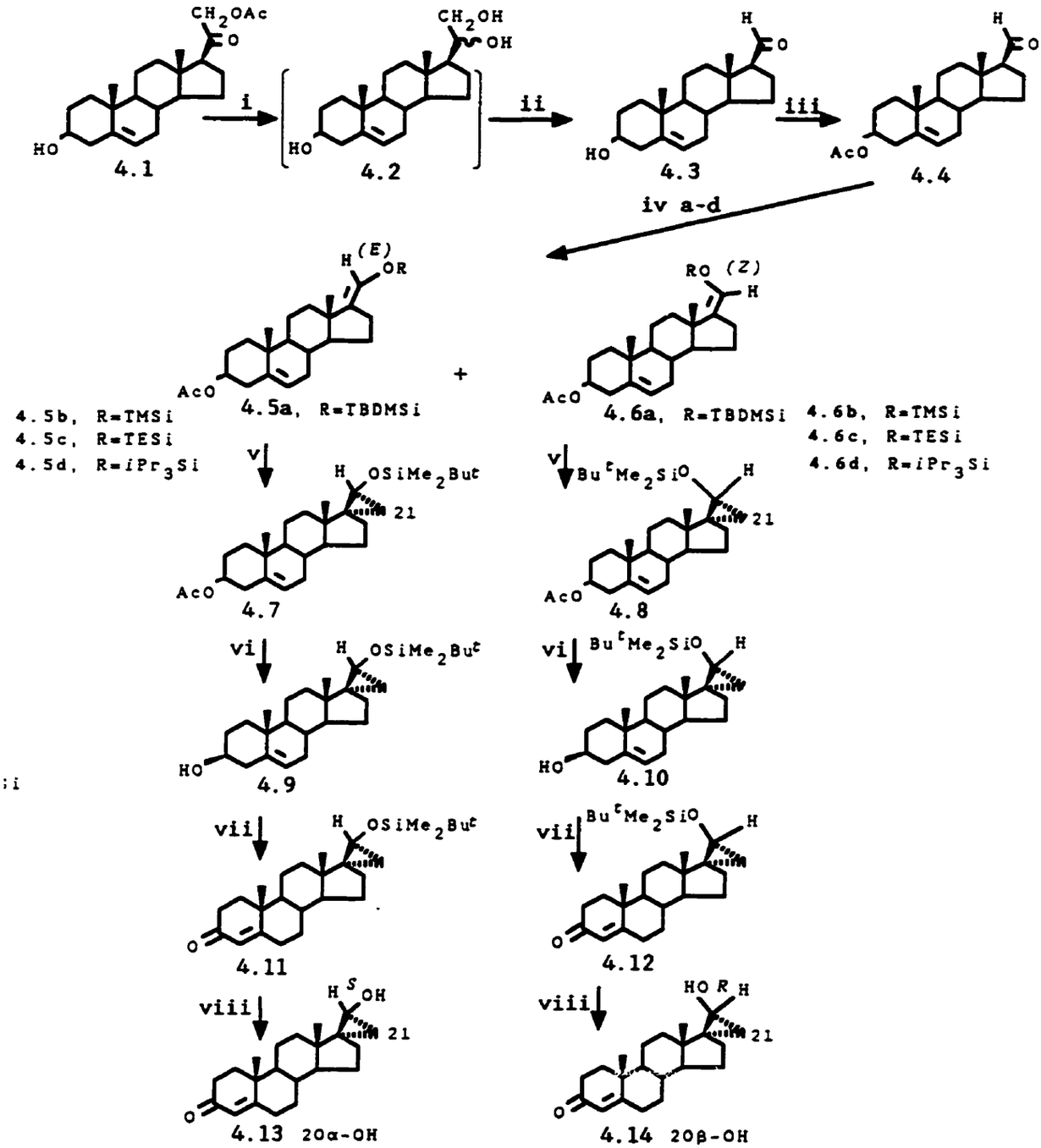
4.1.0 Synthesis of 20α-Hydroxy-17α,21α-cyclopregn-4-en-3-one and 20β-Hydroxy-17α,21α-cyclopregn-4-en-3-one

Introduction

As indicated in Chapter 1, the concept of the 17α,21α-cyclopropanol derivatives acting as irreversible inhibitors has been applied to 20α- and 20β-hydroxy steroid dehydrogenases. Synthesis of two C-17 steroid spirocyclopropanols, 20α- **4.13** and 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one **4.14** have been developed (Scheme 4.1, **4.1-4.14**). As outlined in Scheme 4.1, the key synthetic steps in the total synthesis of compounds **4.13** and **4.14** from 5-pregnan-20-one-3β,21-diol 21-acetate **4.1** involved preparation of the following intermediates: (i) the 17β-aldehyde **4.4**; (ii) the 20(*E*/*Z*)-silyl enol ethers, **4.5a** and **4.6a**; and (iii) the silyl 17α,21α-cyclopropanols, **4.7** and **4.8**.

Two synthetic methods for 3β-hydroxyandrost-5-en-17β-al **4.3**. (21-norpregnenelone) have been reported. A first approach to steroid derivatives having the 17β-aldehyde function involved a partial degradation of the C-17 side chain of pregn-5-ene-3β,20,21-triol with HIO_4 in 1,4-dioxane (Miescher *et al.*, 1940) or $NaIO_4$ in methanol (Eberlein *et al.*, 1974; Gelbart and Thomas, 1978). A second approach to the 17β-aldehyde **4.3** involved the reaction of androst-5-en-17-on-3β-ol with Wittig reagent, methoxymethylenetriphenylphosphine, in DMSO to give 17-methoxymethylene-androst-5-en-3β-ol followed by acid hydrolysis (Danishefsky *et al.*, 1975).

Our initial attempts to repeat the reported Wittig synthesis were unsuccessful. Therefore, our attention was directed toward the former approach, which involved a partial degradation of the pregnane C-17 side chain. This method was also dictated by the availability of



Reagents: i, $LiAlH_4$; ii, $NaIO_4$; iii, Ac_2O, DMAP; iv, Et_3N, (a) TBDMSiOTf; (b) TMSiOTf; (c) TESiOTf; (d) $i\text{-}Pr_3SiOTf$; v, Zn-Ag, CH_2I_2, Et_2O; vi, 0.5 M KOH, MeOH; vii, $Al(i\text{-}PrO)_3$, C_6H_5Me, cyclohexanone; viii, nBu_4NF, THF.

Scheme 4.1 Synthesis of 20α- **4.13** and 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one **4.14**.

a suitable starting material, 5-pregnane-20-one-3β,21-diol 21-acetate (from Griffon Seale). Lithium aluminum hydride reduction of pregn-5-en-20-one-3β,21-diol 21-acetate in tetrahydrofuran afforded the 3β,20ξ,21-triols **4.2**, which were, without separation, oxidatively cleaved with $NaIO_4$-methanol to give 3β-hydroxyandrost-5-en-17β-al **4.3**. Acetylation of **4.3** with acetic anhydride and DMAP as catalyst in CH_2Cl_2 gave the desired intermediate, 3β-acetoxyandrost-5-en-17β-al **4.4**.

Our objective has been to synthesize the 20(*E*/*Z*)-silyl enol ethers **4.5a** and **4.6a**. Silyl enol ethers have been proven to be versatile substrates for a wide variety of synthetic purposes, including acylations (Fleming *et al.*, 1983), aldol reactions (Murata *et al.*, (1980), alkylations (Mukaiyama *et al.*, 1977), brominations (Reuss and Hassner, 1974), [4+2] cycloadditions (Danishefsky, 1981), cyclopropanations (Conia, 1975), hydroborations (Larsen *et al.*, 1975), nitrations, and oxidative processes (Rubottom and Gruber, 1977; Ito *et al.*, 1978). We were interested in synthesizing the 20(*E*/*Z*)-enol silyl ethers **4.5a** and **4.6a**, with a view to preparing the 17α,21α-cyclopropanes, **4.7** and **4.8**, which were envisaged as the intermediates in the synthesis of the target cyclopropanols, **4.13** and **4.14**. Our efforts to prepare the *exo* 20(*E*/*Z*)-silyl enol ethers, **4.5a** and **4.6a** by procedures usually employed to obtain *endo*-silyl enol ethers were unsuccessful, presumably, because of the elevated temperatures and/or basic conditions employed. Trimethylsilyl triflate has been used to produce silyl enol ethers from aldehydes and variety of ketones under very mild conditions with a rate constant of 10^8 relative to trimethylsilyl chloride (Emde *et al.*, 1982). However, because of the instability of trimethysilyl enol derivatives towards chromatography as well as to solvolysis in protic media (in presence of either acid or base), *tert*-

butyldimethylylsilyl derivatives were selected in anticipation of greater stability. The *tert*-butyldimethylsilyoxy group is *ca* 10^4 times more stable than trimethylsilyloxy group (Sommer, 1965). Although *tert*-butyldimethylsilyl triflate (TBDMSiOTf) had been applied with considerable success to the silylation of hindered alcohols (Stewart and Miller, 1980; Corey *et al.*, 1981), in the formation of allylic silyl ethers from epoxides (Murata *at al.*, 1979), and to the formation of silyl enol ethers of a wide range of ketones (Mander and Sethi, 1984), there appeared to be no reports of the formation of enol silyl ethers from aldehydes with this reagent. We have found that TBDMSiOTf with triethylamine furnished a mixture of the 17,20(*E*/*Z*)-*tert*-butyldimethylsilyl enol ethers, **4.5a** and **4.6a**, under very mild conditions and in high yield (**4.5a**:**4.6a**; 3:2). Formation of the silyl enol ethers, **4.5a** and **4.6a**, is stereoselective and provides a mixture favouring the 20(*E*)-isomer **4.5a**. After separation of the silyl enol ethers, **4.5a** and **4.6a**, the subsequent steps toward the target compounds were conducted separately starting from each silyl enol ether.

Introduction of the cyclopropane function into the electron rich 17,20-double bond of the silyl enol ether **4.5a** was performed with the Simmons-Smith reagent. Efforts were made to synthesize the 17α,21α-cyclopropane derivative, **4.7**, by employing a Zn-Cu couple and CH_2I_2 in diethyl ether. Initial experiments were not promising, since the Zn-Cu couple (Simmons and Smith, 1959) proved to be very sensitive to moisture. The use of the more reactive, moisture insensitive, Zn-Ag couple (Denis *et al.*, 1972) was much more effective. Preliminary experiments gave a satisfactory yield of compound **4.7** (45%), and later, with an excess of the reagents, the reaction went to completion as determined by the ^1H NMR spectrum. However, the use of Zn-Au couple

(Majgier-Baranowska and Pitura, 1992)* proved to be exceedingly effective and resulted in the conversion of **4.5a** to **4.7** in 80-100%. The final stages of this synthesis involved deacetylation of **4.7** and **4.8** under basic conditions. Oppenauer oxidation of **4.9** and **4.10** with aluminium triisopropoxide, and desilylation of **4.11** and **4.12** with nBu_4NF (Corey and Venkateswarlu, 1972) yielded 20α-hydroxy-17α,21α-cyclopregn-4-en-3-one **4.13** and 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one **4.14**.

Results and Discussion

Syntheses of the 20α- **4.13** and 20β-hydroxy-17α,21α-cyclopregn4-en-3-one **4.14** isomers *via* the 17β-aldehyde **4.4** and the *E* **4.5** and *Z* **4.6**.) silyl enol-ethers are outlined in Scheme 4.1. The syntheses of **4.13** and **4.14** commence with a suitable starting material pregn-5-en-20-one-3β,21-diol 21-acetate **4.1**, which after treatment with $LiAlH_4$ in tetrahydrofuran gave a mixture of the 20ξ,21-diols **4.2**. Although, it was expected that $LiAlH_4$ reduction of the 20-keto group would result in a mixture of 20α- and 20β-alcohols, no attempt was made at separation. Overlap of the 20α-H and 20β-H protons in the ^1H NMR spectrum did not allow determination of the 20α:20β alcohol ratio. The 17β-aldehyde group was generated by oxidative cleavage of the C_{20}-C_{21} bond of **4.2** with $NaIO_4$ in methanol (Gelbart and Thomas, 1978) to yield compound **4.3**, which after reaction with acetic anhydride and DMAP as catalyst gave the 3β-acetylated 17β-aldehyde **4.4**.

The aldehyde function, in the ^1H NMR spectrum, appeared as doublet at δ 9.84 ppm ($J_{17\alpha,20}$ = 3 Hz). All attempts to prepare the *tert*-buty-

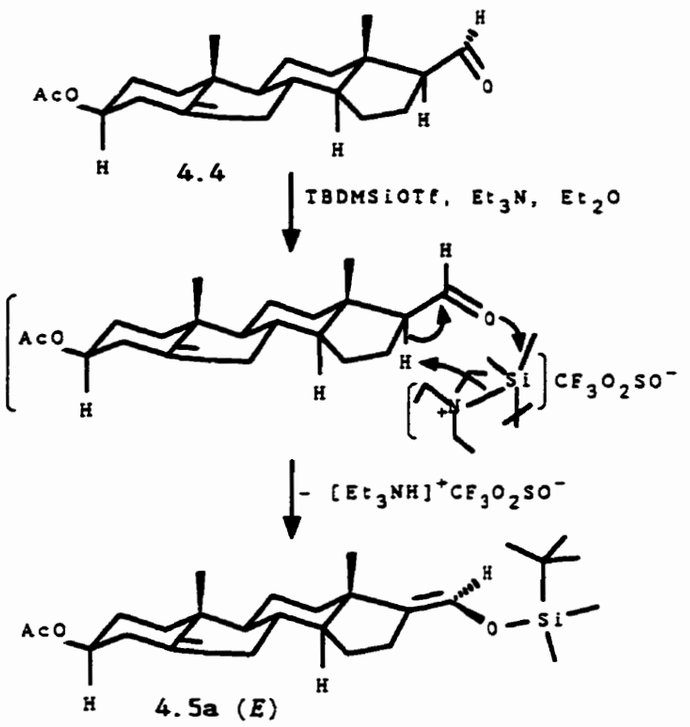
* Discussion between the author and Randy Pitura M.Sc. about improvement of the Simmons-Smith reaction. R. Pitura suggested the use of mercuric acetate [$(CH_3COO)_2Hg$], while the author proposed gold acetate (CH_3COOAu).

ldimethylsilyl enol derivatives **4.5a/4.6a** by using procedures described below were unsuccessful. When the 17β-aldehyde **4.4** was treated with TBDMSiCl and Et_3N (or imidazole) in DMF under reflux, a product showed on TLC with a R_f corresponding to the silyl enol ethers **4.5a/4.6a**. However, before the starting material had been consumed the silyl enol ethers formed also decomposed. Presumably, the 20-silyl enol ethers formed were unstable under the conditions employed and decomposed at a rate greater than the rate of formation. Similar treatment of the 17β-aldehyde **4.4** with TBDMSiCl, imidazole in refluxing DMF or hexame-

Table 4.1 ^1H-^1H NOE enhancements of 20(*E*/*Z*)-silyl enol ethers, **4.5a** and **4.6a**, and 20(α/β)-silyl ethers, **4.11** and **4.12**.

Compound	H's irradiated (δ ppm)	H's enhancement (%)
4.5a	18-CH_3	20-H (1.7)
	20-H	12α-H (1.9)
		12β-H (4.4)
		18-CH_3 (0.6)
4.6a	18-CH_3	20-H (1.0)
	20-H	16α-H (2.0)
		16β-H (1.6)
4.11	18-CH_3	20-H (5.9)
	20-H	12β-H (3.0)
		18-CH_3 (2.5)
	21-H (0.8 ppm)	12α-H (2.0)
	21-H (ca 0.22 ppm) *	
4.12	18-CH_3	20-H (1.2)
	20-H	16β-H (2.5)
		18-CH_3 (0.4)
	21-H (0.7 ppm)	12α-H (8.6)
	21-H 16α-H (3.6) (0.22 ppm)	

* Not available; overlaps with the $SiMe_2$ signals



Scheme 4.2 Proposed mechanism of formation of the (*E*) 17,20-silyl enol ether **4.5a**.

thyldisilyl naphtalene, NaH, and I_2 in benzene or TBDMSiCl and NaH in benzene under reflux or TBDMSiCl and Na_2S in CH_3CN (Olah *et al.*, 1979) were unsuccessful. However, treatment of an ice-water cooledsolution of the 17β-aldehyde **4.4** and triethylamine in diethyl ether with *tert*-butyldimethylsilyl triflate gave the expected mixture of the silyl enol ethers, **4.5a** and **4.6a**, in the ratio *E* : *Z* (1:0.37) based on comparison of their vinyl 20-H signals in the ^1H NMR spectrum of the reaction product. The structures of the silyl enol ethers **4.5a** and **4.6a** were established by the NOE difference spectra (Table 4.1).

A proposed mechanism for formation of one of the 20(*E/Z*) isomers, the 20(*E*)-silyl enol ether **4.5a**, is shown in Scheme 4.2. Presumably, the reaction proceeds *via* a six membered transition state. Initially,

triethylamine reacts with TDMSiOTf to form the "real" reagent, $[TBDMSi\text{-}NEt_3]^+TfO^-$, (Emde *et al.*, 1981). This reagent, because of bulkiness, approaches the 17α-hydrogen and oxygen of the 17β-aldehyde from the less hindered α-side, yielding **4.5a** as the major product. On the other hand, approach of the $[TBDMSi\text{-}NEt_3]^+TfO^-$ species, in similar manner, from the more hindered side must lead to a minor product, the 20(*Z*)-silyl enol ether **4.6a**.

Treatment of the 17β-aldehyde **4.3** or **4.4** with silyl reagents having a different bulk, e.g. trimethylsilyl- (TMSi) or triethylsilyl- (TESi) or triisopropylsilyl (iPr_3Si)triflate gave a mixture of the 17,20(*E*/*Z*)-silyl enol ethers with the ratio *E*:*Z* based on comparison of their vinyl 20-H signal in the ^1H NMR spectra (Table 4.2). Higher selectivity was obtained with the more bulky reagents.

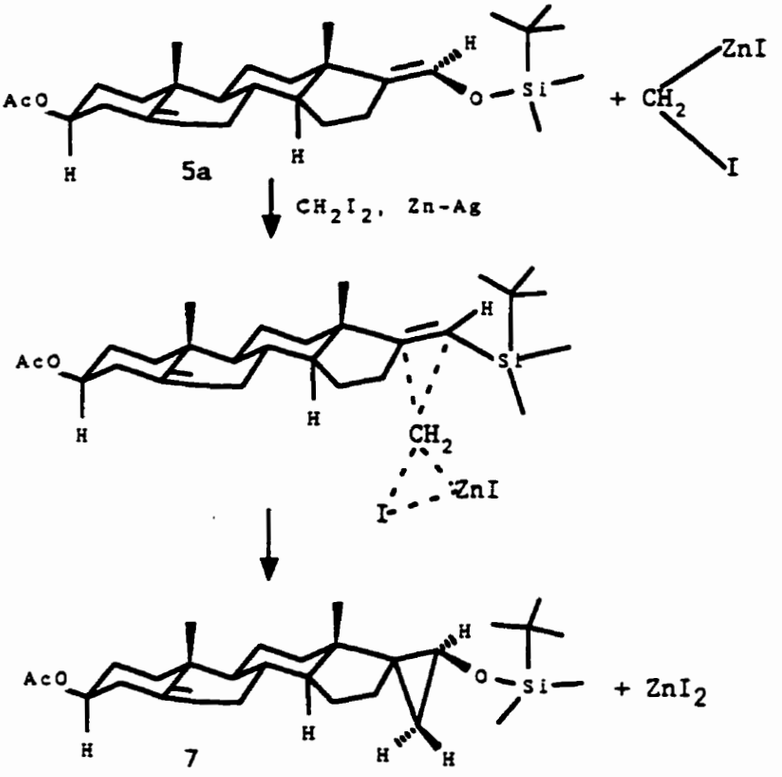
Table 4.2 ^1H NMR chemical shifts (δ ppm) of vinylic 20-H signals of 17,20(*E*/*Z*)-silyl enol ethers, **4.5a-4.5d** and **4.6a-4.6d**,

Silyl group[a]	20(*E*)-silyl enol ethers			20(*Z*)-silyl enol ethers			*E*:*Z* ratio
		δ(ppm)	J(Hz)		δ(ppm)	J(Hz)	
TMSi	**4.5b**	5.94t	2.4	**4.6b**	6.00t	1.8	1:1
TESi	**4.5c**	5.97t	2.4	**6c**	6.02t	1.82	1:0.33
TBDMSi	**4.5a**	5.98t	2.4	**4.6a**	6.03t	1.8	1:0.37
iPr_3Si	**4.5d**	6.07t	2.4	**4.6d**	6.12t	1.75	1:0.37

[a] given in order of increasing bulk of the silyl group.

The two 17,20(*E*/*Z*)-silyl enol isomers, **4.5a** and **4.6a**, were separated by crystallization. The major isomer **4.5a** crystallized from diethyl ether-methanol and after chromatographic separation of the

mother liquor the minor isomer **4.6a** was obtained. Cyclopropanation of the silyl enol ethers was accomplished with an organozinc compound, a carbenoid known as the Simmons-Smith reagent (Simmons *et al.*, 1964). The reagent was generated by the reaction of diiodomethane with zinc to give the organozinc compound, ICH_2ZnI. Preparation of the zinc is critical to good yields in a Simmons-Smith reaction. The zinc surface must be activated. This can be achieved by production of zinc couples.



Scheme 4.3 Proposed mechanism of formation of 3β-acetoxy-20α-tert-butyldimethylsiloxy-17α,21α-cyclopregn-5-ene **4.7** with a Simmons-Smith reagent.

Three different couples, Zn-Cu (Simmons *et al.*, 1964), Zn-Ag (Denis *et al.*, 1972), and Zn-Au were tested. Respectively, the yields of the cyclopropane compounds **4.7** were 0-15%, 45%, and 80-100% when Zn-Cu, Zn-Ag, and Zn-Au couples were employed. Although the best yield was obtained by using the Zn-Au couple, the cost of the reagent was too high (AuOAc $200/1 g). Therefore, instead of the Zn-Au couple an excess of Zn-Ag couple was used. Cyclopropanation of **4.5a** or **4.6a** with ICH_2ZnI yielded the cyclopropyl *tert*-butyldimethylsilyl ethers **4.7** or **4.8** as a single diastereoisomer. While the reaction proceeded, the reaction products were monitored by the ^1H NMR spectrum, because both the starting material **4.5a** or **4.6a** and the 17α,21α-cyclopropyl products, **4.7** or **4.8**, respectively, had the same R_f on TLC. The addition of the CH_2 carbene was stereospecifically *syn*, and a concerted mechanism (Simmons *et al.*, 1964) is likely as shown in Scheme 4.3. The high chemoselectivity observed in the conversion of **4.5a** or **4.6a** to **4.7** or **4.8**, respectively, can be attributed to the faster rate of reaction of the electrophilic zinc carbenoid with the more electron-rich enol ether 17,20-double bond than with the 5,6-double bond. In fact, even with an excess of the reagents monocyclopropanation was only observed as determined by the ^1H NMR spectrum of the crude reaction products. Selective addition occurred from the α-face as shown by X-ray crystallographic analysis (Figure 4.1) and NOE difference spectra (Table 4.2; compounds **4.11** and **4.12**).

α-Face addition was expected to result from steric hindrance by the 13-CH_3 group, because of the *trans* conjunction of rings C and D. Of the two isomers, as observed by TLC and NMR, the *Z* isomer **4.6a**, in which the reaction was completed in 18 h, reacted more readily than the *E* isomer **4.5a**, which required not only more reagent but also a longer

Table 4.3 Crystallographic data for 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one **4.14**.

	Compound **4.14**
Formula	$C_{21}H_{30}O_2$
M_r	314.47
T(°K)	298
Crystal Size (mm)	0.40 x 0.30 x 0.05
Crystal System Orthorombic	
Space Group	$P2_12_12_1$ (no.19)
Cell Dimensions:	
a/Å 23.511 (3)[a]	
b/Å 23.748 (3)	
c/Å 6.285 (6)	
α, β, γ (°)	90/90/90
Cell Volume/Å^3	3509 (3)
Z	8
D_{calc}/g cm^{-3}	1.190
F(000) 1376	
λ/Å (MoKα)	0.71069
$\mu_{(MoK\alpha)}$/cm^{-1}	0.69
Θ min. and max. (°)	8.97-19.03
2Θ max. (°)	50.2
Total Reflections	3575
Observed Reflections (I>2.00σ(I))	1461
Function Minimized	$\Sigma w(\|F_o\| - \|F_c\|)^2$
Least-square Weights	$4F_o^2/\sigma^2(F_o^2)$
R	0.059
R_w	0.031

[a]Estimated standard deviations in parentheses refer to the last digit.

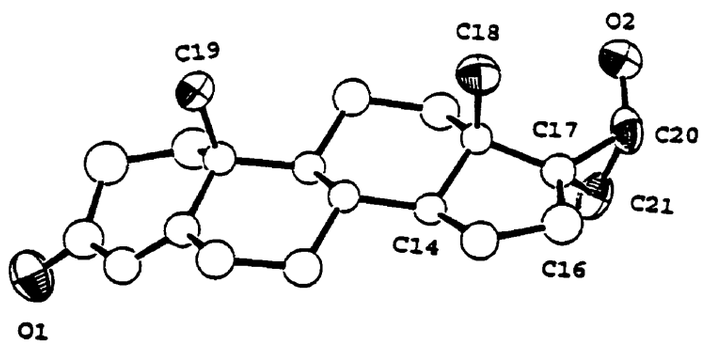
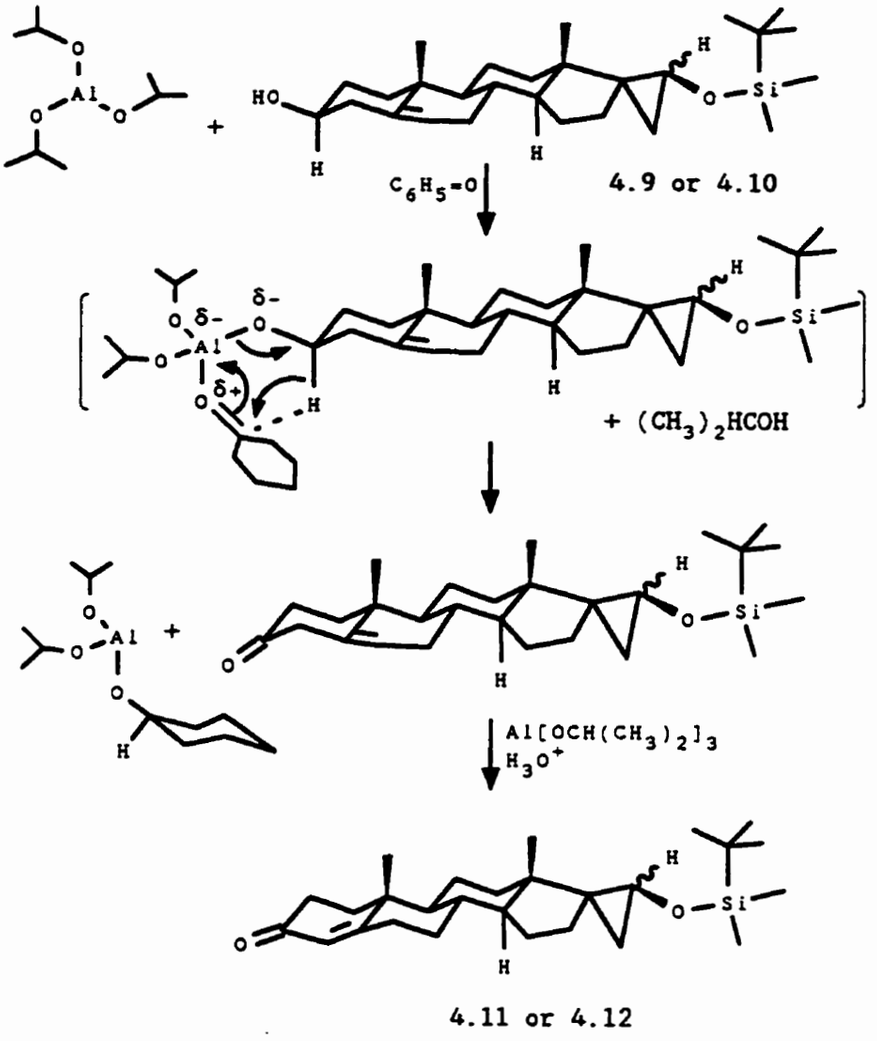


Figure 4.1 PLUTO representation of the 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one **4.14** structure.



Scheme 4.4 Intermediates in the Oppenauer oxidation.

period of time (36 h). Alkaline hydrolysis of the acetates **4.7** and **4.8** gave the alcohols **4.9** and **4.10**, respectively, which on Oppenauer oxidation yielded the conjugated ketone **4.11** and **4.12**.

The proposed mechanism of the Oppenauer reaction is outlined in Scheme 4.4. Initially, aluminium triisopropoxide reacts with the 3β-hydroxy group of compound **4.9** or **4.10** with elimination of isopropyl

alcohol as a good leaving group. The real oxidation reaction involves transfer of a hydride from the oxygen substituted carbon atom of the steroid to a carbonyl group of cyclohexanone. Three factors combine to make this reaction facile: (1) activation of the carbonyl group to cyclohexanone toward nucleophilic addition as a result of coordination of the Lewis acid (alumnium diisopropoxide-steroid species); (2) activation of the secondary C-H bond as a σ donor, by the presence of a good leaving group, in this case the steroid substituent (steroid-O-Al, which resembles -O-); and (3) the opportunity presented by coordination within the complex shown in Scheme 4.4. Desilylation with a fluoride ion yielded the 20α- **4.13** and 20β-cyclopropanols **4.14**.

The structure of compound **4.14** has been confirmed by X-ray crystallographic analysis (Figure 4.1). Crystallographic data are presented in Table 4.3. Of the two 20α- and 20β-hydroxy-cyclopropyl isomers, **4.13** and **4.14**, the 20α-hydroxy-cyclopropyl **4.13** was less stable in an aprotic solvent (CH_2Cl_2), presumably, because of H-bonding occurring not only between two 20α-hydroxyl groups, but also between the 20α-hydroxyl group of one molecule and the carbonyl oxygen of the second molecule. However, its epimer, the 20β-hydroxy-17α,21α-cyclo **4.14**, having more hindered hydroxyl group, was more stable. Indeed, the X-ray analysis of the 20β-hydroxy isomer **4.14** showed that the distances between the 20ξ-hydroxyl group of one molecule and the 3-carbonyl oxygen of the second molecule were in the upper limit of normal H-bonding ($d_{20\beta\text{-}OH\ldots O=C(3)}$ *ca* 2.9 A), (Dr. J. Bridson, personal communucation).

4.1.1 Summary

1. 20α-Hydroxy-17α,21α-cyclopregn-4-en-3-one **4.13** and 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one **4.14** have been synthesized as potential mechanism-based inhibitors of 20α- and 20β-steroid oxidoreductase.
2. Synthesis was accomplished by production of 20(*E*/*Z*)-*tert*-butyldimethylsilyl enol ethers with the *tert*-butyldimethylsilyltriflate reagent having one of the best leaving groups, the trifluoromethylsulphonate or "triflate" anion.
3. The Simmons-Smith reaction on the 20(*E*/*Z*)-silyl enol ether **4.5a** with one portion of the Zn-Au couple gave a higher yield of the cyclopropane derivative **4.7** (80-100%) than with Zn-Ag (45%) or Zn-Cu (0-15%) couple.
4. Instability of one of the 17α,21α-cyclopropanols, the 20α-hydroxy **4.13**, with the exposed hydroxyl group, has been explained by H-bond interactions between the 20α-hydroxyl group of one molecule and the carbonyl group of the second molecule.

4.1.2 Experimental

3β-Hydroxy-21-nor-pregn-5-en-20-one 4.3

A solution of pregn-5-en-20-one-3β,21-diol 21-acetate **4.1** (10.3 g, 27.5 mmol) in dry THF (300 mL) was refluxed with $LiAlH_4$ (2.82 g, 74.3 mmol, 2.7 eq.) for 18 hrs, cooled to room temperature and the excess of the reagent decomposed with H_2O (3 mL), 10% NaOH (3 mL) and H_2O (3 mL), (Fieser & Fieser, 1967), until a creamy precipitate formed. The mixture was filtered, concentrated until the steroid started to precipitate, and then water (500 mL) was added in portions, with stirring. The precipitate was collected on a filter paper, washed with

water, and dried in an oven at 60° C to give a crude product consisting of two 20ξ-diols of 5-pregnen-3β,20ξ,21-triol **4.2** (9.0 g, 27.0 mmol, 98%).

To a stirred solution of the crude reaction product **4.2** was added an aqueous solution of $NaIO_4$ (8 g; dissolved in hot water, 18 mL) over 1-2 min. The mixture was stirred for 1 h when no starting material was detected by TLC. Ethylene glycol (2 mL) was added to destroy an excess reagent. After stirring the mixture for a further 1/2 hr, the $NaIO_4$ formed during the reaction was removed by filtration through Celite, and the solution was concentrated under reduced pressure until a precipitate started to form. The products were extracted with CH_2Cl_2 and the organic layer was washed with water, 5% HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give the 17β-aldehyde **4.3** (6.15 g, 20.3 mmol, 75%), mp 153-157 °C (from EtOAc-LP), (lit., 148-153 °C, sublimed, Miescher *et al.*, 1940; mp 155-157 °C; from EtOH-H_2O; Danishefsky *et al.*, 1975).

3β-Acetoxy-21-norpregn-5-en-20-one 4.4

A suspension of the 3β-alcohol **4.3** (6 g, 20 mmol) in ethyl ether (200 mL) was stirred with acetic anhydride (3.5 mL) and N,N-dimethyl-aminopyridine (DMAP) (2 mg). As the reaction proceeded, the steroid dissolved. After 4 hr, the reaction was complete and TLC showed no starting material. The organic layer was washed with 10% HCl (100 mL), water, brine, dried over Na_2SO_4, filtered, and evaporated to give a colorless residue (5 g, 15 mmol, 76%), which on FCC with 2.5% EtOAc-LP gave **4.4** (4.20 g, 12.6 mmol, 64%), mp 170-172 °C (from CH_2Cl_2-EtOAc), (lit., mp 169-171 °C, from hexane, Miescher *et al.*, 1940).

^{1}H NMR of **4.4** ($CDCl_3$): δ 9.76 (d, J 2.0, 1H, 20-H), 5.37 (d, J 5.2, 1H, 6-H), 4.59 (m, 1H, 3α-H), 2.02 (s, 3H, 3β-OAc), 1.02 (s, 3H,

10-Me), 0.76 (s, 3H, 13-Me).

^{13}C NMR of **4.4** ($CDCl_3$): δ 37.00 (1), 27.71 (2), 73.77 (3), 38.06 (4), 139.66 (5), 122.18 (6), 31.75 (7), 31.40 (8), 50.00 (9), 36.65 (10), 20.53 (11), 38.26 (12), 44.62 (13), 56.39 (14), 24.89 (15), 20.01 (16), 62.78 (17), 13.73 (18), 19.31 (19), 204.75 (20), 170.42 (3-OCOMe), 21.39 (3-OCO**Me**).

3β-Acetoxy-20α- 4.5a and 3β-Acetoxy-20β-*tert*-butyldimethylsiloxy-21-norpregna-5,17-diene 4.6a

With *tert*-butyldimethylsilyl triflate (TBDMSiOTf):

To a stirred and cooled (ice-bath) solution of the aldehyde **4.4** (4.2 g, 9.15 mmol) in ethyl ether (200 mL) and triethylamine (5.1 mL, 36.6 mmol, 4 eq.), under an argon atmosphere, was added, over 5 min, by syringe through a rubber septum, *tert*-butyldimethylsilyl triflate (5.65 mL, 24.6 mmol, 2.7 equin). Stirring was continued at room temperature for 6 h, after which TLC showed no starting material. The reaction mixture was washed with water, brine, dried over Na_2SO_4, filtered, and evaporated to give a mixture of two enols, **4.5a** and **4.6a**, which on recrystallization yielded the 20(*E*)-silyl enol ether **4.5a** (2.35 g, 5.12 mmol, 60%), mp 155-159 °C (from Et_2O-MeOH) (Found: C, 73.1; H, 10.0. $C_{28}H_{46}O_3Si$ (MW 458.763) requires C, 73.3; H, 10.1%). The mother liquor on FCC on elution with 10-20% CH_2Cl_2-LP yielded the 20(*Z*)-enol ether **4.6a** (1.3 g, 2.8 mmol, 31%), mp 96-99 °C (from Et_2O-MeOH) (Found: C, 73.2; H, 10.0. $C_{28}H_{46}O_3Si$ (MW 458.763) requires C, 73.3; 10.1%).

^{1}H NMR of **4.5a** ($CDCl_3$): δ 6.03 (t, J 1.8, 1H, 20-H), 5.30 (d, J 4.6, 1H, 6-H), 4.62 (m, 1H, 3α-H), 2.03 (s, 3H, 3β-OAc), 1.03 (s, 3H, 10-Me), 0.92 (s, 9H, SiC**Me**$_3$), 0.81 (s, 3H, 13-Me), 0.11 (s, 6H, Si**Me**$_2$).

^{13}C NMR of **4.5a** ($CDCl_3$): δ 37.01 (1), 27.78 (2), 73.92 (3), 38.14 (4), 139.68 (5), 122.48 (6), 31.78 (7), 31.54 (8), 50.47 (9), 36.74 (10),

20.92 (11), 24.68 (12), 41.72 (13), 55.64 (14), 24.36 (15), 36.11 (16), 133.95 (17), 19.30 (18), 49.35 (19), 129.85 (20), 170.45 (3-OCOMe$_3$), 21.44 (3-OCOMe$_3$), -5.27 and -5.22 (SiMe$_2$), 18.35 CMe$_3$ (SiCMe$_3$), 25.76 (SiCMe$_3$).

^{1}H NMR of 4.6a ($CDCl_3$): δ δ 6.03 (t, J 1.8, 1H, 20-H), 5.30 (d, J 4.6, 1H, 6-H), 4.62 (m, 1H, 3α-H), 2.03 (s, 3H, 3β-OAc), 1.03 (s, 3H, 10-Me), 0.92 (s, 9H, SiCMe$_3$), 0.89 (s, 3H, 13-Me), 0.09 (s, 6H, SiMe$_2$).

^{13}C NMR of 4.6a ($CDCl_3$): δ 37.00 (1), 27.81 (2), 73.98 (3), 38.16 (4), 139.78 (5), 122.48 (6), 31.87 (7), 31.78 (8), 50.40 (9), 36.75 (10), 21.15 (11), 26.12 (12), 43.27 (13), 56.32 (14), 36.58 (15), 24.91 (16), 129.67 (17), 16.68 (18), 19.31 (19), 130.82 (20), 170.40 (3-OCOMe$_3$), 21.45 (3-OCOMe$_3$), -5.42 and -5.35 (SiMe$_2$), 18.17 CMe$_3$ (SiCMe$_3$), 25.75 (SiCMe$_3$).

3β-Acetoxy-20α- 4.5b and 3β-Acetoxy-20β-trimethylsiloxy-21-norpregna-5,17-diene 4.6b

With trimethylsilyl triflate (TMSiOTf):

Compound **4.3** (100 mg, 0.33 mmol) was treated with Et_3N (0.64 mL, 4.6 mmoL, 14 equiv.), TMSiOTf (651 μL, 4.09 mmol, 12 eq.) in CH_2Cl_2 (3 mL) for 30 min., followed by work-up, as described above, to give a residue (120 mg) consisting of the 20(*E*)- and 20(*Z*)-trimethylsilyl enol ethers, **4.5b** and **4.6b**, respectively, where **4.5b**:**4.6b**=1:1 determined by comparison of the vinylic 20-H signals in their ^{1}H NMR spectra.

3β-Acetoxy-20α- 4.5c and 3β-Acetoxy-20β-triethylsiloxy-21-norpregna-5,17-diene 4.6c

With triethylsilyl triflate (TESiOTf):

Compound **4.4** (100 mg, 0.29 mmol) was treated with Et_3N (130 μL, 0.58 mmol, 2 eq.) and TESiOTf (100 μL, 0.73 mmol, 2.5 eq.) in CH_2Cl_2 (3 mL) for 10 h followed by work-up, as described above, to give an oily

residue (120 mg, 0.26 mmol, 90%) consisting of the 20(*E*)- and 20(*Z*)-triethylsilyl enol ethers, **4.5c** and **4.6c**, where **4.5c**:**4.6c**=1:0.33 as above.

3β-Acetoxy-20α- 4.5d and 3β-Acetoxy-20β-tri-*iso*-tripropylsiloxy-21-nor-pregna-5,17-diene 4.6d

With triisopropylsilyl triflate (*i*-Pr_3SiOTf): (Corey and Hopkins, 1982) Compound **4.4** (200 mg, 0.58 mmol) was treated with Et_3N (1 mL, 7.2 mmol, 12 equiv.) and *i*-Pr_3SiOTf (315 μL, 1.2 mmol, 2 eq.) in CH_2Cl_2 (3 mL) for 10 h followed by work-up, as described above, to give a residue (300 mg, 0.51 mmol, 88%) which consisted of the 20(*E*)- and 20(*Z*)-triisopropylsilyl enol ethers, **4.5d** and **4.6d**, with the ratio **4.5d**:**4.6d**=1:0.37 as above.

3β-Acetoxy-20α-*tert*-butyldimethylsiloxy-17α,21α-cyclopregn-5-ene 4.7

(A) With Zn-Cu couple: compounds **4.5a** and **4.7**

Preparation of a Zn-Cu couple : Zinc powder (1.0 g; Mallinckrodt) and $(CH_3COO)_2Cu$ (56 mg) were ground by mortar and pestle, then transferred to a test tube (25 mL). Boiling acetic acid (10 mL) was poured onto the solid mixture, which was washed with cold acetic acid (2x20 mL), and dry diethyl ether (4x20 mL). While still under ether, the Zn-Cu couple was transferred into a reaction flask filled with dry diethyl ether (10 mL), followed by CH_2I_2 (200 μL). The heterogenous mixture was refluxed with stirring for 1/2h, under an argon atmosphere, and the solid 20(*E*)-silyl enol ether **4.5a** (100 mg) added at once. Stirring and refluxing were continued for 18 hr, and then was cooled to RT. The organic layer was decanted, washed with saturated aqueous $NaHCO_3$, water, brine, dried over Na_2SO_4, filtered, and evaporated to give a white residue (80 mg). The ^1H NMR spectrum showed two products, the starting material (85%) and a new compound **4.7** (15%).

(B) With Zn-Ag couple: compound **4.7**

Small scale reaction: To a Zn-Ag couple (5g), prepared as described by Denis *et al.* (Denis *et al.*, 1972), stirred under argon in diethyl ether (15 mL) was added CH_2I_2 (0.5 mL), over 20 min, by syringe, at a rate sufficient to maintain gentle reflux, followed by the 20(*E*)-silyl enol ether **4.5a** (250 mg). The mixture was stirred for 18 h. The ethereal solution was transfered to a separatory funnel. The couple was washed with diethyl ether and organic washings combined. The organic layer was washed with saturated aqueous $NaHCO_3$, until no precipitate was formed, brine, dried, filtered, and evaporated to give a residue (110 mg). The ^1H NMR spectrum showed the presence of two compounds, the starting material **4.5a** and the 17α,21α-cyclopropyl **4.7** (**4.5a**:**4.7**; 1:1) by comparison of the 20-H signals.

Large scale reaction : To a Zn-Ag couple (10g, 0.153 mmol of Zn), prepared as described previously (Denis *et al.*, 1972), stirred under argon in ether (35 mL) was added CH_2I_2 (4 mL) dropwise (*ca.* 1h), at a rate sufficient to maintain gentle reflux. The solid (20E)-*tert*-butyldimethyl silyl enol ether **4.5a** (2.1 g, 4.58 mmol), was added in one portion and heated under reflux for 18 h. The progress of the reaction was monitored by the ^1H NMR spectrum, because both the product **4.7** and starting material **4.5a** had the same R_f on TLC. The solution was again treated with the same amount of the above reagents; the mixture was then heated under reflux, for a further 18 h. After this, the organic layer was decanted, and the couple washed with diethyl ether. The organic layers were combined and washed with saturated $NaHCO_3$, water, brine, dried over Na_2SO_4, filtered, and evaporated to give a residue, which on recrystallization gave the cyclopropylsilyl ether **4.7** (1.24 g, 2.62 mmol, 57%), mp 154.5-160 °C

(from Et_2O-MeOH), (Found: C, 73.4; H, 10.3. $C_{29}H_{48}O_3Si$ (MW 472.790) requires C, 73.7; H, 10.2%).

^1H NMR of **4.7** ($CDCl_3$); δ 5.38 (d, J 4.8, 1H, 6-H), 4.61 (m, 1H, 3α-H), 3.14 (dd, J 3.5, 7.0, 1H, 20-H), 2.03 (s, 3H, 3β-OAc), 1.02 (s, 3H, 10-Me), 0.89 (s, 9H, $SiCMe_3$), 0.79 (s, 3H, 13-Me), 0.077 and 0.087 (s, 6H, $SiMe_2$).

^{13}C NMR of **4.7** ($CDCl_3$): δ 37.03 (1), 27.77 (2), 73.92 (3), 38.12 (4), 139.59 (5), 122.59 (6), 28.68 (7), 32.20 (8), 50.17 (9), 36.70 (10), 20.53 (11), 34.43 (12), 40.49 (13), 55.63 (14), 24.79 (15), 32.00 (16), 36.27 (17), 16.65 (18), 19.32 (19), 19.40 (20), 52.03 (21), 170.46 ($3-OCOMe_3$), 21.43 ($3-OCOMe_3$), -4.79 and -4.71 ($SiMe_2$), 18.15 CMe_3 ($SiCMe_3$), 25.89 ($SiCMe_3$).

(C) With Zn-Au couple: compound **4.5a** and **4.7**

Preparation of a Zn-Au couple:

CH_3COOAu (54 mg) was added to cold glacial acetic acid (100 mL) and the mixture heated to boiling, with stirring. When the acetic acid started to boil, granular zinc (17 g, 10 mesh, 99%, Aldrich) was added at once. Stirring was continued without heating for a further 2 minutes. The solution was decanted, and the couple washed with CH_3COOH (2x100 mL), dry diethyl ether (5x100 mL), until no acetic acid was detected, and dried in a desiccator. The couple can be stored at room temperature without loss in activity.

Small scale reaction: Similar treatment of the 20(*E*)-silyl enol ether **4.5a** (108 mg, 23 mmol) in diethyl ether (15 mL) with one portion of the Zn-Au couple (2 g) and CH_2I_2 (600 μL) for 18 h, followed by work-up as described above, yielded one product, the 17α,21α-cyclopropane *tert*-butyldimethylsilyl enol **4. 7** (80 mg, 0.17 mmol, 74%), mp as above.

3β-Acetoxy-20β-*tert*-butyldimethylsiloxy-17α,21α-cyclopregn-5-ene 4.8

Similar treatment of the 20(Z)-*tert*-butyldimethylsilyl enol ether **4.6a** (1.12 g, 2.37 mmol) with one portion of Zn-Ag couple (5.5 g) and CH_2I_2 (1.6 mL) for 18 h, as described for **4.5a**, yielded the cyclopropane *tert*-butyldimethylsilyl ether **4.8** (1.0 g, 2.12 mmol, 87%), mp 126-130 °C (from Et_2O-MeOH) (Found: C, 73.5; H, 10.1. $C_{29}H_{48}O_3Si$ requires C, 73.7; H, 10.2%).

^{1}H NMR of **4.8** ($CDCl_3$); δ 5.38 (d, J 4.4, 1H, 6-H), 4.61 (m, 1H, 3α-H), 3.32 (dd, J 3.5, 6.3, 1H, 20-H), 2.03 (s, 3H, 3β-OAc), 1.03 (s, 3H, 10-Me), 0.96 (s, 3H, 13-Me), 0.88 (s, 9H, $SiCMe_3$), 0.72 (dd, J 3.1, 5.3, 1H, 21-H_b), 0.20 (t, J 5.8, 1H, 21-H_a), 0.053 and 0.084 (s, 6H, $SiMe_2$).

^{13}C NMR of **4.8** ($CDCl_3$): δ 37.06 (1), 27.81 (2), 74.00 (3), 38.15 (4), 139.69 (5), 122.58 (6), 31.98 (7), 31.96 (8), 50.22 (9), 36.71 (10), 20.76 (11), 35.83 (12), 41.82 (13), 55.54 (14), 25.86 (15), 34.24 (16), 34.86 (17), 15.18 (18), 19.36 (19), 19.98 (20), 55.70 (21), 170.49 (3-$OCOMe_3$), 21.44 (3-$OCOMe_3$), -5.36 and -4.86 ($SiMe_2$), 18.00 CMe_3 ($SiCMe_3$), 25.86 ($SiCMe_3$).

20α-*tert*-Butyldimethylsiloxy-17α,21α-cyclopregn-5-en-3β-ol 4.9

The crude reaction product **4.7** (1.2 g, 2.54 mmol), from the Simmons-Smith reaction of **4.5a**, was treated with 0.5 m KOH-MeOH (120 mL) for 1 h (TLC), after which the mixture was poured into water and extracted with diethyl ether. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered, and evaporated to give a crude product which on FCC, on elution with 15% EtOAc-LP, yielded fractions of **4.9** (375 mg, 0.87 mml, 34%), mp 170-174 °C (from Et_2O-MeOH), Found: C, 72.4; H, 10.9. $C_{27}H_{46}O_2Si.H_2O$ (MW 430.753) requires C, 73.7; H, 10.2%).

^{1}H NMR of **4.9** ($CDCl_3$); δ 5.37 (d, J 4.4, 1H, 6-H), 3.52 (m, 1H, 3α-H),

3.13 (dd, J 3.5, 7.0, 1H, 20-H), 1.01 (s, 3H, 10-Me), 0.86 (s, 3H, 13-Me), 0.89 (s, 9H, SiCMe$_3$), 0.081 and 0.092 (s, 6H, SiMe$_2$).

^{13}C NMR of **4.9** ($CDCl_3$): δ 37.32 (1), 31.69 (2), 71.77 (3), 42.34 (4), 140.72 (5), 121.68 (6), 32.04 (7), 32.27 (8), 50.30 (9), 36.65 (10), 20.61 (11), 34.50 (12), 40.52 (13), 55.74 (14), 24.83 (15), 28.83 (16), 36.30 (17), 16.68 (18), 19.43 (19), 19.43 (20), 52.06 (21), -4.77 and -4.68 (SiMe$_2$), 18.17 CMe$_3$ (SiCMe$_3$), 25.91 (SiCMe$_3$).

20β-*tert*-Butyldimethylsiloxy-17α,21α-cyclopregn-5-en-3β-ol 4.10

Treatment of **4.8** (200 mg, 0.42 mmol) with 0.5 M KOH-MeOH (20 mL) as described for **4.9** gave **4.10** (145 mg, 0.34 mmol, 81%), mp 210-212 °C (from Et_2O-MeOH), (Found: C, 75,2; H, 10.6. $C_{27}H_{46}O_2Si$ (MW 430.754) requires C, 75.3; H, 10.8%).

^{1}H NMR of **4.10** ($CDCl_3$); δ 5.36 (d, J 5.2, 1H, 6-H), 3.53 (m, 1H, 3α-H), 3.32 (dd, J 3.1, 6.3, 1H, 20-H), 1.02 (s, 3H, 10-Me), 0.97 (s, 3H, 13-Me), 0.88 (s, 9H, SiCMe$_3$), 0.72 (dd, J 3.1, 5.3, 1H, 21-H$_b$), 0.20 (t, J 5.8, 1H, 21-H$_a$), 0.057 and 0.087 (s, 6H, SiMe$_2$).

^{13}C NMR of **4.10** ($CDCl_3$): δ 37.32 (1), 32.01 (2), 71.82 (3), 42.35 (4), 140.80 (5), 121.65 (6), 31.72 (7), 32.01 (8), 50.32 (9), 36.65 (10), 20.82 (11), 35.88 (12), 41.82 (13), 55.55 (14), 29.71 (15), 34.26 (16), 34.87 (17), 15.19 (18), 19.45 (19), 19.98 (20), 55.78 (21), -5.36 and -4.86 (SiMe$_2$), 18.00 CMe$_3$ (SiCMe$_3$), 25.86 (SiCMe$_3$).

20α-*tert*-Butyldimethylsiloxy-17α,21α-cyclopregn-4-en-3-one 4.11

To compound **4.9** (184 mg, 0.43 mmol) in anhydrous toluene (45 mL) under argon was added freshly distilled cyclohexanone (5 mL, 48 mmol); the solution was then distilled until toluene (6.5 mL) has been removed. Aluminium triisopropoxide (400 mg, 1.96 mmol, 4.5 eq.) in toluene (15 mL) was added dropwise (over 0.5 h) to the mixture, and the solution again distilled slowly to remove toluene (15 mL). After 45 min, the

reaction mixture was cooled and subjected to steam distillation. The residue was extracted with CH_2Cl_2 and the organic layer evaporated to give a residue which on FCC, on elution with 10% EtOAc-LP gave **4.11** (150 mg, 35 mmol, 84%), mp 181-185 °C (from Et_2O-MeOH), (Found: C, 75.5; H, 10.4. $C_{27}H_{44}O_2Si$ (MW 428.737) requires C, 75.6; H, 10.3%).

^{1}H NMR of **4.11** ($CDCl_3$); δ 5.73 (s, 1H, 4-H), 3.13 (dd, J 3.5, 7.1, 1H, 20-H), 1.18 (s, 3H, 10-Me), 0.83 (s, 3H, 13-Me), 0.89 (s, 9H, $SiCMe_3$), 0.080 and 0.092 (s, 6H, $SiMe_2$).

^{13}C NMR of **4.11** ($CDCl_3$): δ 35.75 (1), 34.24 (2), 199.48 (3), 123.79 (4), 171.42 (5), 32.96 (6), 32.12 (7), 36.08 (8), 53.88 (9), 36.69 (10), 20.56 (11), 33.98 (12), 40.55 (13), 54.77 (14), 24.64 (15), 28.60 (16), 36.19 (17), 16.75 (18), 17.43 (19), 19.41 (20), 52.00 (21), -4.78 and -4.71 ($SiMe_2$), 18.15 CMe_3 ($SiCMe_3$), 25.88 ($SiCMe_3$).

20β-*tert*-Butyldimethylsiloxy-17α,21α-cyclopregn-4-en-3-one 4.12

Compound **4.10** (216 mg, 0.50 mmol) was treated with cyclohexanone (7 mL) and Al(*i*-PrO)$_3$ (480 mg), as described for **4.9**, to give, on FCC, on elution with Et_2OAc-LP, **4.12** (180 mg, 42 mmol, 84%) mp 134-137 °C (from Et_2O-LP), (Found: C, 75.4; H, 10.5. $C_{27}H_{44}O_2Si$ (MW 428.737) requires C, 75.6; H, 10.3%).

^{1}H NMR of **4.12** ($CDCl_3$); δ 5.72 (s, 1H, 4-H), 3.32 (dd, J 3.08, 6.3, 1H, 20-H), 1.19 (s, 3H, 10-Me), 0.99 (s, 3H, 13-Me), 0.88 (s, 9H, $SiCMe_3$), 0.70 (dd, J 3.1, 5.4, 1H, 21-H_b), 0.21 (t, J 5.9), 0.053 and 0.087 (s, 6H, $SiMe_2$).

^{13}C NMR of **4.12** ($CDCl_3$): δ 35.74 (1), 34.06 (2), 199.60 (3), 123.70 (4), 171.67 (5), 32.93 (6), 32.13 (7), 35.74 (8), 54.78 (9), 38.72 (10), 20.78 (11), 35.68 (12), 41.85 (13), 53.94 (14), 25.67 (15), 32.00 (16), 34.72 (17), 15.91 (18), 17.45 (19), 19.91 (20), 55.44 (21), -4.88 and -5.39 ($SiMe_2$), 17.98 CMe_3 ($SiCMe_3$), 25.84 ($SiCMe_3$).

20α-Hydroxy-17α,21α-cyclopregn-4-en-3-one 4.13

The tert-butyldimethylsilyl ether **4.11** (121 mg, 0.28 mmol) in tetrahydrofuran (5 mL) was treated with 1M nBu$_4$NF-THF (0.7 mL, 0.7 mmol, 2.5 eq.) for 1.5 h at room temperature. The mixture was poured into water, and products were extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and evaporated to give a residue, which on FCC, on elution with 10% EtOAc-LP, gave **4.13** (78 mg, 0.25 mmol, 88%), mp 163-167 °C (from CH_2Cl_2-LP), (Found: C, 79.1; H, 9.4. $C_{21}H_{30}O_2$x1/4 H_2O (MW 314.472) requires C, 79.1; H, 9.6%).

^{1}H NMR of **4.13** ($CDCl_3$); δ 5.73 (s, 1H, 4-H), 3.28 (dd, J 3.5, 7.2, 1H, 20-H), 1.18 (s, 3H, 10-Me), 0.85 (s, 3H, 13-Me), 0.90 (dd, J 3.8, 7.2, 1H, 21-H_b), 0.22 (dd, J 3.5, 5.7, 1H, 21-H_a).

^{13}C NMR of **4.13** ($CDCl_3$): δ 35.74 (1), 33.96 (2), 199.56 (3), 123.82 (4), 171.39 (5), 32.93 (6), 32.07 (7), 36.06 (8), 53.85 (9), 38.70 (10), 20.53 (11), 34.09 (12), 40.69 (13), 54.59 (14), 24.63 (15), 28.19 (16), 37.30 (17), 117.42 (19), 19.62 (20), 51.51 (21).

20β-Hydroxy-17α,21α-cyclopregn-4-en-3-one 4.14

Treatment of the tert-butyldimethylsilyl ether **4.12** (152 mg, 0.35 mmol) in THF (5 mL) with 1M nBu$_4$NF-THF (0.8 mL, 0.8 mmol, 2.3 eq.), as described for **4.11**, gave a residue, which on FCC, on elution with 25% EtOAc-LP, yielded **4.14** (69 mg, 0.22 mmol, 63%), mp 182-192 °C (decomp.) (from CH_2Cl_2-LP), (Found: C, 79.05; H, 9.6. $C_{21}H_{30}O$x1/4 H_2O (MW 314.472) requires C, 79.1; H, 9.6%).

^{1}H NMR of **4.14** ($CDCl_3$); δ 5.73 (s, 1H, 4-H), 3.51 (dd, J 3.1, 6.6, 1H, 20-H), 1.20 (s, 3H, 10-Me), 1.02 (s, 3H, 13-Me), 0.84 (dd, J 3.8, 7.2, 1H, 21-H_b), 0.33 (t, J 6.1, 1H, 21-H_a).

^{13}C NMR of **4.14** ($CDCl_3$): δ 35.75 (1), 33.75 (2), 199.60 (3), 123.77

(4), 171.50 (5), 32.96 (6), 32.07 (7), 35.75 (8), 54.84 (9), 38.69 (10), 20.71 (11), 35.96 (12), 41.85 (13), 53.88 (14), 25.60 (15), 33.80 (16), 36.58 (17), 16.22 (18), 17.45 (19), 20.18 (20), 55.41 (21).

4.2.0 Inhibition Activity Assay for 20α- and 20β-Hydroxydehydrogenases

Inhibition activity assay for 20α- and 20β-hydroxysteroid dehydrogenases was carried out in collaboration with Dr. J.C. Orr, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland.

3α,20β-Hydroxysteroid dehydrogenase from *Streptomyces hydrogenases* was obtained from Boehringer-Mannheim and used without further purification. Protein concentrations were determined by a modification of the method (Lowry *et al.*, 1951) involving precipitation with sodium deoxycholate and trichloroacetic acid (Peterson, 1977). Bovine serum albumin (BSA, from Sigma) was used as a standard.

Cortisone reductase activity (Edwards and Orr, 1978) was measured in a Beckmann DU-8 spectrophotometer as loss of absorbance at λ 350 nm per minute upon exposure of enzyme to 150 μM cortisone and 180 μM NADH in 500 μL 50 mM NaH_2PO_4 buffer pH 8.0 (ϵ_{350} = 5300 L mol^{-1} cm^{-1}) based on the following equation:

enzyme + cortisone + NADH → enzyme + 4-pregn-17α,20β,21-triol-3,11-dione + NAD^+

During enzymatic reduction of cortisone to 4-pregn-17α,20β,21-triol-3,11-dione, NADH is converted to NAD^+. But, NAD^+ contrary to

NADH is not seen after exposure to UV. Loss in absorbance observed is due to the disappearance of NADH.

Inactivation kinetics were performed with the enzyme (enough to have control values of ΔA_{350}/min of *ca* - 0.040) and NAD^+ (150 μM) placed in 0.95 mL of 100 mM $NaCO_3$ buffer (pH 9.2) and inhibitor at the appropriate concentration. After vortexing, 25 μL aliquots were removed at various times and assayed for cortisone reductase activity. Controls containing EtOH without inhibitor retained >90% activity at all time points (Edwards and Orr, 1987).

Enzyme evaluation studies showed that neither 20α- **4.13** nor 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one **4.14** was effective as an inhibitor of steroid 17β,20α- and 3α,20β-oxidoreductase, respectively (Dr. J.C. Orr, personal communication).

CHAPTER 5

C O N C L U S I O N S

The research in this thesis was carried out with the following five objectives:

1. Synnthesis and inhibitory activity for 20α- or 20β-hydroxysteroid dehydrogenases of the spirocyclopropanol isomers, 20α- and 20β-hydroxy-17α,20-cyclopregn-4-en-3-one, **4.13** and **4.14**. respectively.
2. Synthesis and inhibitory activity for aromatase of 19(R)- **2.12** and 19(S)-acetoxy-5β,19-cycloandrost-1-ene-3,17-dione **2.15** as well as 19(R)- and 19(S)-1β,19-cyclo-5α-androst-4-ene-3,17-dione and their synthetic intermediates **2.32**, **2.29**, **2.28**, **2.27**.
3. Synthesis and inhibitory activity for aromatase of the 19,19-bis substituted cyclopropyl steroid derivatives **2.71** (Scheme 2.33)
4. Synthesis and inhibitory activity for aromatase of 19(R/S)-amino-5β,19-cycloandrostane-3,17-dione **2.79a** (Schemes 2.41).
5. Improved synthesis of 4-hydroxyestrone **3.15a** and 4-hydroxyestradiol **3.16a** (Schemes 3.12 and 3.16), the natural metabolite of estrone and estradiol, and their derivatives **3.15b-3.15e**, **3.16a-3.16b**.

The results obtained are summarized as follows:

1 Two 17-spirocyclopropanol isomers, 20α- and 20β-hydroxy-17α,20-cyclopregn-4-en-3-one were synthesized. The syntheses were carried out from 5-pregnane-20-ene-3β,21-diol 21-acetate *via* the key intermediates: 3β-acetoxyandrost-5-en-17-al **4.4**, 20(E/Z)-enol *tert*-butyldimethylsiloxy isomers, **4.5b** and **4.6a**, and 17α,21α-cyclopropanes, **4.11** and **4.12**. Introduction of the silyl enol function was achieved by treatment of 3β-acetoxyandrost-5-en 17-al **4.4**, with TBDMSiOTf and triethylamine in diethyl ether. The ratio of *E* or *Z*

isomer depends on the bulk of the reagent: (1) TMSiOTf *E*:Z=1:1; TESiOTf, TBDMSiOTF, and i-Pr_3SiOTf *E*:*Z* = 1 : 0.4. Cyclopropanation of the *exo* 17,20-*tert*-butylsilyloxy double bond was performed by the Simmons-Smith method using the reported Zn-Ag couple or by an improved method using the Zn-Au couple.

Preliminary enzyme evaluation studies showed that neither 20α- **4.13** nor 20β-hydroxy-17α,20-cyclopregn-4-en-3-one **4.14** was effective as an inhibitor of steroid 17β,20α- and 3α,20β-oxidoreductase, respectively.

2 The unsaturated 5β,19-cyclosteroid derivatives, 19(R)- **2.12** and 19(S)-acetoxy-5β,19-cycloandrost-1-ene-3,17-dione **2.15** were synthesized. The 5β,19-cyclopropanol function was achieved by reductive cyclization of androstane-4-ene-3,17-dion-19-al both with zinc in 50% aqueous acetic acid and Li-NH_3. Both reduction conditions gave the same products, where 19(R)-hydroxy-5β,19-cycloandrost-4-ene-3,17-dione **2.4** was the major one, 19(R):19(S)=1:0.3. The mechanism of the reaction-reductive cyclization has been proposed. The 1,2-unsaturation in ring A was introduced *via* oxidation of the 3-trimethylsilyl enol ether derivatives, **2.11a** and **2.14a**, with an equimolar amount of palladium acetate in acetonitrile.

Syntheses directed toward 19(R/S)-1β,19-cyclo-5α-androst-4-ene-3,17-diones were carried out *via* the unsaturated intermediate, 19-siloxy-5α-androst-1-ene-3,17-dione **2.25**, which was obtained by treatment of 19-siloxy-5α-androstane-3,17-dione with Ph_2Se_2, 3-iodoxybenzoic acid, and camphorsulphonic acid in tetrahydrofuran to give the androst-1-ene-3,17-dione **2.25** in 70%, yield.

Reductive cyclization of androst-1-ene-3,17-dion-19-al **2.28** in Li-NH_3 reversed the ratio of 19(*R*/*S*)-hydroxy-1β,19-cycloandrostane-

3,19-dione products, 19(*R*):19(*S*)=0.4:1, compared to reductive cyclization under Zn-50% aqueous acetic acid conditions, 19(*R*):19(*S*)=1:0.3. Furthermore, unlike reductive cyclization of androst-4-ene-3,17-dion-19-al with Zn-50% acetic acid which gave the less stable isomer **2.4**, androst-1-ene-3,17-dion-19-al **2.28** under the same conditions yielded the more stable isomer **2.28**. However, reductive cyclization with Li-NH_3 of androst-4-ene-3,17-dion-19-al and androst-1-ene-3,17-dion-19-al led to the formation of the less stable isomers, the 19(R)-hydroxy-5β,19- **2.4** and the 19(S)-hydroxy-1β,19- **2.31**, respectively, as the major products.

Attempts to introduce the 4,5-saturation in ring A of the 19(R)-1β,19-cyclo-5α-androstane-3,17-dione **2.29** *via* the 3-silyl enol ether derivatives were not successful. The synthetic approach failed because enolization occurred only toward C-2. The outcome of this research calls for a different synthetic approach toward 19(R/S)-1β,19-cyclo-5α-androst-4-ene-3,17-diones, particularly for the introduction of 4,5-unsaturation. It may be achievable *via* 4,5-epoxy-5ξ-androst-1-ene-3,17-dion-19-al or 5β-androst-1-ene-3,17-dion-19-al or androst-1,5-diene-3,17-dion-19-al.

In the course of the enol formation, a new approach to the synthesis of acetoxy enols was discovered: treatment of 19(*R*)-acetoxy-1β,19-cyclo-androstane-3,17-dione **2.32** with acetic anhydride and one equivalent of $BF_3.Et_2O$ in CH_2Cl_2 gave the 2-acetoxy enol **2.52**. However, treatment of 19(*R*)-acetoxy-1β,19-cyclo-androstane-3,17-dione **2.32** with an excess of $BF_3.Et_2O$ led to acylation at C-2 and formation of the cyclic steroid-BF_2 derivative **2.53**. The structure of the BF_2 steroid derivative **2.53** was determined by X-ray crystallographic analyis (Figure 2.2).

Aromatase evaluation studies.

Several steroid-based compounds were synthesized on the premise they could exert an inhibitory activity upon aromatase of the metabolic steroid pathway: hydroxylation, water elimination or the carbonyl group formation (Scheme 1.32).

Although some steroid and also non-steroid based inhibitors of these enzymes are known, the goal of the present research was to synthesize the compounds which would be more active and selective than those already available.

Using reductive cyclization methods, three 19(R/S)-hydroxy cyclosteroids **2.4**, **2.5**, and **2.29** were prepared from steroid precursors. These reactions allowed introduction, simultaneously, of the following functionalities at the steroid skeleton: 5β,19- or 1β,19-cyclopropane ring and 19(R/S)-hydroxy group. Their presence has been determined by ^{1}H, ^{13}C NMR spectra. The *in vitro* biological activity of these compounds was studied in multicentre collaborative studies using an aromatase inhibition assay performed in a system of human placental microsomes which manifested $^{3}H_2O$, released upon the convertion of [1β-^{3}H]androst-4-ene-3,17-dione (specific activity 15-30 Ci/mmol) to estrone (Thomas and Siitteri, 1974).

It was shown that in the range of the concentrations used, (1-20 μm) three compounds exerted an inhibitory activity ranging from more than 40% to 50%. Some other compounds, i.e. **2.28** and **2.27**, exerted lower inhibitory activity ranging from 24-30%, whereas two compounds were very weak inhibitors of *ca* 30% at 20 μM.

We postulated that the presence of the 19-hydrogen, in **2.32**, **2.12**, and **2.29**, projected toward the centre of the enzyme active side, is

responsible for this effect. Moreover, the structural geometry of **2.4** but not **2.5** supports the above conclusion. Compound **2.12** has a structural geometry different from its saturated analogue **2.4**. Introduction of the 1,2-unsaturation closely resembles the structure of the natural substrate of aromatase, androstanedione. Compounds **2.32** and **2.29**, even without 4,5-unsaturation, however, with structures close to substrate, show promising inhibitory activity.

On the basis of the results obtained it seems to be of importance that the putative inhibitors had both a structural geometry and an activated hydrogen exposed to the aromatase active side, whereas the noninhibitory **2.4** and **2.5** compounds, were lacking either the proper structure **2.4** or/and the activated hydrogen **2.5** not properly pointed toward the aromatase active side.

In conclusion, it may be postulated that the steroid based compounds obtained by reductive cyclization may be of use for *in vivo* inhibition *via* the suicide or "suicide" like mechanism.

3 An attempt to synthesize the 19,19-disubstituted 5β,19-cyclosteroid derivatives by reductive cyclization of methyl androst-4-ene-3,19-dion-19-oate failed and led to the 3,3'-dimeric steroid derivative **2.67**. The product was determined by the ^{1}H , ^{13}C, and T_1 of ^{13}C NMR spectra, EIMS and FABMS.

4 Attempts to synthesize 19(R/S)-amine-5β,19-cycloandrostane-3,17-dione **2.79b** *via* reductive cyclization of the androst-4-ene-3,17-dione 19-oxime **2.79** as well as its derivatives either with zinc in acetic acid or Li-NH_3 failed. However, reaction of Li-NH_3 with androst-4-ene-3,17-dione 19-TBDMSioxime **2.81** led to a reductive

elimination of the γ substituent. The keto conjugated system versus δ-derivitized oxime led to the fragmentation of the C-10 substituent yielding the β,γ-unsaturated ketones **2.3** and **2.7** (Scheme 2.43). This could be a general relationship, and the new reaction could be added to the list of oxime fragmentations. This experimental result suggests additional investigations of similar fragmentations of androst-4-ene-3,17-dion-19-al 19-oxime as well as other substituents (RN=CH- where R= MeO, $C_6H_5CH_2O$, R_2N- *etc.*) may be justified.

5 4-Hydroxyestrone **3.15a** has been synthesized by acidic aromatization of 4β,5β-epoxyestr-1-ene-13,17-dione **3.14b**. Introduction of the unsaturation at C-1-C-2 was achieved with Ph_2Se_2, camphorsulfonic acid, and 3-iodylbenzoic acid in a good yield (50-79%). It was found that estr-4-ene-3,17-dione can be converted into 4α,5α-epoxyestra-3,17-dione in 80% yield in a one-step procedure by treatment of estr-4-ene-3,17-dione with 1,1,1-trifluoromethyl(methyl)-dioxirane (Mello reagent) *in situ*. Mello reagent was also found to be the only convenient reagent to introduce an oxygen function into a weakly nucleophilic 3,4-double bond of 4-chloro-estra-4-ene-3,17-dione **3.13c** (Sceme 3.16) to give 4β-chloro-4α,5α-epoxyestra-3,17-dione **3.14c** in 80% yield. 4-Hydroxyestrone was obtained also upon pyrolysis of compound **3.14c**, however, with a lower yield (*ca* 30%) than under acidic aromatization of compound **3.14b** (73%). It was noted that pyrolysis of compound **3.14c** or **3.14d** at *ca* 180 °C gave three products (4-hydroxyestrone **3.15a**, 4-hydroxyestra-4,6-diene-3,17-dione **3.15c**, and estra-5,7,9-triene-3,17-dione **3.15e**) and pyrolysis at *ca* 240 °C gave two products (4-hydroxyestrone **3.15a** and a new compound, estr-5,7,9-triene-3,17-dione **3.15e**).

General Experimental Techniques

Products of organic reactions were monitored by TLC in the following solvent systems on silica gel (Merck type 60H): acetone-light petroleum ether (35-60 °C) (LP), diethyl ether-LP, and ethyl acetate-LP; compounds were visualized with a UV lamp where appropriate and then by dipping the plates in 8% concentrated sulphuric acid in ethyl alcohol followed by heating on a hot plate at *ca* 120 °C where colour changes could be observed. Anhydrous Na_2SO_4 was used as a drying agent for solvents during work-up of a reaction mixture. Flash column chromatography (FCC) was carried out on silica gel (Therochem silica gel 20-45 microns for column chromatography). Melting points were determined on an Electro-thermal Kohler-type apparatus and are uncorrected. Elemental analyses were done by Mr. W. Baldeo, School of Pharmacy, University of London, England.

<u>^1H, ^{13}C, COSY, NOE, HSQC, HMBQC, T_1 of ^{13}C NMR spectra</u> (performed by T. Foniak, T. Wolowiec, and K. Marat, University of Manitoba)

^1H and ^{13}C NMR spectra were recorded on a Bruker AM300 instrument operating at 300 MHz for hydrogen and 75 MHz for carbon. Carbon spectra were classified as to multiplicity with the DEPT technique (Doddrell *et al.*, 1982).

All ^1H and ^{13}C chemical shift data are presented in the experimental sections. Homonuclear correlation (COSY), (Aue *et al.*, 1976), heteronuclear correlation (HSQC), heteronuclear multiple-bond proton-detected quantum correlation (HMBQC), and nuclear Overhauser effect (NOE) difference spectra (Kinns and Sanders, 1984) were recorded on a Bruker AM500 spectrometer. Samples were measured as *ca* 50 mmol/mL solutions in 5 mm sample tubes in $CDCl_3$, CD_3COCD_3 or $CDCl_3$:CD_3OD (1:1) as indicated. For samples in $CDCl_3$, the residual $CHCl_3$ peak in the

solvent (δ_H = 7.26 ppm; δ_c = 77.0 ppm) was used as the internal reference for both proton and carbon spectra. For the remaining solvents $SiMe_4$ was used as reference standard.

Mass Spectrometry (performed by W. Buchannon, University of Manitoba).

EI and FAB Mass spectra were determined on a VG-7070-HF instrument at 70 eV. Xenon gas was used for recording the FAB mass spectra.

Molecular modelling (MMX).

MMX molecular mechanics modelling program, Version 4.0, was used to study structures of synthesized compounds and their dynamics (PCMODEL, Molecular Modeling Software, Serena Software, 1990).

X-ray Crystallographic Analysis (performed by Dr. J. Bridson, Memorial University of Newfoundland).

X-Ray crystallographic data for selected compounds, **3.14c** and **4.14**, were collected on a Rigaku AFC6S diffractometer with a graphite monochromator MoKα (λ = 0.71069 A) radiation. Crystallographic data are summarized in Tables 3.3 and 4.1. Cell constants and an orientation matrix for data collection were obtained by least squares using the setting angles for **3.14c** 18 or **4.14** 23 reflections in the 2θ ranges **3.14c** 9.08-23.39° or **4.14** 8.97-19.03°. Data collection used the ω-2θ scan technique. Omega scans of several intense reflections, made before data collection, had an average scan width at half-height of **3.14c** 0.36° or **4.14** 0.33° with a take off angle of 6°. Scans of **3.14c** (1.47 + 0.35 tan θ°) or **4.14** (0.94 + 0.30 tang θ°) were made at a speed of 4°/min or 2°/min, respectively (in ω). The weak reflections $1<10.0\sigma(1)$ were rescanned for **3.14c** (maximum of 4 rescans) or **4.14** (maximum of 2 rescans), and the counts accumulated to assure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peaks counting time to

background counting time was 2:1. Three reference reflections, measured every 150 reflections remained constant and decay correction was applied. Intensities were corrected for Lorentz and polarization effects; a correction for absorption was applied based on azimuthal scans of several reflections. The structure was solved using direct methods (Gilmore, 1984; Beurskens, 1984). Full matrix least-squares refinement with anisotropic factors given to all non-H atoms converged to for ($R = 0.042$, $R_w = 0.038$, $S = 2.22$) **3.14c** or to ($R = 0.059$, $R_w = 0.031$, $S = 1.62$) **4.14**. The weighting scheme was based on counting statistics. The maximum shift/error in the final cycle 0.00. The largest peaks in the final difference map were 0.21 and -0.25 e^-/A^3 for **3.14c** and (0.21 and -0.20 e^-/A^3 for **4.14**. Atomic scattering factors were from International Tables for X-ray crystallography (Cromer and Weber, 1974). Anomalous dispersion effects were included in F_{calc} and values were taken from Cromer (Cromer, 1974). All calculations were made with the TEXSAN crystallographic software package (TEXAN-TEXRAY, 1985). Figures 3.1 and 4.1 are represented in PLUTO (Motherwell and Clegg, 1978). Tables of atomic coordinates, bond length and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

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