STUDIES IN THE CHEMISTRY OF MEDICINALLY ACTIVE STEROIDS:

POTENTIAL AROMATASE INHIBITORS AND STEREOCHEMISTRY OF KETONE REDUCTION

by
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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE

FACULTY OF PHARMACY
THE UNIVERSITY OF MANITOBA
WINNIPEG, MANITOBA

August 1991

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ISBN 0-315-76909-2

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BY

ANNE M. FRIESEN

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ACKNOWLEDGEMENTS

I would like to thank Dr. John F. Templeton for his encouragement and patience during the research for this thesis as well as for his guidance and assistance in its preparation. I would also like to acknowledge Dr. S. Kumar for his helpful discussion in the identification of the catechol estrogens.

I am grateful to the following people for their help: Mr. K. Marat who performed the ^{1}H NMR, Mr. W. Buchanan who did the mass spectroscopy work (Department of Chemistry, University of Manitoba), and Mrs. D. Smith who performed the HPLC analyses (Faculty of Pharmacy, University of Manitoba). Technical assistance from Mr. D. Neufeld and Mr. F. Friesen is also gratefully acknowledged.

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SECTION I

INTRODUCTION

Many of the chemical processes that take place in living organisms are the result of enzymatic activity. These enzymes and their cofactors are responsible for ensuring the continued growth and health of their hosts. Certain disease states, however, are the result of inappropriate enzyme activity.

Enzymes are of particular importance medically because they serve as potential sites of intervention. Biological responses to various substrates can be modified by subtly changing the structure of the activating molecule. This substrate would then be called an agonist if it produced the same physiological effect as the naturally occurring substance or an antagonist if it prevented this from exerting its full effect.

The process of substrate binding can use any one of the different chemical interactions. These include ionic interactions, hydrogen bonding, and van der Waals forces. Any one of these chemical bonds, or a combination, can play a part in substrate-receptor affinity.

Covalent bonding produces the strongest link. This interaction is usually of a more permanent nature than the first three and results in prolonged enzyme inactivation. It is also generally more specific than the temporary chemical interactions listed above. This promises to be an exciting new direction to pursue in the area of drug design.

RECEPTOR ANTAGONISTS

Competitive antagonism of a natural receptor substrate by a drug molecule occurs when the reaction between the receptor and the drug is reversible (usually when the attractive forces are relatively weak). In this type of antagonism the drug's inhibitory effects can be overcome by increasing the concentration of the normal substrate until the maximal effect is obtained. Both molecules bind to the same receptor site but a greater number of substrate molecules can overwhelm the drug molecule and hamper its inhibitory effect.

A noncompetitive antagonist is generally one that binds irreversibly to the receptor. There is no competition with the substrate since increasing the substrate concentration will not prevent inhibition. Instead, the noncompetitive antagonist, once it has attached itself to the receptor, blocks any effect that the substrate might have. The antagonist does not need to bind directly to the active site to exert its effect. It can attach itself to another part of the receptor molecule and induce a structural change in the active site such that the substrate no longer fits.

A. Mechanism-Based Inactivation (Suicide Inhibition)

The subject of mechanism-based enzyme inactivators or "suicide substrates" has been reviewed in detail by Walsh (1982) and is of particular interest because of its applicability to the formation of enzyme-specific drugs. Two generations of suicide substrates are described. The first generation includes compounds that are structural analogs of the natural substrate but contain some reactive group that is

involved in a displacement reaction with a nucleophilic amino acid side chain in the active site of a target enzyme. The disadvantage with this group of compounds is that they are too reactive and are usually more useful as tools for structural mapping of purified enzyme active sites. Cyclophosphamide, however, is an example of a first generation suicide substrate that is used extensively in clinical settings to treat various types of cancer.

The second generation of suicide substrates are compounds that contain a latent reactive group that is only uncovered after binding to the enzyme. This ensures a high degree of specificity with a relatively low degree of indiscriminate reactivity.

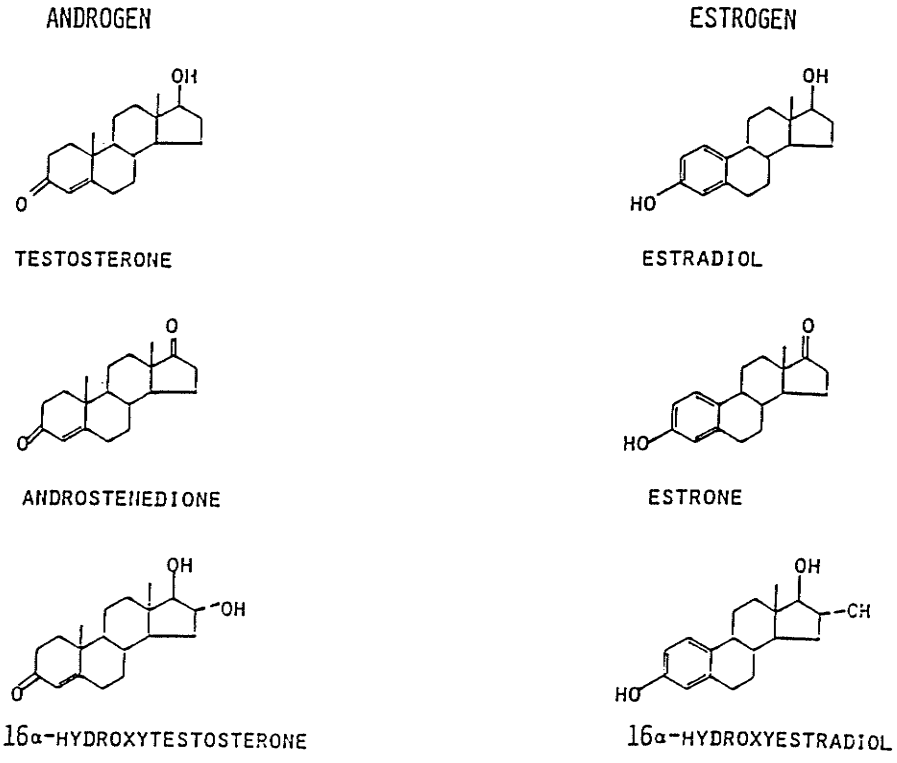
In general the suicide substrate will be an electrophilic species after activation at the enzyme active site and requires a nucleophilic amino acid side chain with which to react. A variety of functional group activations are possible. These are listed in Table 1.

TABLE 1: Types of Functional Groups Available for Activation

Acetylenes
Olefins
Halogenated Substrates
Cyclopropanes
Quinones
Carbonium Ion Precursors
Penicillin Analogs
Thionosulfur Compounds

B. Estrogen Synthetase (Aromatase)

The aromatization of suitable androgens is accomplished by an enzyme complex known as estrogen synthetase or aromatase. Composed of two parts, aromatase cytochrome P450 and nicotinamide adenosine dinucleotide (NADPH) P450 reductase, this complex requires three moles of both oxygen and NADPH for every mole of estrogen produced (Carr, 1986). The reaction, generally, is a series of hydroxylations occurring at C-19 of any aromatizable androgen resulting in the formation of an estrogen, water, and formic acid (Nicholls et al., 1986; Fishman and Goto, 1981; Fishman and Raju, 1981; Kellis and Vickery, 1987). Androgens suitable for aromatization include testosterone, androstenedione, and 16α-hydroxytestosterone which are transformed into estradiol, estrone, and 16α-hydroxyestradiol, respectively, the three principal human estrogens.



Estrogens are largely responsible for the actions of the female reproductive cycle and the physical changes that occur at puberty. These hormones are also important in less tangible ways such as forming feminine behaviour patterns. Combined with the actions of progesterone, follicle stimulating hormone, and luteinizing hormone, estrogens form part of a necessary but complex series of events that are aimed at reproduction as the goal (Murad and Haynes, 1980).

Aromatase is most active in the ovaries during the reproductive years of a woman's life (Kuehnel et al., 1986; Santen, 1986). Ovarian thecal cells are important in forming androgen precursors while granulosa cells convert these androgens into estrogens in reponse to various impulses sent by the follicle stimulating hormone and the luteinizing hormone (Tonetta et al., 1986).

After menopause, however, the greatest area of aromatization seems to be adipose tissue (Simpson et al., 1983; Folkerd and James, 1983), especially in obese women (Newton et al., 1986). Enzyme activity remains unchanged in obesity but there is more tissue in which aromatization can occur. While ovarian aromatase activity is controlled by the gonadotropins the mechanism of most peripheral enzyme activity is unknown. Studies have shown that glucocorticoids such as dexamethasone will increase aromatase activity in vitro (Simpson et al., 1983; Folkerd and James, 1983) but have little effect in vivo (Newton et al., 1986). Progesterone has also been implicated in the control of fat aromatase but by an inhibitory action. The increase in peripheral estrogen formation after menopause

may be a result of decreased progesterone production by the ovaries (Newton et al., 1986).

Aromatase is present in other tissues as well. These include the liver, brain, muscle, skin, and hair follicles (Santen, 1986; Chabab et al., 1986). Its existence has also been demonstrated in normal and malignant breast tissue although the significance of this has not yet been established (Vermeulen et al., 1986). Aromatase activity is shown to be significantly higher in cancerous tissue but the concentration of androgen precursors is lower than in normal breast tissue. There is a theory that the majority of mammary estrogen is a result of estrone sulfate being taken up into the cell where it undergoes hydrolysis to estrone (Vermeulen et al., 1986; Brodie et al., 1986). However, even though breast cancer tissues can have a 10-50 fold higher level of estrogens than plasma, a correlation between aromatase activity, estrogen concentration, and estrogen receptor status has not been demonstrated (Santen, 1986).

Because aromatase is active in peripheral tissues, estrogens are formed at target organs. They can then bind to certain receptors and exert their effect. Estrogens interact with receptors in the pituitary to affect the release of gonadotropins. Very generally, estrogens exert negative feedback activity on the formation of gonadotropin releasing hormone. This eventually slows down the release of follicle stimulating hormone and, to a lesser extent, luteinizing hormone. With a decrease in gonadotropin release follows a decreased production of estrogen

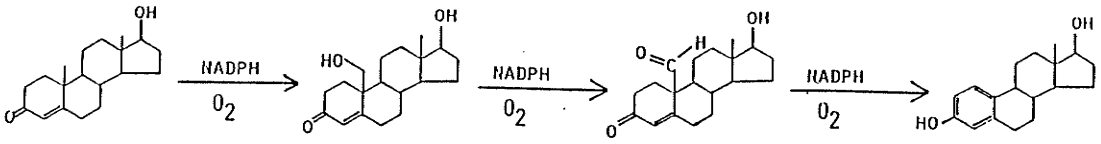
and progesterone in the ovary (Murad and Haynes, 1980).

At a cellular level estrogens can bind to specific estrogen receptors in the cytoplasm. This complex is then translocated to the nucleus where it is responsible for the rapid synthesis of mRNA and, consequently, various proteins (Wittliff, 1984). A general increase in RNA synthesis and protein production occurs a few hours later and DNA synthesis takes place after that (Murad and Haynes, 1980).

Steroid transport in the plasma is achieved by three different proteins: albumin, testosterone-estradiol binding globulin, and corticosteroid binding globulin. Estrogens can enter a cell by passive diffusion (Wittliff, 1984) but, as previously mentioned, most target organs are also capable, to some degree, of manufacturing estrogens with their own aromatase.

The structure of aromatase has not yet been elucidated and one of the problems associated with the study of this enzyme is that it is very difficult to purify (Muto and Tan, 1986). This is due to the membrane-bound nature of the enzyme and also because solubilized aromatase is rapidly inactivated by atmospheric oxygen. Therefore any conclusions drawn from a preparation of "purified" enzyme have to be considered carefully to avoid attributing an effect to aromatase when another steroid-metabolizing enzyme might be contaminating the system.

A recent review by Cole and Robinson (1990) details the aromatization process. It seems likely that all three steps occur at the same active site of the same enzyme complex. Generally, the aromatase reaction is as follows:



The first step is a "classical" cytochrome P450 hydroxylation occurring at C-19 and involves 1 molar equivalent of NADPH and oxygen. The second step, which also requires 1 molar equivalent of NADPH and oxygen, involves stereospecific removal of the C-19 pro-R hydrogen to produce the 19-oxo compound (Cole and Robinson, 1990; Fishman and Goto, 1981). The aldehyde is formed either by direct dehydrogenation of the 19-alcohol group or by stereospecific dehydration of a gem-diol, which is formed by a second "classical" hydroxylation. The latter mechanism is currently favoured (Cole and Robinson, 1990).

The third step of the reaction has not been fully elucidated as yet. The most likely theory features attack of an enzyme-bound ferric peroxide on the 19-aldehyde group to form an unstable geminal hydroxyferric peroxide. This intermediate could collapse to estrogen and formic acid by hydride shift, proton transfer, or free-radical pathways (Cole and Robinson, 1990). Earlier proposed mechanisms required a third hydroxylation at the 1α- or 2ß-postion followed by dehydration, thereby introducing the required degree of unsaturation to form the aromatic ring (Muto and Tan, 1986).

More work needs to be done to fully and accurately determine the mechanism of estrogen synthesis. This work will become easier when a purified

enzyme preparation can be achieved.

C. Aromatase Inhibitors

1. Rationale

There are a number of reasons for attempting to create an aromatase inhibitor that will act as a suicide substrate. Firstly these agents could provide an alternative to chemotherapy commonly used for estrogen dependent neoplasms (Brodie et al., 1986). Agents such as 5-fluorouracil, methotrexate and cyclophosphamide usually produce severe side effects due to their relatively non-specific cytotoxic actions. A specific irreversible aromatase inhibitor could potentially decrease the incidence of adverse reactions by focussing on the production of estrogens, a propagating factor in approximately 30% of malignant breast tumours (Simon et al., 1984), without affecting the rest of the body.

Secondly, aromatase inhibitors could be used in place of organ ablative surgery (Santen, 1986). The latter procedure can stop the production of estrogens in specific organs (e.g. the ovaries) but will not prevent peripheral aromatase activity in the way that an inhibitor would. This would be especially important for postmenopausal women who produce much of their estrogen in adipose and other peripheral tissues.

Thirdly, specificity for the aromatase enzyme would prevent interference in other steps of steroid synthesis. Aromatase is the last enzyme in the estrogen pathway and inhibiting it alone would have very little effect on the production of

glucocorticoids, mineralocorticoids, or androgens (Santen, 1986).

Besides their potential efficacy in neoplastic diseases that are estrogen dependent, aromatase inhibitors could be used in other situations. These include contraception, gynecomastia, endometriosis (Brodie et al., 1983), and a syndrome called MIEHA or menometrorrhagia, infertility, elevated serum estradiol, and hyperprolactinemia resulting from increased aromatase activity (Odell and Meikle, 1986). This syndrome is a result of increased aromatase activity. Estradiol overproduction is responsible for the associated infertility and heavy menses and it has been suggested that aromatase inhibition might be appropriate in treating this and similar conditions (Brodie et al., 1983; Odell and Meikle, 1986).

Another reason for developing aromatase inhibitors is more fundamental in that these structures can help to elucidate the functions of estrogens in various physiological processes, leading to a better understanding of the effects of the sex hormones.

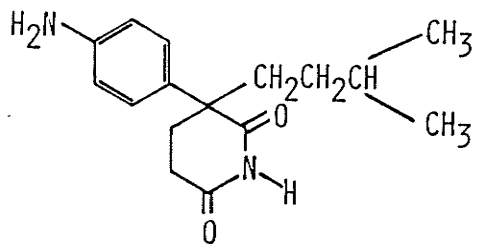
2. Known Aromatase Inhibitors

Aminoglutethimide is the most extensively used aromatase inhibitor and is effective in 55% of women with estrogen receptor positive tumours (Santen, 1986). It acts primarily by reducing the amount of estrogen produced peripherally (Hartmann and Batzl, 1986) and must be used in conjunction with hydrocortisone in therapy because of its lack of aromatase specificity. Glucocorticoid synthesis is impaired in the adrenals by aminoglutethimide resulting in a reflex rise in

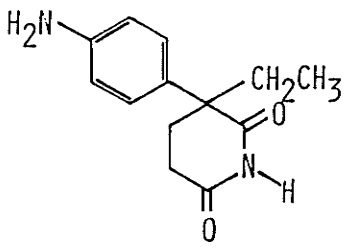
adrenocorticotrophic hormone (ACTH) and overstimulation of the adrenals. Hydrocortisone administration prevents this reflex action.

The response of tumours to aminoglutethimide/hydrocortisone therapy is similar to antiestrogen therapy with tamoxifen but is less often prescribed because of side effects. Aminoglutethimide/hydrocortisone treatment can result in nausea and vomiting, lethargy, skin rash, orthostatic hypotension, ataxia, and drug fever. Occasional hematologic toxicities have also been reported (Compendium of Pharmaceuticals and Specialties, 1991).

The severity of side effects with aminoglutethimide as well as the lack of response in some tumours have prompted research in synthesizing an aminoglutethimide analogue that would be more active against aromatase and less active in inhibiting the other steroid-producing enzymes. The most potent aromatase inhibitor produced to date is 3-isopentyl-3-(4-aminophenyl)-piperidine-2,6-dione (Hartmann and Batzl, 1986).



3-ISOPENTYL-3-(4-AMINOPHENYL)-PIPERIDINE-2,6-DIONE



AMINOGLUTETHIMIDE

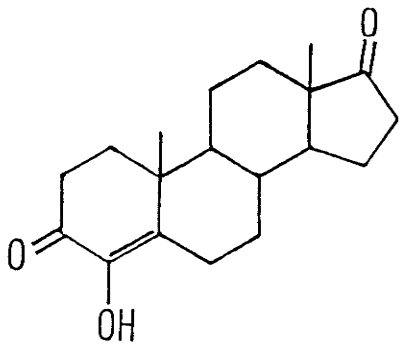
The increased inhibition of aromatase activity is thought to be a result of this analogue's increased lipophilicity over aminoglutethimide. This has been suggested

(Hartmann and Batzl, 1986) since previously designed, more polar analogues (possessing amine and hydroxyl groups) demonstrated less aromatase inhibition.

Although this compound is more potent than aminoglutethimide in aromatase inhibition, its effects on other steroid-metabolizing enzymes is not proportionately increased. For example, its inhibitory effect on desmolase (the enzyme responsible for converting cholesterol into pregnenolone) is similar to that of aminoglutethimide. Because of this, equivalent aromatase inhibiting doses of the isopentyl derivative and aminoglutethimide demonstrate different effects on desmolase with the new compound having less effect on this enzyme.

A caution with this compound is that greater lipophilicity can enhance its penetration of the blood brain barrier. Since aminoglutethimide was used in the past as an anticonvulsant, increased levels of a similar compound in the central nervous system might have some adverse central effects. This would be an important factor in assessing clinical results with this compound.

The most successful candidate for aromatase suicide inhibition thus far has been 4-hydroxyandrostenedione (Goss et al., 1986).



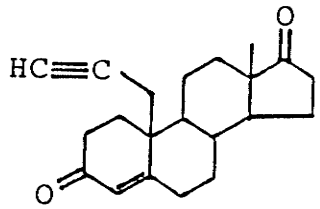
4-HYDROXYANDROSTENEDIONE

The exact mechanism has not been determined as yet but the kinetics of the reaction indicate that addition of androstenedione, the naturally occurring substrate, slowed the rate of inactivation. This competitive inhibition is followed by gradual, time-dependent loss of enzyme activity which occurs only in the presence of NADPH. Enzyme activity is not recovered after the residual inhibitor is removed by washing the preparation (Brodie et al., 1986; Brodie et al., 1983).

Irreversible inhibition is also indicated by the fact that enzyme inactivity is maintained long after the compound has left the blood. Even though 4-hydroxyandrostenedione is rapidly cleared from circulation via metabolism to the glucuronide, plasma levels of estradiol have been consistently below control levels a week after the last dose (Goss, Jarman et al., 1986).

This compound has been found to be approximately 60 times more potent than aminoglutethimide and is more selective for aromatase. Therefore corticosteroid supplementation is not required since other steroid-metabolizing enzymes are not affected. In fact in clinical trials 4-hydroxyandrostenedione has demonstrated the same degree of efficacy without the adverse effects of aminoglutethimide (Brodie et al., 1986; Brodie et al., 1983). Although large studies of a longer duration need to be conducted, 4-hydroxyandrostenedione promises to be a useful adjunct in the treatment of metastatic breast carcinoma. This will become apparent when optimal route of administration, formulation, and dosage are determined.

Another compound designed as an irreversible aromatase inhibitor makes use of an acetylenic group to bind to the enzyme.



10ß-PROPARGYL-4-ENE-3,17-DIONE

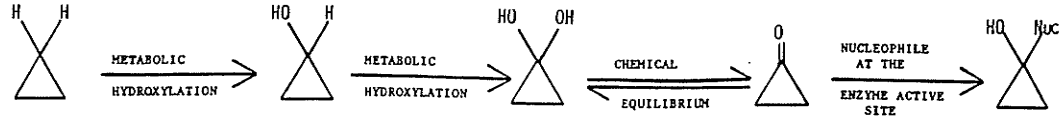
This compound is a potent inhibitor of aromatase but its mechanism of action is not clear. The first mechanism suggests that the 19-methylene group is oxidized to a 19-carbonyl, resulting in an α,ß-acetylenic ketone. A nucleophile at the enzyme active site would be attracted to the electrophilic end of the chain (carbon 21) and form a covalent, irreversible bond (Cole and Robinson, 1990; Covey et al., 1981).

The second mechanism involves iron-oxo attack on the acetylenic functional group to produce an oxirene species. This unstable group could either directly, or after rearrangement, react with an active site grouping in the aromatase enzyme (Cole and Robinson, 1990; Metcalf et al., 1981).

There are many more possible structures to investigate as potential suicide inhibitors of the aromatase enzyme complex. The purpose of these studies is to develop a potential aromatase inactivator using a cyclopropane ring as the latent electrophilic species.

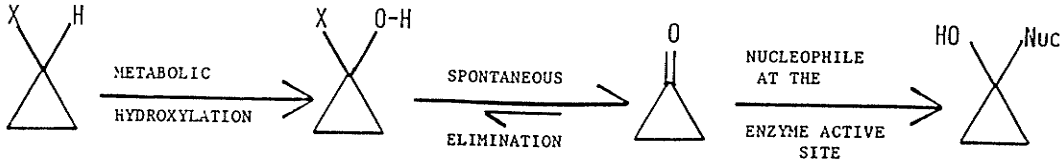
DISCUSSION

A cyclopropane ring could act as a mechanism-based-inhibitor of a dehydrogenase in this manner:



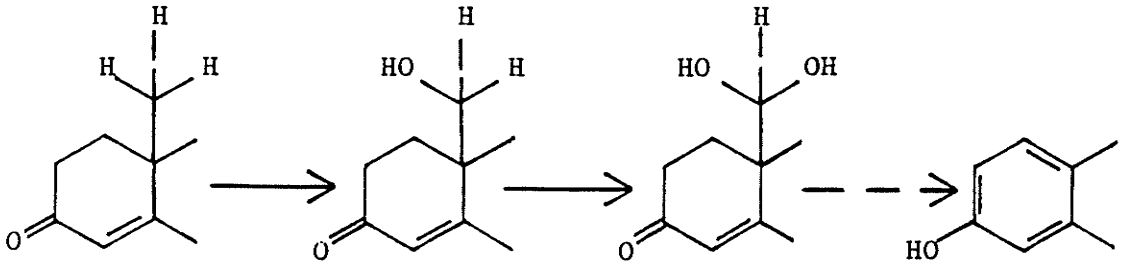
The formation of a diol after two consecutive enzymatic hydroxylations allows an equilibrium to be established between the diol and cyclopropanone. A covalent bond is formed when a nucleophilic section of the enzyme active site attacks the compound in the ketone state.

However, metabolic hydroxylation of the cyclopropane ring does not occur readily (Templeton and Kim, 1976). This problem may be overcome by the presence of a substituted cyclopropane ring thereby activating the adjacent C-H bond for metabolic oxidation. Therefore the hypothesis is that a cyclopropane ring containing an electronegative substituent (X) can be oxidized more readily at the substituted position.

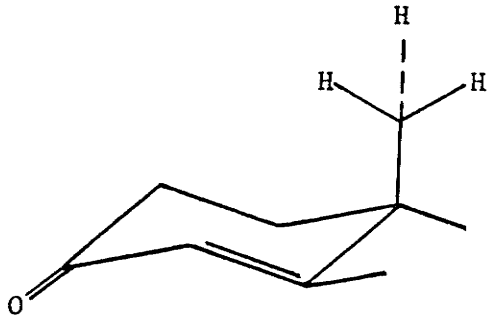


X = OH, OR, OCOR, F, Cl, Br

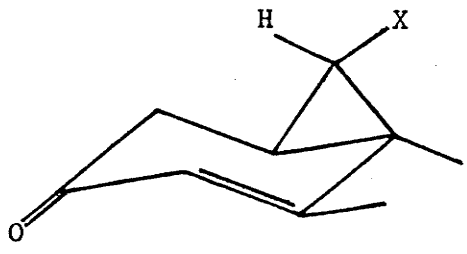
The formation of a steroid with a C-1(10) cyclopropane ring with a C-19 Pro-S leaving group would be the type of compound most likely to interact with estrogen synthetase. As outlined earlier the first step in the testosterone to estradiol conversion is hydroxylation of one of the C-19 hydrogens. It has been shown that the Pro-R hydrogen is the hydrogen which is enzymatically oxidized (Beusen et al., 1987).



Although the three hydrogens on testosterone's C-19 methyl group are able to rotate, thereby changing their orientations, Dreiding models show that the methylene hydrogens are in a sterically favourable position for reaction depending on the absolute specificity of the enzyme.



TESTOSTERONE



SUBSTITUTED CYCLOPROPANE COMPOUND

Figure 1 demonstrates the proposed mechanisms of enzyme inactivation (1,2,3). With the electronegative group in the Pro-S position oxidation takes place at the Pro-R hydrogen. In mechanisms 1 and 2 spontaneous elimination results in cyclopropanone formation. A nucleophilic portion of the aromatase enzyme can attack the electrophilic carbon and form a covalent bond. In mechanism 3 spontaneous rearrangement is responsible for the formation of a carbonyl group and subsequent nucleophilic attack. The bonds formed in mechanisms 2 and 3 are covalent and irreversible. Although mode 1 represents the least stable bond formation, the equilibrium nevertheless favours it. The enzyme is effectively inactivated by all three mechanisms.

MECHANISM 1

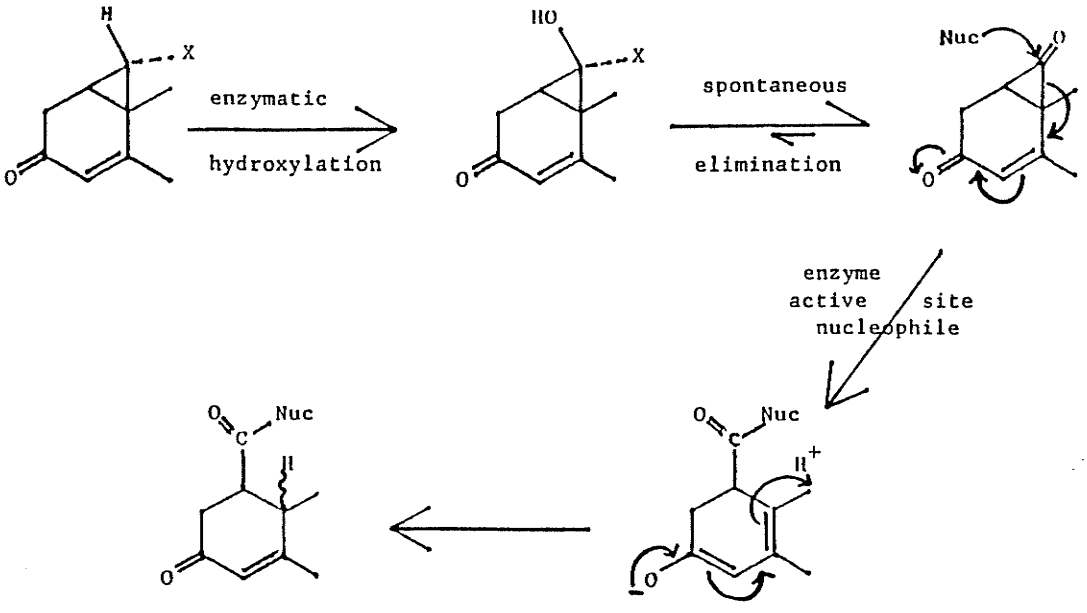
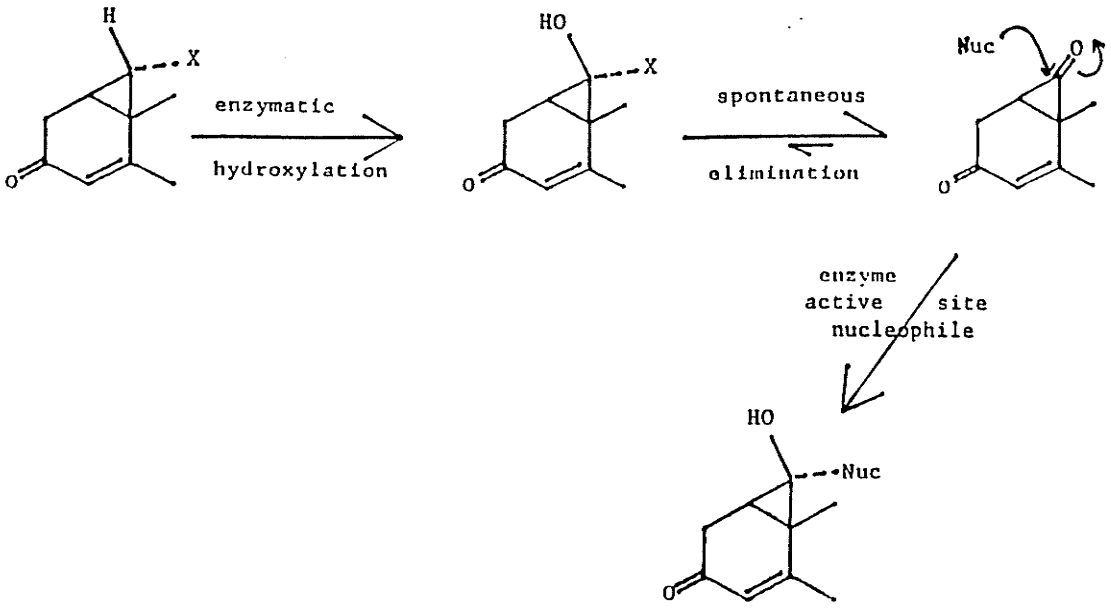


FIGURE 1

MECHANISM 2



MECHANISM 3

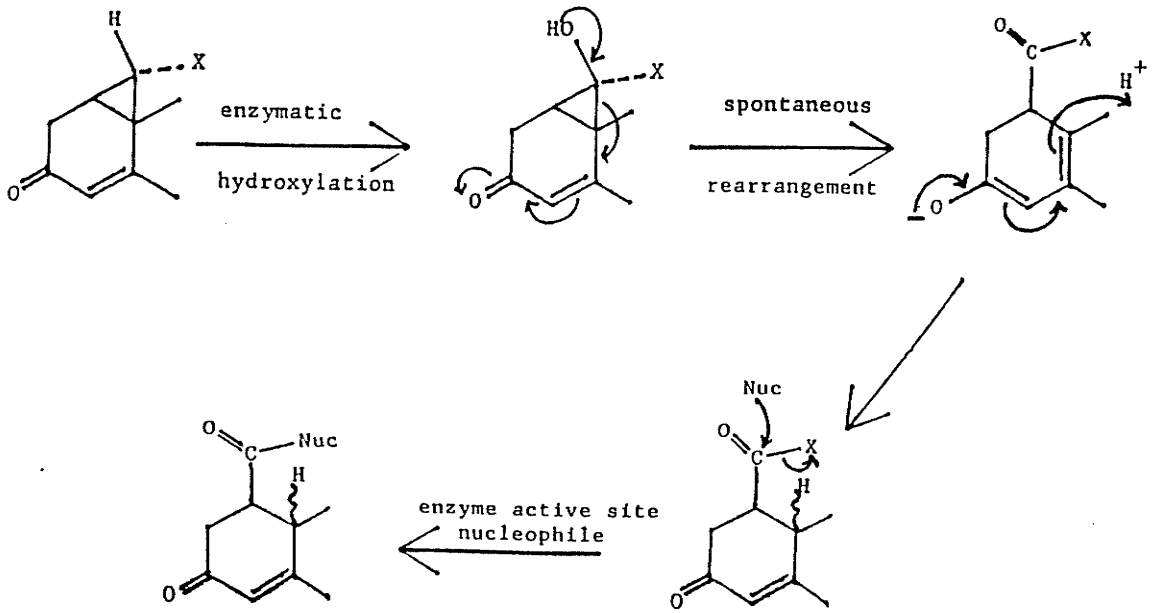
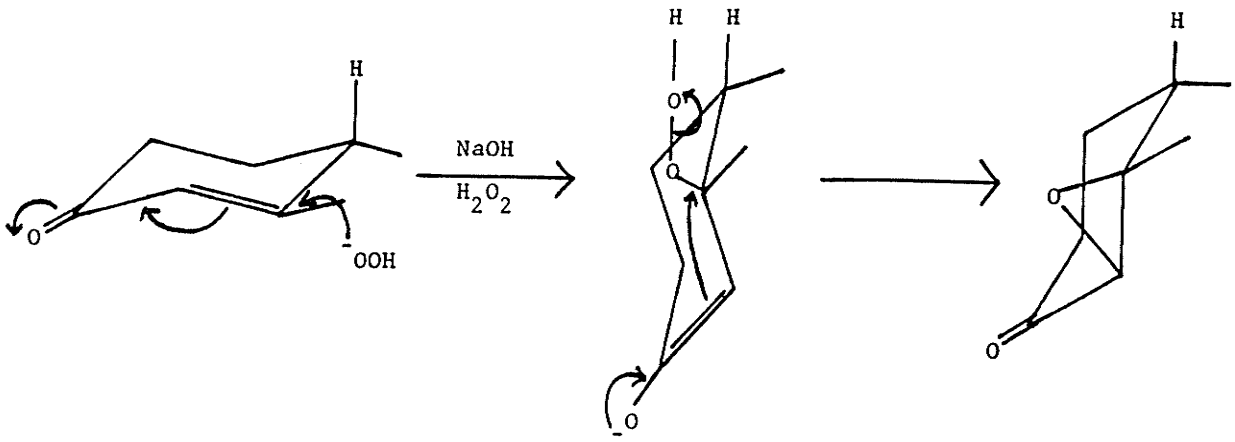


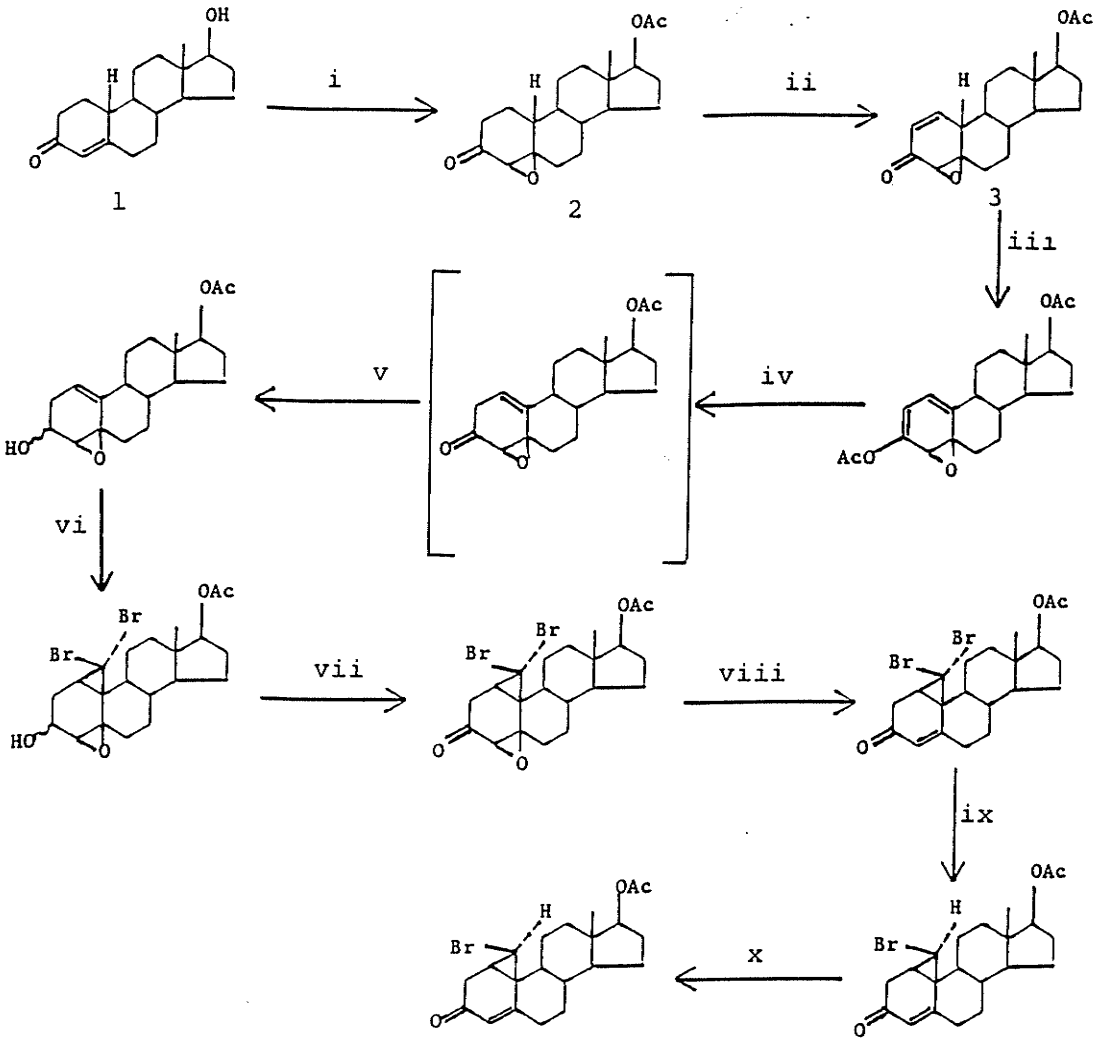
FIGURE 1 continued

The following Schemes for the synthesis of a mechanism-based-inactivator of the estrogen synthetase (aromatase) enzyme were considered.

Scheme 1 uses 19-nortestosterone (1) as the starting material. Hydrogen peroxide and sodium hydroxide are added to produce the epoxide at carbons 4 and 5. Proton NMR showed that approximately 90% of the product was the 4ß,5ß-epoxide, unlike the 19-methyl steroids where a reaction under the same conditions yields a 3:1 ratio of α to ß (Kirk and Hartshorn, 1968a; Matthews and Hassner, 1972). In these molecules the peroxide adds predominantly from the bottom because of the steric hindrance of the C-19 methyl group which points upward from the steroid plane. In 19-nortestosterone, however, the reaction would proceed mainly from the top of the molecule (equatorial attack) as follows:



After acetylation of the 17ß-hydroxy group with acetic anhydride and pyridine (2), dehydrogenation with selenium dioxide in acetic acid results in a conjugated double bond at carbon 1 (3) (Le Quesne et al., 1986). Dienol acetylation of the

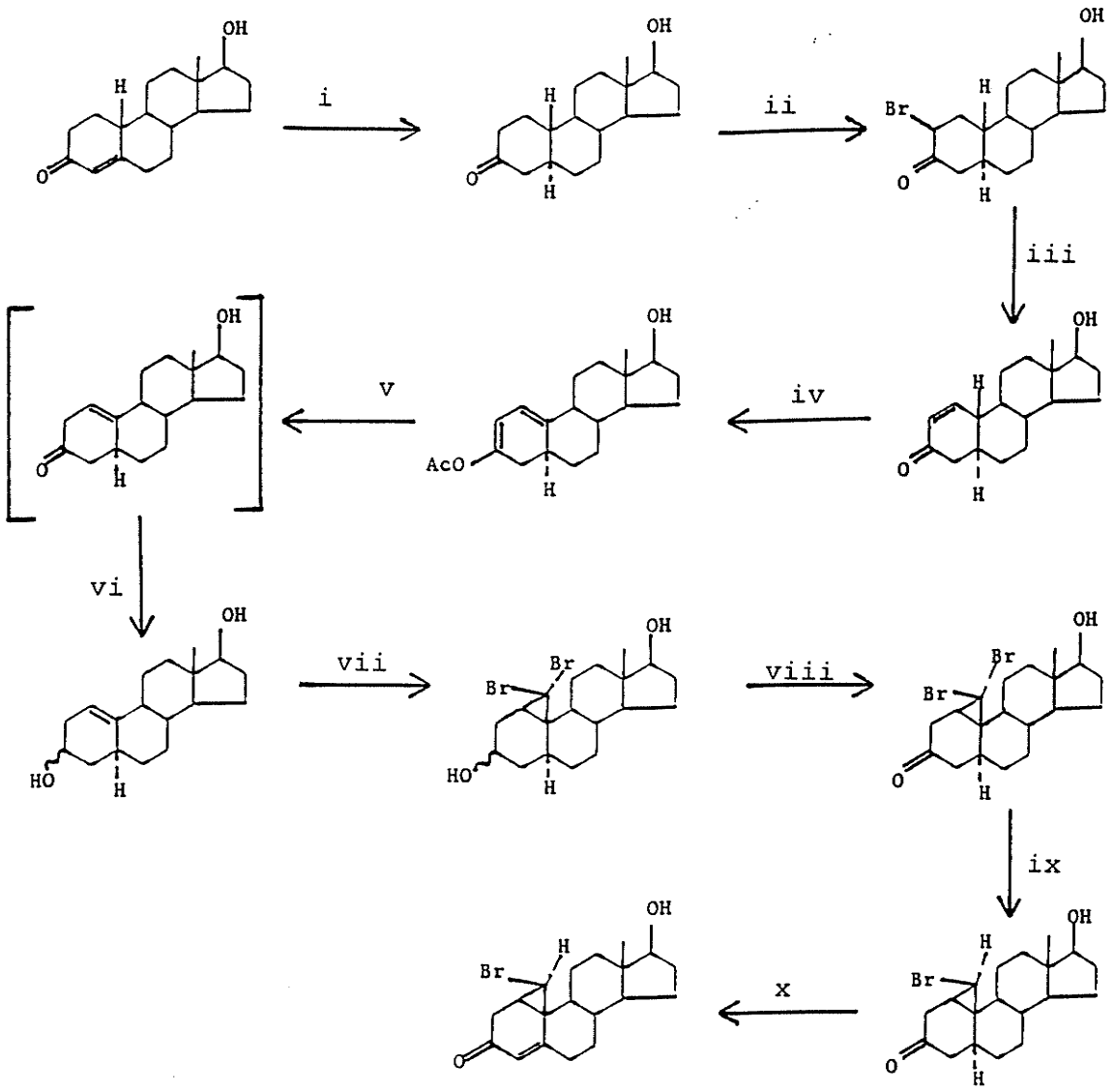


SCHEME 1

i. H_2O_2/NaOH; Ac_2O/pyridine; ii. SeO_2/dry t-BuOH/glacial acetic acid; iii. TMSiCl; iv-v. $NaBH_4$; vi. carbene attack; vii. [O]; viii. Zn/HOAc; ix. nBu_3SnH; x. hydrolysis/[O].

C-3 ketone shifts the double bond around the ring to the 1(10) position. Sodium borohydride reduction conditions hydrolyse the acetate group and quickly reduce the carbonyl group, forming the 3-hydroxy compound. Because the double bond has been shifted to the 1(10)-position where it is trisubstituted, we anticipate it will not revert as protonation should occur at the less hindered C-2 position. This double bond is then available for carbene attack, most probably from the ß-face of the molecule since it is less sterically hindered based on Dreiding models. After oxidation back to the C-3 ketone, mild zinc and acetic acid treatment or an equivalent reagent, e.g. ferric tetracarbonyltetramethylurea (Alper and Des Roches, 1977), can be used to reduce the protecting epoxide and regenerate the C-4 double bond. Halide reduction with e.g. tri-n-butyltin hydride forms a compound useful for in vitro testing, while further hydrolysis and oxygenation forms the potentially active compound for in vivo use.

Scheme 2 again uses 19-nortestosterone as the starting material. Hydrogenation with sodium in liquid ammonia results in reduction of the A ring to the thermodynamically more stable trans (5α) ring junction (Bowers et al., 1958). Treatment with bromine in acetic acid yields both the 2- and 4-bromo compounds because enolization of the 3-ketone occurs equally towards both C-2 and C-4 but the 2ß-bromo predominates (Villotti et al.,1960). After separation of the 2-bromo steroid, elimination results in a double bond at carbon 1. Enol acetylation of the conjugated ketone may produce both the 1(10), 2-dienol and the 1,3-dienol but the

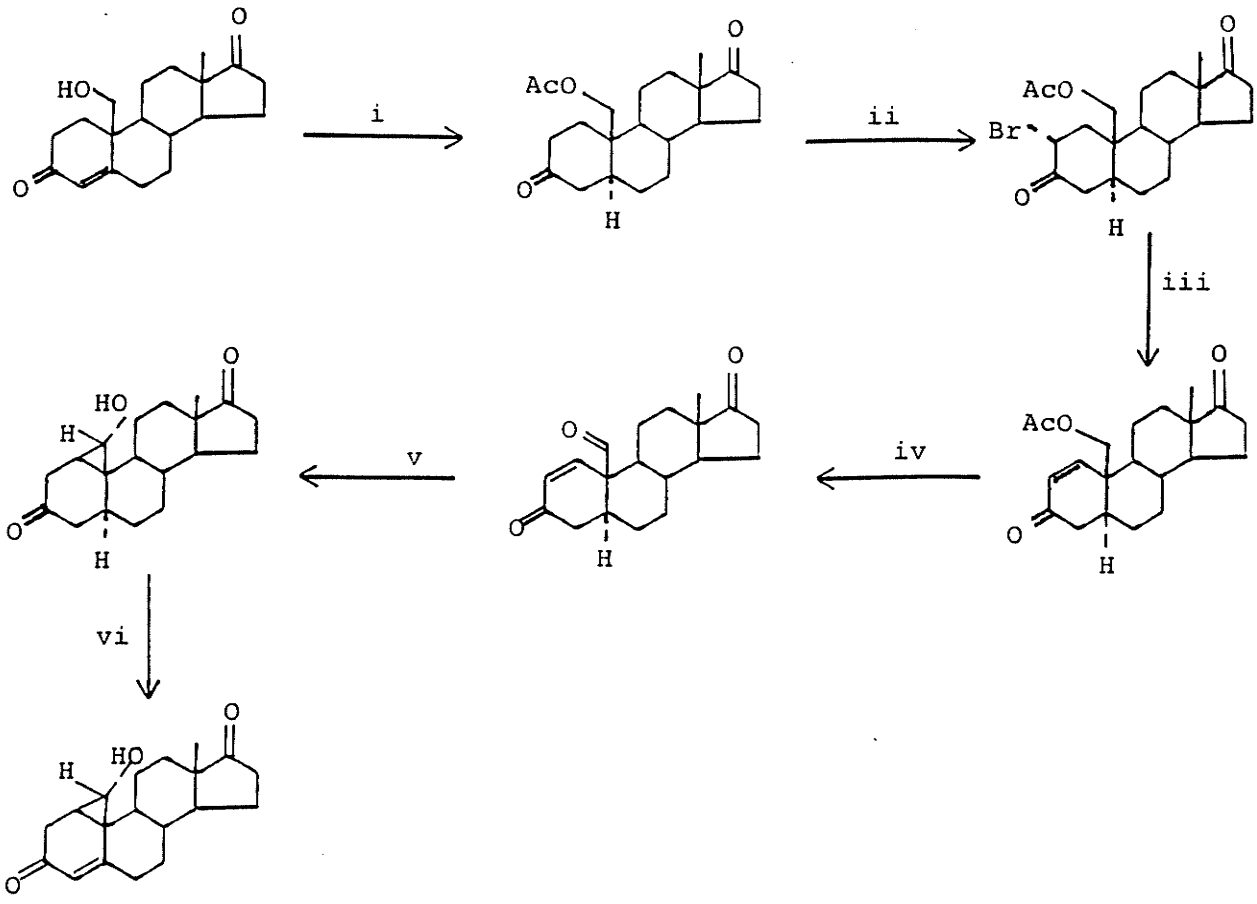


SCHEME 2

i. Ac_2O/pyridine; Na/NH_3; ii. Br_2/HOAc; iii. -HBr; iv. TMSiCl; v-vi. $NaBH_4$; vii. carbene attack; viii. [O]; ix. nBu_3SnH; x. DDQ.

former is probably the preferred product because of the trisubstitution of the C-1(10)-double bond. Also the non-bonded electrons on oxygen have a longer conjugated system for delocalization in the 1(10), 2-dienol. Sodium borohydride can again be used to reform, briefly, the ketone and cause reduction to the 3-hydroxy compound (Amar et al., 1969). As in Scheme 1 the next steps are carbene attack of the C-1(10) double bond followed by C-3 oxidation back to the ketone and selective reduction of one of the bromine atoms from the cyclopropane ring. The final step in this Scheme would be rejuvenation of the C-4 double bond with p-dichlorodicyanobenzoquinone (DDQ).

The third Scheme suggested for the synthesis of a potential aromatase inhibitor uses 19-hydroxytestosterone as the starting material (Scheme 3). After hydrogenation with sodium in liquid ammonia, the C-19 hydroxyl group is protected by acetylation. Bromination and elimination follow (as outlined in Scheme 2) and, after the acetate group is removed, the C-19 hydroxyl group is oxidized to the aldehyde. This carbon is then open to nucleophilic attack. Zinc acts as an electron donor to push electrons along the conjugated system so that the C-1(10) cyclopropane ring can be formed. The final step, which may require protection of the cyclopropanol, is reforming the C-4 double bond with p-dichlorodicyanobenzoquinone (DDQ).

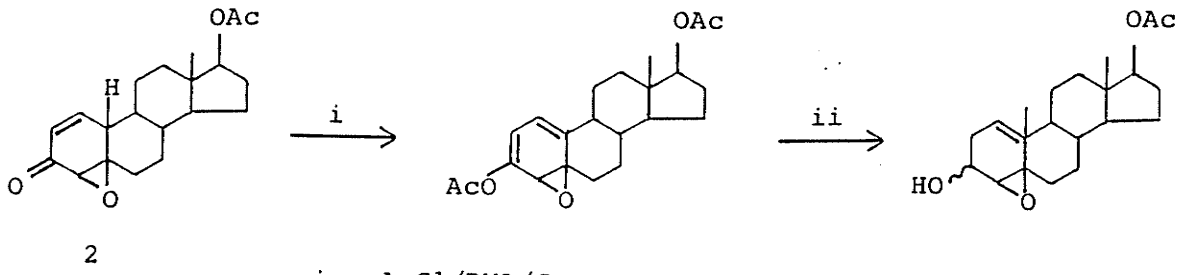


SCHEME 3

i. Na/NH_3; Ac_2O/pyridine; ii. Br_2/HOAc; iii. -HBr; iv. [O]; v. Zn; vi. DDQ.

It had been observed that treatment of the 4-en-3-one system (in testosterone and progesterone) with acetyl chloride and calcium carbonate in dimethylformamide gave the dienol acetate (Templeton et al., personal communication). Since the usual conditions for dienol acetylation are strongly acidic (Kirk and Hartshorn, 1968b), this milder method was employed with the epoxide, 17ß-acetoxy-4ß,5ß-epoxyandrost-1-en-3-one, as the third step in the proposed Scheme 1, shown again in Scheme 4. This epoxide on treatment with acetyl chloride and calcium carbonate in dimethylacetamide gave a crystalline product which was identified as 4-chloroestradiol or 4-chloro-estra-1,3,5(10)-triene-3,17ß-diol diacetate (3) based on the following evidence. Mass spectroscopy indicated the presence of one chlorine atom. A COSY correlation was performed and the results are given in Table 2. This correlation is in complete agreement with the assigned structure which may be formed as shown by the mechanism in Figure 2 where enolization and elimination lead to the formation of an aromatic ring.

When the reaction was carried out in the absence of acetyl chloride, 4-hydroxyestradiol or estra-1,3,5(10)-triene-3,4,17ß-triol 17-acetate (4) was formed. Besides the mp and ^{1}H NMR, which are in agreement with literature values (Gelbke et al., 1973), the ^{13}C NMR has been assigned by analogy with the chlorophenol. Further confirmation of the structure was possible after acetylation to produce estra-1,3,5(10)-triene-3,4,17ß-triol triacetate, a known compound (Kirk



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i. $AcCl/DMA/CaCO_3$; ii. $NaBH_4$

SCHEME 4

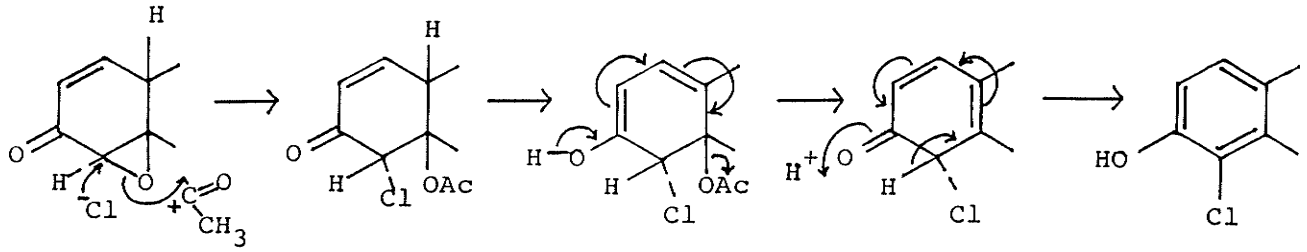


FIGURE 2: Possible mechanism for the formation of 4-chloroestradiol

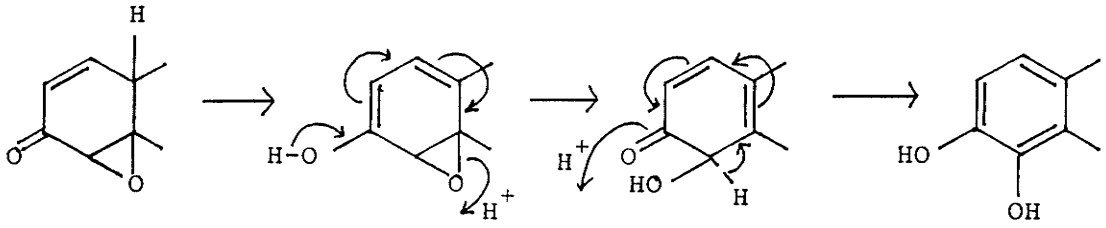


FIGURE 3: Possible mechanism for the formation of 4-hydroxyestradiol

TABLE 2: C-H (COSY) Correlation for

4-chloro-estra-1,3,5(10)-triene-3,17ß-diol diacetate[a,b].

Carbon	^{13}C (ppm)	^{1}H (ppm)
1	124.31	7.22
2	120.05	6.91
3	144.9	-
4	126.79	-
5	136.36	-
6	27.95	α3.01, ß2.72
7	26.85	α1.35, ß1.99
8	37.39	1.43
9	49.80	1.22
10	139.99	-
11	26.21	α2.30, ß1.48
12	36.83	α1.38, ß1.89
13	42.78	-
14	44.19	2.25
15	23.24	α1.43, ß1.77
16	27.58	α1.58, ß2.22
17	82.59	4.70
18	12.00	0.819
17ß-OAc	171.20, 21.18	2.06
3-OAc	168.96, 20.69	2.34

[a]Observed NOE's (Nuclear Overhauser Effect): H_1 to H_2; H_1 to $H_{11\alpha}$; $H_{6\alpha}$ to $H_{6\beta}$; $H_{12\alpha}$ to $H_{12\beta}$; $H_{12\beta}$ to 13-Me; 3-OAc to H_2; 3-OAc to H_1 ($H_{11\alpha}$ overlaps the 3-OAc and there is probably an NOE from $H_{11\alpha}$); 13-Me to $H_{17\alpha}$; 17-OAc to $H_{17\alpha}$.
[b]The 3-OAc, 4-Cl substitution is further confirmed by the ^{13}C shift of C-2. A Cl has a very small (~0) ortho substituent chemical shift (SCS) while OAc has a substantial negative SCS (~6 ppm).

and Slade, 1982). The ^{1}H NMR was in agreement with their reported values for this compound. A possible mechanism for its formation is outlined in Figure 3, similar to that for the 4-chloro derivative.

This regiospecific synthesis of 4-hydroxyestradiol offers an advantage in that catechol estrogens are difficult to purify from their estrogenic precursors (MacLusky et al., 1981). Catechol estrogens have been the objects of considerable interest as they are naturally occurring metabolites of estradiol (MacLusky et al., 1981; Purdy et al, 1982; Martucci, 1983; Vandewalle et al., 1985).

Despite the recently reported synthesis of 17ß-acetoxy-4ß,5ß-epoxyandrost-1-ene-3-one and 17ß-acetoxy-4α,5α-epoxyandrost-1-ene-3-one, repetition of the procedure proved to be very difficult and much smaller yields were obtained than those reported (Le Quesne et al., 1986). Because these compounds were key intermediates and, therefore, necessary in sufficient quantities to complete the Schemes outlined, work on these synthetic approaches was discontinued.

SECTION II

REDUCTIONS OF C-2 SUBSTITUTED STEROID C-3 KETONES

Sodium borohydride and lithium tri-tert-butoxyaluminum hydride are established metal hydride reagents for the reduction of ketones. The stereochemistry of reduction, particularly among steroid ketones has been studied extensively (Wheeler and Wheeler, 1972; Fajbos, 1959).

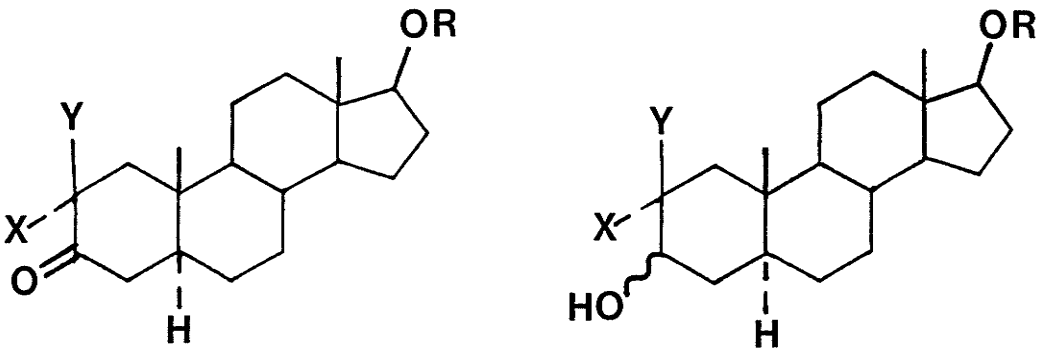
A number of rationalizations for the stereochemistry observed have been reviewed by Wigfield (1979). However, the early formulation by Dauben et al. (1956) based on the concepts of "steric approach control" and "product development control" remains a plausible hypothesis.

Potassium and lithium tris-(R,S-1,2-dimethylpropyl)-borohydride (K-Selectride and L-Selectride respectively) are relatively new reagents which show the reverse stereochemistry for ketone reduction to that found with the above reagents (Göndös and Orr, 1982; Templeton et al., 1987).

RESULTS

Sodium borohydride and lithium tris-(R,S-1,2-dimethylpropyl)-borohydride were used to reduce 17ß-acetoxy-5α-androstan-3-one (7), 17ß-acetoxy-2α-chloro-5α-androstan-3-one (8), 2α-methyl-17ß-propionyloxy-5α-androstan-3-one (9), and 17ß-acetoxy-2,2-dichloro-5α-androstan-3-one (10) as part of a study of the stereoselectivity of these reagents to α-substituted ketones. The results of this study are shown in Figure 4 and Table 3. Based on the overall data given in Table 4 the

FIGURE 4: Reduction Products of α-Substituted C-3 Ketones



7 R = Ac, X = Y = H
8 R = Ac, X = Cl, Y = H
9 R = Prop, X = Me, Y = H
10 R = Ac, X = Y = Cl

7a R = Ac, X = Y = H αOH
7b R = Ac, X = Y = H ßOH
8a R = Ac, X = Cl, Y = H αOH
8b R = Ac, X = Cl, Y = H ßOH
9a R = Prop, X = Me, Y = H αOH
9b R = Prop, X = Me, Y = H ßOH
10a R = Ac, X = Y = Cl ßOH

TABLE 3: Stereoselective Reduction of Steroid C-3 Ketones with Borohydride Reagents

5α-Androstane-3-one derivative	L-Selectride		$NaBH_4$	
	%3α-OH	%3ß-OH	%3α-OH	3ß-OH
2-unsubstituted (17ß-OAc) (7)	100 (7a)	*	10 (7a)	90 (7b)
2α-chloro (17ß-OAc) (8)	100 (8a)	*	40 (8a)	60 (8b)
2α-methyl (17ß-Prop) (9)	100 (9a)	*	24 (9a)	76 (9b)
2,2-dichloro (17ß-OAc) (10)	*	100 (10a)	*	100 (10a)

*Not detected by ^1H NMR or HPLC.
Relative percentages of C-3 epimers were estimated from ^1H NMR.

TABLE 4: ^{1}H NMR Spectra of 5α-Androstane Derivatives[a]

No.	13-Me	10-Me	17ß-OAc	17α-H	Other
8	0.81	1.10	2.04	4.59, dd, J = 7.4, 9.4 Hz	2.54, dd, J = 6.3, 12.8 Hz (1ß-H); 4.57, dd, J = 6.0, 13.3 Hz (2ß-H)
9	0.81	1.07		4.58, dd, J = 7.8, 9.2 Hz	1.00, d, J = 6.4 Hz (2α-Me); 1.13, t, J = 7.5 Hz ($OCOCH_2\underline{CH_3}$); 2.31, dd, J = 7.5, 15.0 Hz ($OCO\underline{CH_2}CH_3$)
10	0.81	1.21	2.03	4.59, dd, J = 7.0, 9.0 Hz	2.31, d, J = 15.0 Hz (1α-H); 3.05, d, J = 15.0 Hz (1ß-H)
7a	0.78	0.79	2.03	4.59, dd, J = 7.8, 9.1 Hz	4.05, m, (3ß-H)
7b	0.78	0.81	2.03	4.58, dd, J = 7.8, 9.1 Hz	3.59, m, (3α-H)
8a	0.78	0.83	2.03	4.58, dd, J = 7.8, 9.3 Hz	4.00, W ½ = 7 Hz (3ß-H); 4.23, octet, J = 2.7, 4.6, 12.7 Hz (2ß-H)
8b	0.78	0.86	2.03	4.58, dd, J = 7.8, 9.0 Hz	3.88, sextet, J = 5.1, 10.3, 10.3 Hz (3α-H); 3.94, octet, J = 4.6, 9.7, 12.4 Hz (2ß-H)
9a	0.77	0.79		4.59, dd, J = 7.8, 9.0 Hz	0.92, d, J = 6.9 Hz (2α-Me); 1.13, t, J = 7.5 Hz ($OCOCH_2\underline{CH_3}$); 2.31, dd, J = 7.5, 15.0 Hz ($OCO\underline{CH_2}CH_3$); 3.77 (3ß-H)
9b	0.77	0.82		4.58, dd, J = 7.8, 9.2 Hz	0.97, d, J = 6.3 Hz (2α-Me); 1.13, t, J = 7.5 Hz ($OCOCH_2\underline{CH_3}$); 2.32, dd, J = 7.5, 15.0 Hz ($OCO\underline{CH_2}CH_3$); 3.13, sextet, J = 4.9, 10.4, 10.4 Hz (3α-H)
10a	0.79	1.05	2.03	4.58, dd, J = 7.2, 9.0 Hz	2.04, d, J = 15.0 Hz (1α-H); 2.87, d, J = 15.0 Hz (1ß-H); 3.86, dd, J = 8.0, 15.0 Hz (3α-H)

[a]Spectra were recorded in $CDCl_3$ on a Bruker AM300 instrument.

following conclusions were drawn. The 2α-chloro- (8) and 2α-methyl- (9) derivatives of 17ß-acetoxy-5α-androstan-3-one (7) undergo stereoselective reduction with L-Selectride to the axial (3α) alcohol as seen for the unsubstituted ketone (Göndös and Orr, 1982). Sodium borohydride reductions of these compounds yield a mixture of alcohols but the equatorial (3ß) alcohol is the major product in all three cases. Reduction of the 2,2-dichloro-derivative, however, results in the equatorial (3ß) alcohol with both L-Selectride and sodium borohydride.

Products from the L-Selectride reduction of the 2α-methyl- and 2α-chloro-ketones were identified by their ^{1}H NMR coupling constants. Thus a proton signal at 3.77 ppm with a W ½ 7 Hz was assigned as the 3ß (equatorial) hydrogen in the 2α-methyl-3α-alcohol (9a). Similarly a proton signal at 4.00 ppm with a W ½ 7 Hz in the 2α-chloro-3α-alcohol (8a) was designated as the 3ß (equatorial) hydrogen. A proton signal at 4.23, octet, J = 2.7, 4.6, 12.7 Hz was assigned as the 2ß (axial) hydrogen.

The reduction products of 17ß-acetoxy-2,2-dichloro-5α-androstan-3-one (10) with both L-Selectride and sodium borohydride showed a signal at 3.86, dd, J = 8, 15 Hz, consistent with a 3α (axial) hydrogen in a 3ß-alcohol (10a) (Bhacca and Williams, 1964; Göndös and Orr, 1982). The presence of the ß-chloro substituent was demonstrated by the downfield shift of the 10-methyl group (1.06 Hz) as compared with the unsubstituted 3ß-alcohol (0.81 Hz) resulting from a 1,3-diaxial interaction between the 2ß-chloro and the 10-methyl substituent.

Sodium borohydride reduction products of the 2α-methyl (9) and 2α-chloro (8) C-3 ketones were also identified by their ^{1}H NMR coupling constants. In the reduction product of 2α-methyl-17ß-propionyloxy-5α-androstan-3-one (9) a proton signal at 3.13, sextet, J = 4.9, 10.4, 10.4 Hz was consistent with a 3α (axial) proton in a 3ß-alcohol (9b). Similarly a proton signal at 3.88, sextet, J = 5.1, 10.3, 10.3 Hz in the reduction product of 17ß-acetoxy-2α-chloro-5α-androstan-3-one (8) was consistent with the presence of a 3α (axial) hydrogen in a 3ß-alcohol (8b).

DISCUSSION

A remarkable difference was demonstrated between the two reducing agents (Table 3). Reduction with sodium borohydride resulted predominantly in the 3ß-hydroxysteroid (the equatorial product) and was consistent with previously reported results for the unsubstituted C-3 ketone (Wheeler and Wheeler, 1972), 2α-chloro (Wong et al., 1978), 2α-methyl (Cross et al., 1963), and 2,2-dichloro (Templeton et al., 1984) C-3 ketones.

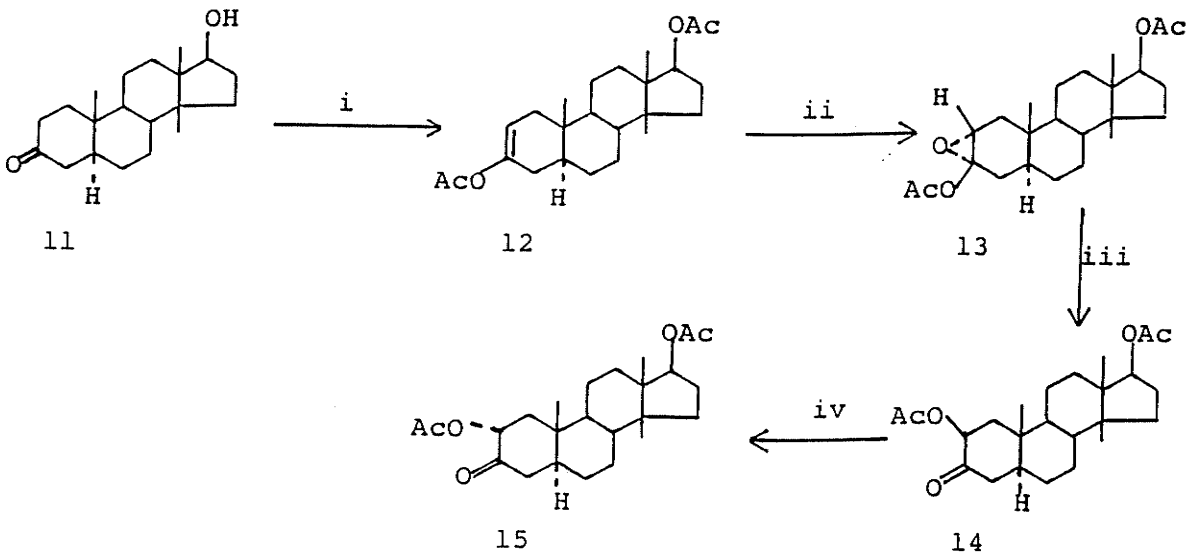
This relative stereoselectivity is understandable if one considers that the 19-methyl group lay above the steroid ring, creating steric hindrance for attack of the reagent from the ß-face. The ratio between epimeric alcohols will depend entirely on the nature of the 2α-substituent. Thus in the unsubstituted steroid the product is almost entirely the 3ß-alcohol, which indicates attack from the α-face. With a methyl group in the 2α (equatorial) position there is a slightly higher proportion of

3α-alcohol but the size of the methyl group is not enough to prevent most of the sodium borohydride from attacking from the reverse face of the molecule. This is not true for the 2α-chloro C-3 ketone, however. The ratio between the two epimeric alcohols is nearly equal because of the steric hindrance offered by the electronegative 2α (equatorial) chloro substituent. The 3ß-alcohol is still the major product and is the exclusive product when the 2,2-dichloro compound is treated with sodium borohydride. All attack by the reagent is from the α-face because of the two large substituents (10-methyl and 2ß-chloro) above the steroid ring.

L-Selectride, on the other hand, stereoselectively produced the 3α (axial) hydroxysteroid exclusively for the unsubstituted, 2α-methyl-, and 2α-chloro-steroids indicating that increasing the size of the alkyl substitution on boron leads to the formation of the less stable epimer with high stereoselectivity (Brown and Krishnamurthy, 1972; Brown, 1973). These results also indicate that the presence of a 10-methyl group has no effect on the ability of this reagent to attack from the ß-face of the steroid. A clear rationalization for this stereoselectivity has not been formulated (Ganem, 1975). However, when reduction was attempted on the 2,2-dichloro-compound, the product was exclusively 3ß-hydroxysteroid indicating that the introduction of an axial substituent on C-2 reverses the stereochemistry of the reaction.

SYNTHESIS AND METAL HYDRIDE REDUCTION OF ACETOXYKETONES

The synthesis of 2α,17ß-diacetoxy-5α-androstane-3-one (15) and 2ß,17ß-diacetoxy-5α-androstan-3-one (14) is shown in Scheme 5.



SCHEME 5

i. $Ac_2O/POCl_3$; ii. $mCPBA/CH_2Cl_2$; iii. heat; iv. HBr/HOAc

5α-Dihydrotestosterone acetate (11) was treated with acetic anhydride and phosphorus oxychloride to yield the 3-enol acetate (12) (Templeton et al., 1976) which on peroxidation with m-chloroperbenzoic acid gave the α-epoxide (13) as a result of stereoselective addition on the less hindered α-face. Stereospecific thermal rearrangement of the α-epoxide gave the 2ß (axial) acetoxy derivative (14) as described previously for the analogous cholestane compounds (Williamson et al., 1967). Following their method the 2ß-acetate was epimerized to the more stable equatorial isomer with hydrobromic acid in acetic acid. The melting points and ^1H NMR spectra of 12 and 13 were consistent with literature values (Ohta et al., 1968; Matsui and Kinuyama, 1977; Templeton et al., 1983).

Reduction of the 2α-acetoxy- and 2ß-acetoxy-3-ketone isomers with L-Selectride and sodium borohydride led to a mixture of products (Figure 5). To rationalize the origins of these products we must keep in mind the equilibrium shown in Figures 6 and 7. Figure 6 shows the mechanism for intramolecular rearrangement of the enolate anion from either the 2α- or 2ß-acetoxy-derivatives. Figure 7 shows the four possible kinetic and thermodynamic ketones resulting from protonation of the enolate anions formed in Figure 6. Reduction products may arise from any of the four possible ketones (i.e. 2α-acetoxy, 2ß-acetoxy, 3α-acetoxy, 3ß-acetoxy) shown.

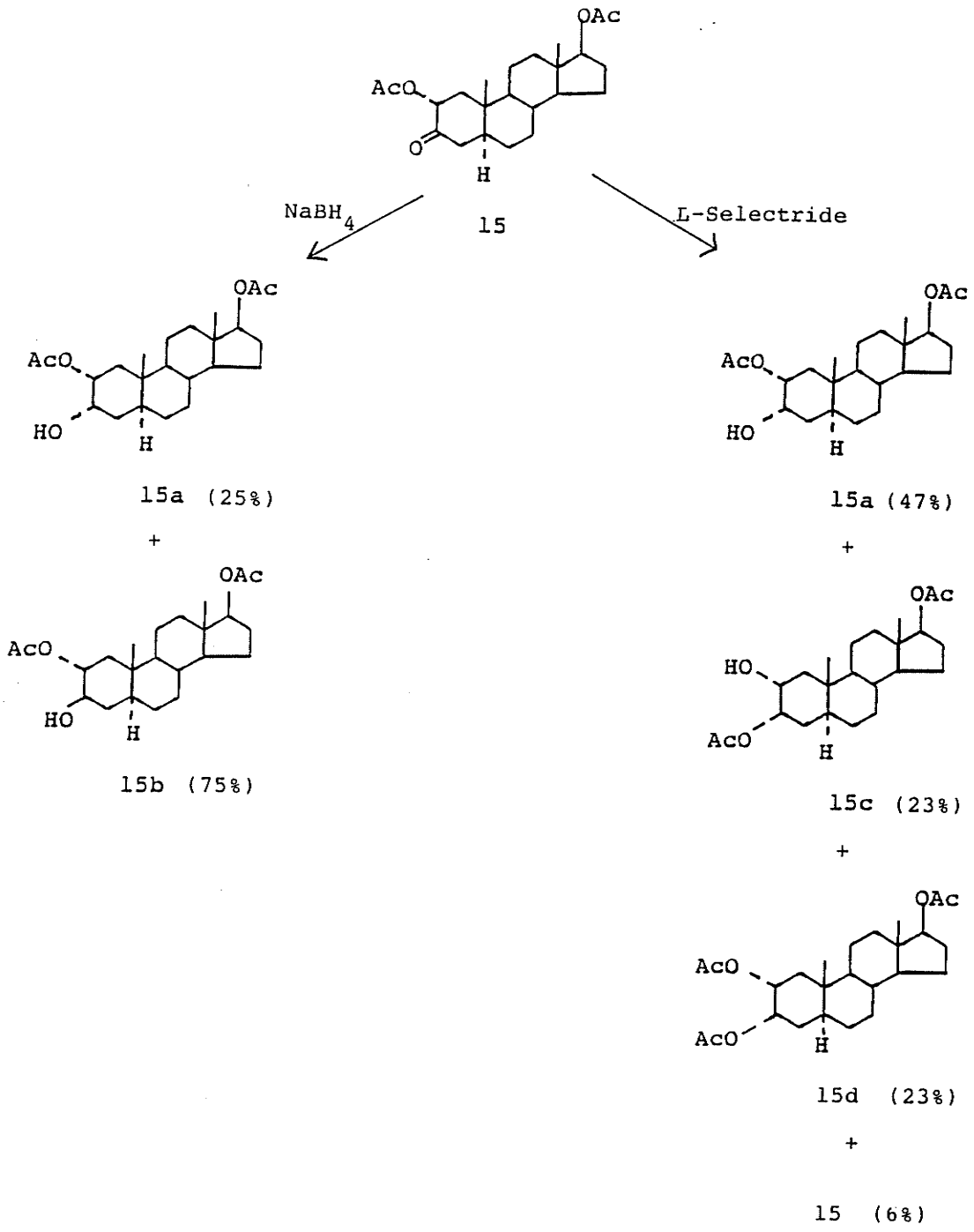


FIGURE 5: Reduction Products of 2α- and 2ß-Acetoxy C-3 Ketones (Percentages obtained were estimated from HPLC and ^{1}H NMR)

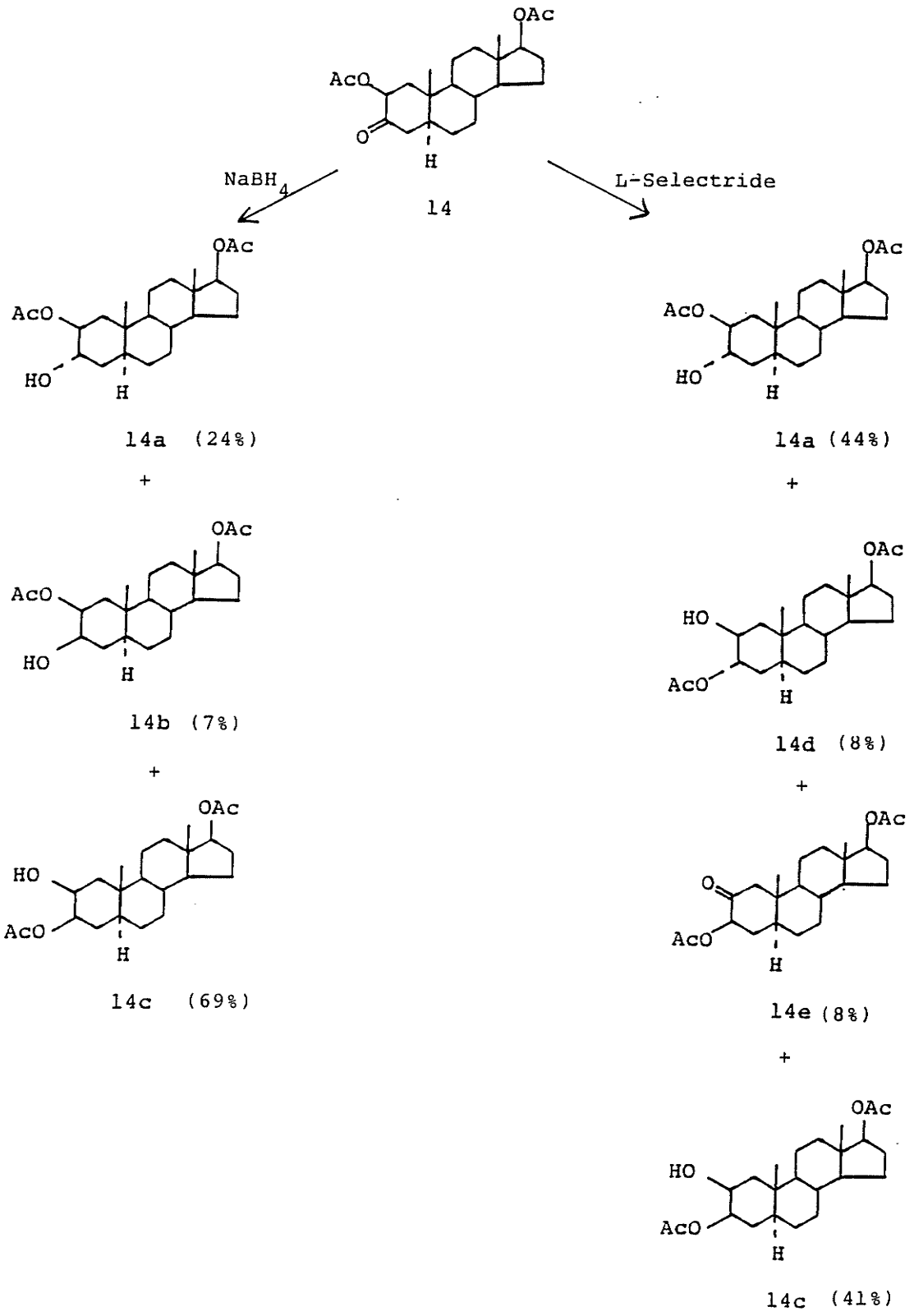


FIGURE 5 continued

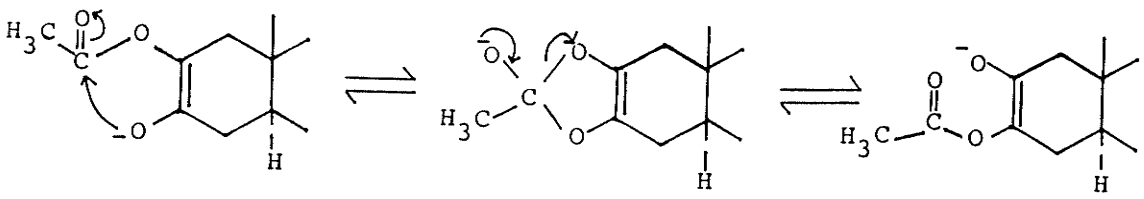


FIGURE 6: Intramolecular Rearrangement of the Enolate Ion

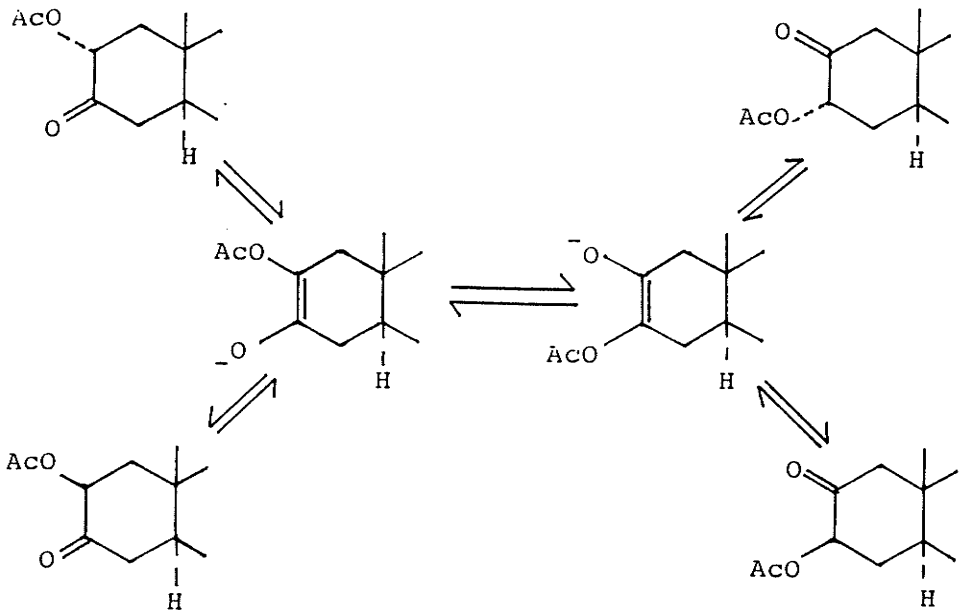


FIGURE 7: Possible Ketones Resulting from Enolic Equilibrium

2α-ACETOXY REDUCTIONS

Reduction of the 2α-acetoxy-derivative (15) with sodium borohydride gave the expected mixture (Matsui and Kinuyama, 1977) of 3α-hydroxy- (15a) and 3ß-hydroxy- (15b) isomers with the 3ß (equatorial) isomer as the major product indicating that reduction had occurred before enolization and rearrangement of the C-3 ketone.

L-Selectride reduction of the 2α-acetoxy-derivative (15) showed the following distribution of products. As expected the 3α-hydroxy-compound (15a) was the major product which is consistent with the anticipated mode of reduction with the reagent. The formation of the rearranged isomer, 5α-androstane-2α,3α,17ß-triol 3,17-diacetate (8), can best be explained as an interaction occurring between the acetyl and enolate oxygen (i.e. the acetyl is exchanged), and resulting in reduction of the C-2 ketone to give the 2α-(equatorial) alcohol. This is opposite to that seen for sodium borohydride reduction of the C-2 ketone (Wheeler and Wheeler, 1972). The triacetate (15d) can only have occurred through intermolecular reaction possibly during decomposition of an organometallic intermediate. However, if an intermolecular reaction had occurred and the triacetate had been formed, then an equivalent amount of a diol product must be formed. More work is required to clarify this result.

2ß-ACETOXY REDUCTIONS

Reduction of the 2ß-acetate (14) with sodium borohydride produces both α-

and ß-alcohols (14a and 14b) but the 3α-hydroxy compound predominates. This is unexpected as ß-face attack by the reagent would be sterically hindered by the 2ß-acetoxy and 10-methyl groups. However, ring A is probably not in the normal chair conformation but rather nearer a boat because of the 1,3-diaxial interaction between the 2ß-acetate and 10-methyl groups (Marat et al., 1987). Therefore the most favourable stereochemical approach of the reagent to the carbonyl group as well as the product's stability are not comparable to those of the unsubstituted ketone (7). The major product, 5α-androstane-2ß,3ß,17ß-triol 3,17-diacetate (14c), must result from initial enolization and rearrangement to the 3ß-acetoxy-2-ketone (14e) which gives the 2ß-hydroxy compound (14c) on reduction. The 2ß-hydroxy-compound is known to predominate from sodium borohydride reduction of the C-2 ketone, despite the formation of the thermodynamically less stable axial product, because ß-face attack by the reagent is hindered by the 10-methyl group (Wheeler and Wheeler, 1972).

Reduction of 2ß,17ß-diacetoxy-5α-androstan-3-one (14) with L-Selectride also gives a mixture of products. The major product is the same 3α-hydroxy-compound (14a) obtained from the sodium borohydride reduction. However, the 3α-hydroxy-compound (14b) is not present. Isolation of 3ß,17ß-diacetoxy-5α-androstan-2-one (14e) shows that enolization and rearrangement of the acetyl group does occur and that 5α-androstane-2ß,3ß,17ß-triol 3,17-diacetate (14c), also a major product, is formed by reduction of 14e. The minor product, 5α-androstane-2ß,3α,17ß-triol 3,17-

diacetate (14d), can most readily be rationalized as a result of rapid reduction of the kinetic intermediate 3α,17ß-diacetoxy-5α-androstan-2-one (16). This is not in complete agreement with a similar rationalization for the formation of 15c above where reduction of the C-2 ketone leads to the 2α-alcohol but the different ketone environment may be responsible for this. That the enolate rearrangement can occur is seen from the isolation of the C-2 ketone (14e) which accounts for the alcohol (14c).

Of the eight possible isomers (Figure 8) only 5α-androstane-2α,3ß,17ß-triol 3,17-diacetate (17) was not obtained.

The identification of the previously unreported isomers 14a and 14e was achieved by comparison of their ^{1}H NMR spectra. Figure 9 outlines the methine proton signals. The methine proton attached to the acetoxy-carbon is approximately 1 ppm downfield from the corresponding alcohol (Bridgeman et al., 1970). The equatorial protons, which are coupled to both axial and equatorial protons (J~2 Hz) overlap to give a broad singlet (W ½ ~ 7 Hz). The axial protons are also coupled to equatorial and axial protons, but the larger axial-axial coupling gives an even broader signal (W ½ ~ 21 Hz). These differences in W ½ allow for easy identification of axial and equatorial protons. The 1,3-diaxial interaction between the 2ß-substituent and 10-methyl causes a downfield shift in the latter signal of 0.12 ppm thereby allowing identification of the C-2 axial substituent. This comparison together with previously established structures allows the

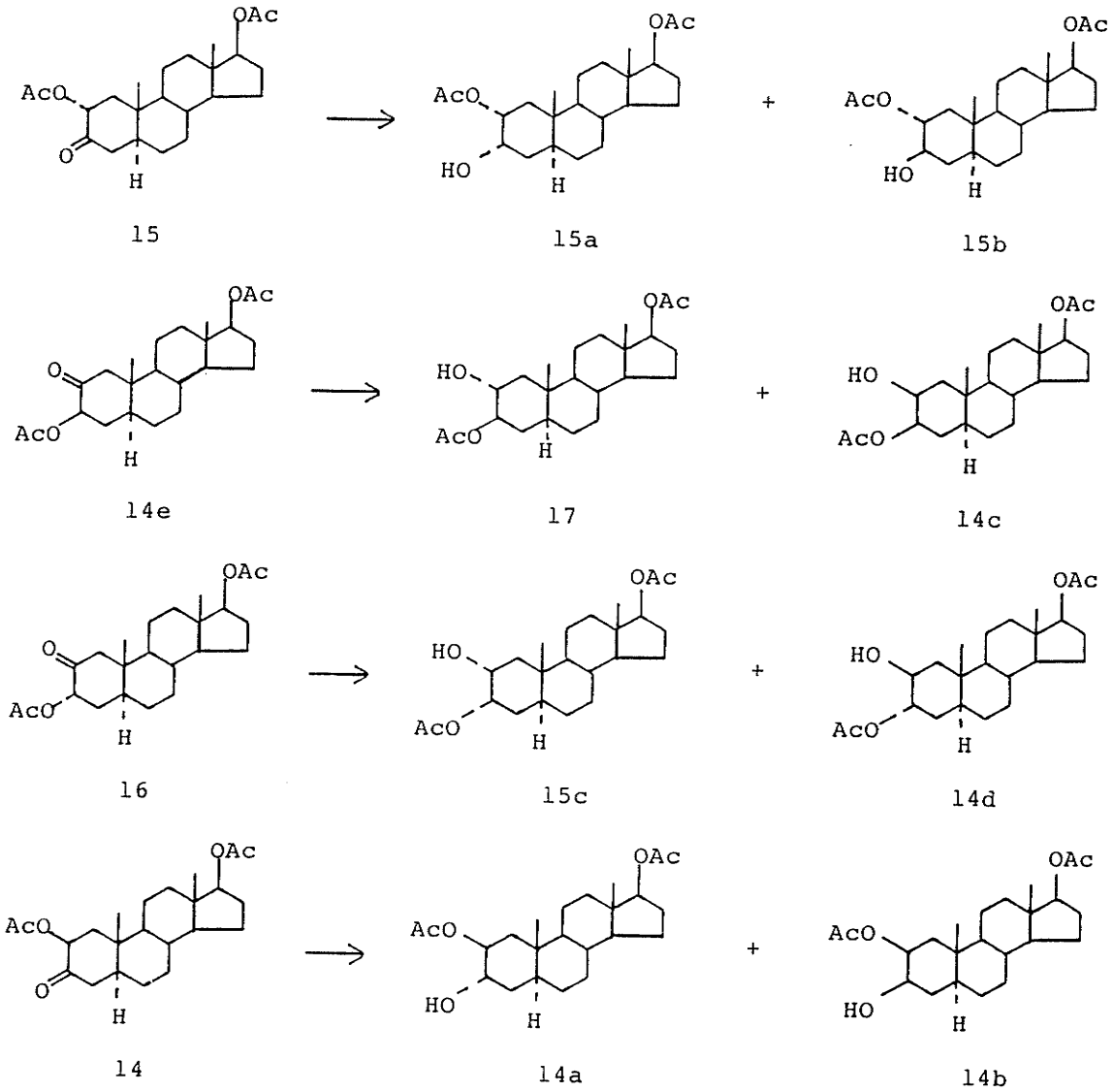


FIGURE 8: Possible Reduction Products from Possible Ketone Rearrangements

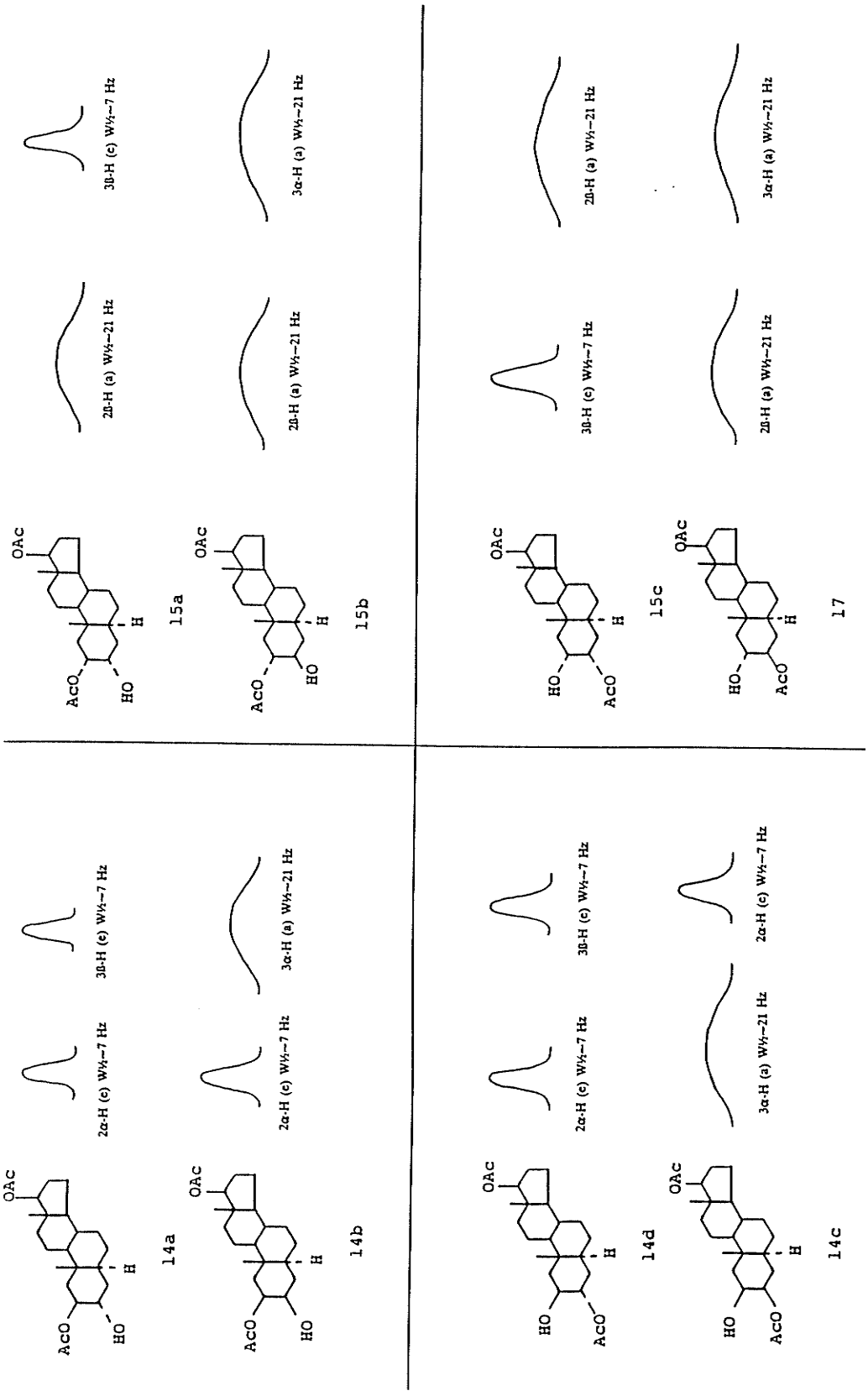


FIGURE 9: Methine Proton Signals Expected from 8 Possible Isomers

identification of 14a and 14c.

DISCUSSION

Reduction of 2α,17ß-diacetoxy-5α-androstan-3-one (15) and 2ß,17ß-diacetoxy-5α-androstan-3-one (14) was complicated by the enolic equilibrium shown in Figure 6. This involved rearrangement of the acetoxy function from C-2 to C-3 resulting in four possible acetoxy ketone isomers (Figure 7). Such equilibration occurring under reduction conditions could have led to the formation of eight possible isomers as shown in Figure 8.

When the rates of reduction and enolization are similar, a mixture of products can be formed. A difference in the ratio between the α and ß (or axial and equatorial) isomers is expected as enolization requires the removal of either an axial or an equatorial hydrogen. A lack of knowledge of the relative rates of enolization in and between the sodium borohydride and L-Selectride reduction conditions together with the rates of formation of kinetic and thermodynamic products makes an overall rationalization of the products speculative.

The ^{1}H NMR spectra of these isomers for the C-2 and C-3 protons are also shown schematically in Figure 9. These proton spectra take into consideration the fact that equatorial-equatorial and equatorial-axial couplings are relatively small (J~3 Hz) compared with that seen for axial-axial couplings (J~10 Hz) resulting in unresolved, broad singlets differentiated on the basis of their width at half-height (W ½). The W ½ is approximately 7 Hz for equatorial-equatorial and equatorial-

axial couplings and 21 Hz for axial-axial couplings thereby allowing the configurational assignment of the alcohols (Bhacca and Williams, 1964; Göndös and Orr, 1982). This allows the isomers to be differentiated into four possible pairs.

Three pairs of isomers were distinguished from the products obtained when the spectra for the C-2 and C-3 protons were examined. Compounds 14a and 14d were distinguished by the difference in the 1,3-diaxial shift induced in the 10-methyl group by a 2ß-hydroxy substituent (0.25 ppm) compared with a 2ß-acetoxy group (0.15 ppm). Compound 15a was distinguished from 14c by the difference in the chemical shift of the 10-methyl group induced by the 2ß-hydroxyl group in 14c. In compound 15c the 10-methyl group resonates at 1.03 ppm which distinguishes it from the chemical shift induced by the 2ß-acetoxy group in 14b. For the remaining pair, 15b and 17, the former has previously been identified (Matsui and Kinuyama, 1977) whereas the latter was not isolated from the reductions.

Reductions with L-Selectride and sodium borohydride were carried out to compare the structures of the obtained products with the possible rearrangement products. Structures have been assigned on the basis of the coupling constants of the C-2 and C-3 protons and are indicated schematically in Figure 9. Figure 5 shows the reduction products and the relative percentages obtained. All products can be rationalized in terms of the equilibrium shown in Figure 6 except products 14d and 15d. Enolization of 15 can result in the formation of the 3α-acetoxy-2-one and its subsequent reduction to 15c. Even though the enolate from the 2α-

acetoxy-3-one is expected to form preferentially a product with an equatorial acetoxy group (i.e. 3ß-acetoxy-2-one) rather than an axial acetoxy group (i.e. 3α-acetoxy-2-one), L-Selectride reduction of the 3ß-acetoxy-2-one is slow (as indicated by its isolation in the reduction of 14). This allows a faster reduction of the less favoured ketone (3α-acetoxy-2-one) to occur. Unexpected products are 15d and 14d. Formation of 15d can only occur through an intermolecular process which might be invoked to rationalize the formation of 14d also.

EXPERIMENTAL

INSTRUMENTS

Melting points (mp) were determined with a Kofler hot plate and are uncorrected. Proton magnetic resonance (^{1}H NMR) spectra were recorded using deuteriochloroform ($CDCl_3$) as the solvent and tetramethysilane (TMS) as the internal standard using the Bruker AM (300 MHz) instrument at the Department of Chemistry, University of Manitoba, Winnipeg, Manitoba. The following designations are used in characterizing ^{1}H NMR signals: singlet (s), doublet (d), triplet (t), doublet of doublet (dd), multiplet (m) and width at half height (W ½). Mass spectra (MS) were recorded on a Finnigan Quadrupole Model 1015 mass spectrophotometer at 70 eV using the direct probe method at the Department of Chemistry, University of Manitoba. Infrared spectra were run on a Perkin-Elmer Model 267 instrument. Elemental analyses were performed at the Microanalytical Laboratory, School of Pharmacy, University of London, England. High pressure liquid chromatography (HPLC) was carried out on a Waters Scientific Limited M45G solvent delivery system with U6K Universal liquid chromatogram injector using RCM-100 Radial Compression Separation System (RCSS), and with μ-Porasil column (10 μ) (7.8 mm x 30 cm) and RCSS silica guard pak. The solvent system was 2% ethanol/dichloromethane. The detector used was R 401 refractive index detector-differential refractometer.

MATERIALS

Thin-layer chromatography (TLC) was carried out on Analtech 25 precoated silica gel GHLF plates 0.25 mm thick using various liquid phases. The TLC plates were viewed under an ultraviolet (UV) source and/or by spraying with a 4% v/v solution of concentrated sulfuric acid in 95% ethanol followed by heating at approximately 100°C in an oven for 5 to 10 minutes to produce a color. Column chromatography was carried out with either alumina (Brockmann activity II aluminum oxide for chromatographic adsorption analysis, BDH Chemicals Limited) or silica gel (Merck Silica Gel 60H for TLC).

17ß-Acetoxy-4ß,5ß-epoxyestran-3-one (**2**)

To a stirred solution of 19-nortestosterone (**1**) (1.0 g) in methanol (100 mL), cooled to 5°C in an ice/water bath, was added 6% hydrogen peroxide (5 mL) and 4N sodium hydroxide (5.5 mL). This method is a slight modification of the work by Mihailovic et al. (1977). Thin layer chromatography showed that the reaction was completed within 1 hour (solvent system of 25% ethyl acetate in petroleum ether) and was quenched with glacial acetic acid (1 mL) and the volume concentrated in vacuo (to approximately 20 mL). Distilled water was added to the flask and the product was extracted with ethyl acetate. The organic layer was washed with aqueous, saturated sodium bicarbonate, and cold, distilled water, and dried over anhydrous sodium sulfate to remove excess water. After filtering, the

organic layer was evaporated to dryness in vacuo to produce an oily residue. This residue was dissolved in pyridine (10 mL) and acetic anhydride (5 mL) and allowed to stand at room temperature for 24 hours. Excess acetic anhydride was converted into acetic acid by the addition of ice cold distilled ice water and the acetylated product was extracted with ether. The organic layer was washed with cold distilled water (3 times), excess dilute hydrochloric acid, cold distilled water, aqueous saturated sodium bicarbonate, cold distilled water, and aqueous saturated sodium chloride. It was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to form a pale yellow, crystalline substance (0.82 g) with mp 108-113°C (lit. mp 108°C, Mihailovic et al., 1977); ^{1}H NMRδ: 0.83 (13-Me), 2.04 (17ß-OAc), 3.03 (4α-H), 4.61, dd, J = 7.8, 9.15 Hz (17α-H).

17ß-Acetoxy-4ß,5ß-epoxyestr-1-en-3-one (**3**)

To a stirred solution of 17ß-acetoxy-4ß,5ß-epoxyestran-3-one (**2**) (750 mg) in dry 2-methyl-2-propanol (35 mL) under argon was added selenium dioxide (753 mg) and glacial acetic acid (3.75 mL) as described by Le Quesne et al. (1986). The reaction mixture was refluxed for 16 hours after which TLC showed the reaction to be complete. After cooling, precipitated selenium was removed by filtration through celite on a sintered glass funnel with negative pressure. The filter was washed with ethyl acetate and the filtrate evaporated in vacuo. Extraction was achieved with ethyl acetate and the organic layer was washed with aqueous saturated sodium bicarbonate (5 times) and cold distilled water (3 times), dried

over anhydrous sodium sulfate, filtered and evaporated to dryness leaving a dark brown residue (800 mg). Purification of the product was by flash chromatography on silica gel (approximately 12 g) with a solvent system 1:3 ethyl acetate/hexane to yield a crystalline product (280 mg) mp 102-110°C (lit. mp 114-115°C, Le Quesne et al., 1980); ^{1}H NMRδ: 0.87 (13-Me), 2.04 (17ß-OAc), 2.59, dd, J = 5.25, 10.9 Hz (10α-H), 3.24, dd, J = 1.4, 1.8 Hz (4α-H), 4.60, dd, J = 7.65, 9.15 Hz (17α-H), 5.95, ddd, J = 1.35, 1.35, 10.6 Hz (2-H), 6.74, dd, J = 5.3, 10.6 Hz (1-H).

4-Chloroestradiol diacetate (4-chloroestra-1,3,5(10)-triene-3,17ß-diol diacetate) (**4**)

To a stirred solution of 17ß-acetoxy-4ß,5ß-epoxyestr-1-en-3-one (**3**) (330 mg) in dimethylacetamide (70 mL) and calcium carbonate (26.7 mg) under argon was added dropwise acetyl chloride (10 mL). The reaction mixture was refluxed for 2 hours when TLC (solvent system 25% ethyl acetate/hexane) showed the reaction to be complete. The contents of the flask were poured slowly into cold, aqueous, saturated sodium bicarbonate and extracted with ether. The organic layer was washed successively with cold distilled water (7 times) and aqueous saturated sodium chloride, dried over anhydrous sodium sulfate, filtered and evaporated in vacuo to form a light brown solid. Recrystallization from dichloromethane/methanol gave a white crystalline solid (91 mg) mp 146-148.5°C; ^{1}H NMRδ: 0.82 (13-Me), 2.06 (17ß-OAc), 2.34 (3-OAc), 4.70, dd, J = 9.1, 7.7 Hz (17α-H), 6.91, d, J = 8.5 Hz (2-H), 7.22, d, J = 8.4 (1-H).

4-Hydroxyestradiol (estra-1,3,5(10)-triene-3,4,17ß-triol 17-acetate) (**5**)

A stirred solution of 17ß-acetoxy-4ß,5ß-epoxyestr-1-en-3-one (**3**) (330 mg) in dimethylacetamide and calcium carbonate (26.4 mg) was refluxed under argon for 24 hours when TLC (solvent system 25% ethyl acetate/hexane) showed the reaction to be complete. The reaction mixture was filtered through Celite on a sintered glass funnel with negative pressure to remove excess calcium carbonate and the filter was washed with aqueous saturated sodium bicarbonate solution. After extraction with ether, the organic layer was washed with cold distilled water (3 times) and aqueous saturated sodium chloride (2 times), dried over anhydrous sodium sulfate, filtered and evaporated to give a brown residue. Recrystallization (4 times) with dichloromethane/methanol yielded a white crystalline solid (30 mg) mp 212-215°C (lit. mp 214-216°C, Gelbke et al., 1973); ^{1}H NMRδ: 0.82 (13-Me), 2.06 (17ß-OAc), 2.62, m, (6ß-H), 2.86, dd, J = 5.6, 16.8 Hz (6α-H), 4.69, dd, J = 7.7, 9.2 Hz (17α-H), 5.14 (3-OH), 5.16 (4-OH), 6.69, d, J = 8.3 Hz (1-H), 6.76, d, J = 8.3 Hz (2-H).

4-Hydroxyestradiol triacetate (estra-1,3,5(10)-triene-3,4,17ß-triol triacetate) (**6**)

To a solution of 4-hydroxyestradiol (**5**) (~8 mg) in pyridine (20 drops) was added acetic anhydride (10 drops). The solution was allowed to stand for one hour when TLC showed the reaction to be complete. Ice water was added to the reaction flask to convert excess acetic anhydride to acetic acid. After extraction with ether, the organic layer was washed with distilled water, dilute hydrochloric

acid, distilled water, aqueous saturated sodium bicarbonate, distilled water, and aqueous saturated sodium chloride, dried over anhydrous sodium sulfate, filtered and evaporated to give a white, solid residue. ^{1}H NMRδ: 0.82 (13-Me), 2.04 (17ß-OAc), 2.27 (3-OAc), 2.30 (4-OAc), 2.55, m (6ß-H), 2.75, dd, J = 17.6, 5.3 Hz (6α-H), 4.68, dd, J = 7.8, 9.1 Hz (17α-H), 6.97, d, J = 8.5 Hz (1-H), 7.20, d, J = 8.5 Hz (2-H). ^{1}H NMR is in agreement with reported results (Kirk and Slade, 1982).

General Reduction Method With L-Selectride:

To a stirred solution of ketone (1 mmol) in freshly distilled tetrahydrofuran (THF, 60 mL) cooled in an acetone/dry ice bath (approximately -55°C) under argon was added lithium tris-(R,S-1,2-dimethylpropyl)-borohydride (1.1 mL of a 1 M tetrahydrofuran solution, L-Selectride, Aldrich Chemical Company, Milwaukee, WI) by a metal syringe. The reaction was monitored by TLC and was stopped at the times indicated below when no starting material was detected on TLC. The reaction mixture was poured into a distilled water/ice bath containing an excess of hydrochloric acid and extracted twice with ether. The ether extracts were combined and washed with ice water (3 times), saturated sodium chloride solution (2 times) and dried over anhydrous sodium sulfate. The ether extract was then filtered and evaporated to dryness in vacuo.

General Reduction Method With Sodium Borohydride:

To a stirred solution of ketone (1 mmol) in 95% ethanol (17 mL) at room temperature was added sodium borohydride (1 mmol). After the reaction was complete (progress was monitored by TLC), it was poured into a distilled water/ice bath containing an excess of hydrochloric acid and extracted twice with ether. The ether extracts were combined and washed with ice cold distilled water (3 times), saturated sodium chloride solution (2 times), and dried over anhydrous sodium sulfate. The ether extract was then filtered and evaporated to dryness in vacuo.

Reduction of 17ß-acetoxy-5α-androstan-3-one (**7**).

L-Selectride: The steroid (332 mg) was treated with L-Selectride and the reaction was over within 20 minutes as determined by TLC (solvent system of 25% ethyl acetate in petroleum ether). The product was recrystallized from dichloromethane/methanol to give 17ß-acetoxy-5α-androstan-3α-ol (**7a**), mp 184-185°C (lit. mp 186-187°C; ApSimon et al., 1970); ^{1}H NMRδ: 0.78 (13-Me), 0.79 (10-Me), 2.03 (17ß-OAc), 4.05, m (3ß-H), 4.59, dd, J = 7.8, 9.1 Hz (17α-H).

Sodium Borohydride: The steroid (500 mg) was treated with sodium borohydride and the reaction was over within 10 minutes as determined by TLC (solvent system of 25% ethyl acetate in petroleum ether). The product (300 mg) was recrystallized from dichloromethane/methanol to give 17ß-acetoxy-5α-androstan-3ß-ol (**7b**), mp 149-151°C (lit. mp 150-151°C; DeVivar and Romo, 1959);

^{1}H NMRδ: 0.78 (13-Me), 0.81 (10-Me), 2.03 (17ß-OAc), 3.59, m (3α-H), 4.58, dd, J = 7.8, 9.1 Hz (17α-H).

Reduction of 17ß-acetoxy-2α-chloro-5α-androstan-3-one (**8**).

L-Selectride: The steroid (100 mg) was treated with L- Selectride and the reaction was over within 3 hours as determined by TLC (solvent system 25% ethyl acetate/petroleum ether). The product was recrystallized from dichloromethane/methanol to give 17ß-acetoxy-2α-chloro-5α-androstan-3α-ol (**8a**), mp 202-206.5°C (lit. mp 206-209°C; Wong et al., 1978); ^{1}H NMRδ: 0.78 (13-Me), 0.83 (10-Me), 2.03 (17ß-OAc), 4.00, W ½ = 7 Hz (3ß-H), 4.23, octet, J = 2.7, 4.6, 12.7 Hz (2ß-H), 4.58, dd, J = 7.8, 9.3 Hz (17α-H).

Sodium Borohydride: The steroid (100 mg) was treated with sodium borohydride and the reaction was over within 10 minutes as determined by TLC (solvent system 25% ethyl acetate/petroleum ether). The product (16.4 mg) was recrystallized from dichloromethane/methanol to give 17ß-acetoxy-2α-chloro-5α-androstan-3ß-ol (**8b**), mp 198-201°C (lit. mp 193-196°C; Wong et al., 1978); ^{1}H NMRδ: 0.78 (13-Me), 0.86 (10-Me), 2.03 (17ß-OAc), 3.88, sextet, J = 5.1, 10.3, 10.3 Hz (3α-H), 3.94, octet, J = 4.6, 9.7, 12.4 Hz (2ß-H), 4.58, dd, J = 7.8, 9.0 Hz (17α-H).

Reduction of 2α-methyl-17ß-propionyloxy-5α-androstan-3-one (**9**).

L-Selectride: The steroid (52 mg) was treated with L-Selectride and the reaction was over within 4 hours as determined by TLC (solvent system 25% ethyl

acetate/petroleum ether). The product (12.5 mg) was recrystallized from dichloromethane/methanol to give 2α-methyl-17ß-propionyloxy-5α-androstan-3α-ol (**9a**), mp 167-169°C (lit. mp 168-170°C; Cross et al., 1963); ^{1}H NMRδ: 0.77 (13-Me), 0.79 (10-Me), 0.92, d, J = 6.9 Hz, (2α-Me), 1.13, t, J = 7.5 Hz ($OCOCH_2\underline{CH_3}$), 2.31, dd, J = 7.5, 15.0 Hz ($OCO\underline{CH_2}CH_3$), 3.77 (3ß-H), 4.59, dd, J = 7.8, 9.0 Hz (17α-H).

Sodium Borohydride: The steroid (500 mg) was treated with sodium borohydride and the reaction was over within 10 minutes as determined by TLC (solvent system 25% ethyl acetate/petroleum ether). The product (250 mg) was recrystallized from dichloromethane/methanol to give 2α-methyl-17ß-propionyloxy-5α-androstan-3ß-ol (**9b**), mp 161-163°C (lit. mp 162-163°C; Cross et al., 1963); ^{1}H NMRδ: 0.77 (13-Me), 0.82 (10-Me), 0.97, d, J = 6.3 Hz (2α-Me), 1.13, t, J = 7.5 Hz ($OCOCH_2\underline{CH_3}$), 2.32, dd, J = 7.5, 15.0 Hz ($OCO\underline{CH_2}CH_3$), 3.13, sextet, J = 4.9, 10.4, 10.4 Hz (3α-H), 4.58, dd, J = 7.8, 9.2 Hz (17α-H).

<u>Reduction of 17ß-acetoxy-2,2-dichloro-5α-androstan-3-one (**10**).</u>

L-Selectride: The steroid (60 mg) was treated with L- Selectride and the reaction was over within 20 minutes as determined by TLC (solvent system 25% ethyl acetate/petroleum ether). The product was recrystallized from dichloromethane/methanol to give 17ß-acetoxy-2,2-dichloro-5α-androstan-3ß-ol (**10a**), mp 165-167°C (lit. mp 165-167°C; Templeton et al., 1984); ^{1}H NMRδ: 0.79 (13-Me), 1.05 (10-Me), 2.03 (17ß-OAc), 2.04, d, J = 15.0 Hz (1α-H), 2.87, d, J = 15.0

Hz (1ß-H), 3.86, dd, J = 8.0, 15.0 Hz (3α-H), 4.58, dd, J = 7.2, 9.0 Hz (17α-H).

Sodium Borohydride: The steroid (60 mg) was treated with sodium borohydride and the reaction was over within 10 minutes as determined by TLC (solvent system 25% ethyl acetate/petroleum ether). The product was identified as 17ß-acetoxy-2,2-dichloro-5α-androstan-3ß-ol (**10a**) by comparison with the L-Selectride product.

5α-Androstan-2-ene-3,17ß-diol diacetate (**12**)

To a stirred solution of 17ß-hydroxy-5α-androstan-3-one (**11**) (12.5 g) in dichloromethane (100 mL) was added acetic anhydride (20 mL) and the mixture cooled in an ice/water bath. Phosphorus oxychloride (4 mL) was added dropwise and the solution was allowed to stir at room temperature overnight (16 hours) when TLC showed the reaction to be complete (solvent system 25% ethyl acetate/petroleum ether). After adding crushed ice to the reaction flask, the steroid was extracted with ether and the organic layer was washed with cold, distilled water until neutral (5 times), aqueous, saturated sodium bicarbonate, cold, distilled water, and aqueous, saturated sodium chloride, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in vacuo. Recrystallization with dichloromethane/methanol yielded a white, crystalline product (9.89 g) mp 169-173°C (lit. mp 168-171°C; Templeton, et al., 1978 and references therein); IR (CCl_4) ν_{max} 1750 (vinyl O<u>CO</u>CH_3), 1735 (17ß-O<u>CO</u>CH_3) cm^{-1}; ^1H NMRδ: 0.85

(10-and 13-Me), 2.02 (17ß-OAc), 2.08 (3ß-OAc), 4.26, t, J = 7 Hz (17α-H), 5.25, m (vinyl H).

2α,3α-Epoxy-5α-androstane-3ß,17ß-diol diacetate (**13**)

To a stirred solution of 85% m-chloroperbenzoic acid (305 mg) in dichloromethane (10 mL) with a phosphate buffer* pH 7.8 (150 mL) was added a solution of 5α-androstan-2-ene-3,17ß-diol diacetate (**12**) (374 mg) in dichloromethane (5 mL). TLC (solvent system 25% ethyl acetate/petroleum ether) showed the reaction to be over at 18 hours at room temperature and the mixture was diluted with dichloromethane and the layers separated. The organic layer was washed with 10% aqueous sodium sulfite, 10% aqueous sodium carbonate (2 times), and cold, distilled water, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in vacuo. Recrystallization from dichloromethane/methanol yielded a white, crystalline substance (150 mg) mp 155-160.5°C; IR (KBr) ν_{max} 1734 (17ß-OAc), 1753 (3ß-OAc) cm^{-1}; ^1H NMR δ: 0.77 (13-Me), 0.96 (10-Me), 2.03 (17ß-OAc), 2.07 (3ß-OAc), 3.32, d, J = 5.5 Hz (2ß-H), 4.57, dd, J = 8.0, 9.0 Hz (17α-H).

Anal.: C, 70.60; H, 8.70, $C_{23}H_{34}O_5$ requires C, 70.74; H, 8.78%.

*Phosphate buffer prepared from potassium dihydrogen phosphate (6.8 g) and sodium hydroxide (1.6 g) in 1 L distilled water.

2ß,17ß-Diacetoxy-5α-androstan-3-one (**14**)

A flask containing 2α,3α-epoxy-5α-androstane-3ß,17ß-diol diacetate (**13**) (471

mg) was attached to a vacuum and placed in a preheated oil bath (168-170°C) for 5 minutes until the steroid melted. Recrystallization from dichloromethane/methanol yielded a white, crytalline substance (219 mg), mp 169.5-174.5°C; ^{1}H NMRδ: 0.78 (13-Me), 0.86 (10-Me), 2.02 (17ß-OAc), 2.14 (2ß-OAc), 4.61, dd, J = 7, 9 Hz (17α-H), 5.37, dd, J = 7, 10 Hz (2α-H).

Anal.: C, 70.79; H, 8.67, $C_{23}H_{34}O_5$ requires C, 70.74; H, 8.78%.

2α,17ß-Diacetoxy-5α-androstan-3-one (**15**)

To a stirred solution of 2ß,17ß-diacetoxy-5α-androstan-3-one (**14**) (2.5 g) in glacial acetic acid (60 mL) was added 48% hydrobromic acid (1.5 mL) and the reaction mixture was stirred at room temperature for 19 hours when TLC showed the reaction to be complete (solvent system 25% ethyl acetate/petroleum ether). Evaporation in vacuo left a brown semi-crystalline residue that was washed with aqueous, saturated sodium bicarbonate. Purification was achieved by running the compound through an alumina column (18 g). The desired steroid was retrieved by using 50-70% benzene/hexane as eluting solvents and the fractions combined to yield a white crystalline product (493 mg), mp 203-207°C (lit. mp 200-201°C; Gardi et al., 1962); IR (KBr) ν_{max} 1734 (17ß-OAc), 1746 (ketone), 1758 (2α-OAc) cm^{-1}; ^{1}H NMRδ: 0.80 (13-Me), 1.13 (10-Me), 2.04 (17ß-OAc), 2.14 (2α-OAc), 4.60, dd, J = 7.8, 9.2 Hz (17α-H), 5.29, dd, J = 6.7, 12.9 Hz (2ß-H).

Reduction of 2ß,17ß-diacetoxy-5α-androstan-3-one (**14**).

L-Selectride: The steroid (75 mg) was treated with L- Selectride as described above and the reaction was over within 90 minutes as demonstrated by TLC (solvent system 25% ethyl acetate/petroleum ether). The product was purified by HPLC from which 4 fractions were collected.

Fraction 1 (44%) gave 5α-androstane-2ß,3α,17ß-triol 2,17- diacetate (**14a**), mp 180-183°C from dichloromethane/methanol. ^{1}H NMRδ: 0.77 (13-Me), 0.91 (10-Me), 2.03 (17ß-OAc), 2.04 (2ß-OAc), 3.85, W ½ = 8 Hz (3ß-H), 4.58, dd, J = 7.8, 9.2 Hz (17α-H), 4.87, W ½ = 10 Hz (2α-H).

Anal.: C, 68.79; H, 9.08, $C_{23}H_{36}O_5 \cdot$ ½ H_2O requires C, 68.80; H, 9.29%.

Fraction 2 (8%) gave 5α-androstane-2ß,3α,17ß-triol 3,17-diacetate (**14d**), melting point was in agreement with reported values (lit. mp 177-179°C; Kwok and Wolff, 1963); ^{1}H NMRδ: 0.78 (13-Me), 1.01 (10-Me), 2.03 (17ß-OAc), 2.06 (3α-OAc), 3.92, W ½ = 8 Hz (2α-H), 4.58, dd, J = 7.7, 9.1 Hz (17α-H), 4.85, W ½ = 8 Hz (3ß-H).

Fraction 3 (8%) gave 5α-androstan-2-one-3ß,17ß-diol diacetate (**14e**), melting point was in agreement with reported values; ^{1}H NMRδ: 0.78 (13-Me), 0.78 (10-Me), 2.03 (17ß-OAc), 2.15 (3ß-OAc), 4.59, dd, J = 7.8, 9.1 Hz (17α-H), 5.21 (3α-H).

Fraction 4 (41%) gave 5α-androstane-2ß,3ß,17ß-triol 3,17-diacetate (**14c**), mp 173-180°C from dichloromethane/methanol. ^{1}H NMRδ: 0.77 (13-Me), 1.04 (10-Me), 2.03 (17ß-OAc), 2.08 (3ß-OAc), 4.09, W ½ = 7 Hz (2α-H), 4.58, dd, J = 7.7,

9.2 Hz (17α-H), 4.77, octet, J = 3.4, 4.8, 11.8 Hz (3α-H).

Anal.: C, 70.42; H, 9.17, $C_{23}H_{36}O_5$ requires C, 70.38; H, 9.24%.

Sodium borohydride: The steroid (100 mg) was treated with sodium borohydride as described above and the reaction was over within 15 minutes as demonstrated by TLC (solvent system 25% ethyl acetate/petroleum ether). The product was purified by HPLC from which 3 fractions were collected.

Fraction 1 (24%) gave 5α-androstane-2ß,3α,17ß-triol 2,17-diacetate (**14a**), mp 180-183°C from dichloromethane/methanol. ^{1}H NMRδ: 0.77 (13-Me), 0.91 (10-Me), 2.03 (17ß-OAc), 2.04 (2ß-OAc), 3.85, W ½ = 8 Hz (3ß-H), 4.58, dd, J = 7.8, 9.2 Hz (17α-H), 4.87, W ½ = 10 Hz (2α-H).

Anal.: C, 68.79; H, 9.08, $C_{23}H_{36}O_5 \cdot$ ½ H_2O requires C, 68.80; H, 9.29%.

Fraction 2 (7%) gave 5α-androstane-2ß,3ß-17ß-triol 2,17-diacetate (**14b**), melting point was in agreement with reported values. ^{1}H NMRδ: 0.76 (13-Me), 0.92 (10-Me), 2.02 (17ß-OAc), 2.08 (2ß-OAc), 3.68, sextet, J = 4.3, 4.4, 11.1 Hz (3α-H), 4.57, dd, J = 7.9, 8.9 Hz (17α-H), 5.31, W ½ = 8 Hz (2α-H).

Fraction 3 (69%) gave 5α-androstane-2ß,3ß,17ß-triol 3,17-diacetate (**14c**), mp 173-180°C from dichloromethane/methanol. ^{1}H NMRδ: 0.77 (13-Me), 1.04 (10-Me), 2.03 (17ß-OAc), 2.08 (3ß-OAc), 4.09, W ½ = 7 Hz (2α-H), 4.58, dd, J = 7.7, 9.2 Hz (17α-H), 4.77, octet, J = 3.4, 4.8, 11.8 Hz (3α-H).

Anal.: C, 70.42; H, 9.17, $C_{23}H_{36}O_5$ requires C, 70.38; H, 9.24%.

Reduction of 2α,17ß-diacetoxy-5α-androstan-3-one (**15**).

L-Selectride: The steroid (352 mg) was treated with L-Selectride as described above and the reaction was over within 4 hours as demonstrated by TLC (solvent system 25% ethyl acetate/petroleum ether). The product was purified by HPLC from which 4 fractions were collected.

Fraction 1 (47%) gave 5α-androstane-2α,3α,17ß-triol 2,17-diacetate (**15a**); melting point and ^{1}H NMR were found to be in agreement with reported values (see Fraction 1 of sodium borohydride reduction below).

Fraction 2 (23%) gave 5α-androstane-2α,3α,17ß-triol 3,17-diacetate (**15c**), as determined by ^{1}H NMR.

Fraction 3 (23%) gave 5α-androstane-2α,3α,17ß-triol triacetate (**15d**), mp 188-190°C from acetone/petroleum ether; ^{1}H NMRδ: 0.78 (13-Me), 0.88 (10-Me), 1.98 (2α-OAc), 2.03 (3α-OAc), 2.09 (17ß-OAc), 4.59, t, J = 8.5 Hz (17α-H), 4.88, ddd, J = 4.1, 7.8, 12.4 Hz (2ß-H), 5.27, m (3ß-H)

Fraction 4 (6%) gave 2α,17ß-diacetoxy-5α-androstan-3-one (**15**), mp 203-207°C; ^{1}H NMR is in agreement with that of the starting material.

Sodium borohydride: The steroid (39 mg) was treated with sodium borohydride as described above and the reaction was over within 30 minutes as demonstrated by TLC (solvent system 25% ethyl acetate/petroleum ether). The product was purified by HPLC from which 2 fractions were collected.

Fraction 1 (25%) gave 5α-androstane-2α,3α,17ß-triol 2,17-diacetate (**15a**), mp 231-

233°C from acetone/petroleum ether (lit. mp 230-236°C; Matsui and Kinuyama, 1977); ^{1}H NMRδ: 0.77 (13-Me), 0.86 (10-Me), 2.03 (2α-OAc), 2.07 (17ß-OAc), 4.12, m, (3ß-H), 4.58, dd, J = 7.9, 9.0 Hz (17α-H), 4.94, ddd, J = 3.0, 4.7, 12.2 Hz (2ß-H).

Fraction 2 (75%) gave 5α-androstane-2α,3ß,17ß-triol 2,17-diacetate (**15b**), mp 200-208°C from acetone/petroleum ether (lit. mp 201-208°C; Matsui and Kinuyama, 1977); ^{1}H NMRδ: 0.77 (13-Me), 0.90 (10-Me), 2.03 (2α-OAc), 2.07 (17ß-OAc), 3.59, m (3α-H), 4.58, dd, J = 7.9, 9.0 Hz (17α-H), 4.80, ddd, J = 4.8, 9.5, 14.3 Hz (2ß-H).

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