STUDIES IN THE SYNTHESIS AND BIOLOGICAL PROPERTIES OF CYCLOPROPANE CONTAINING STEROIDS

by

Chwi Wan Wie

A Thesis Submitted to the

Faculty of Graduate Studies and Research

of the University of Manitoba

In Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy

January, 1976

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ABSTRACT

The studies outlined in this thesis are directed towards the synthesis of cyclopropane and cyclopropanol containing steroids derivatives in order to determine the effect of the cyclopropane structure on the biological properties of the steroid ring system.

The synthesis of cyclopropane containing steroids has been prompted by the character of the cyclopropane structure which is unique in its physical and chemical properties. The epimeric 2β,4β-cyclo-5α-androstane-3α,17β- and 3β,17β-diol 17-acetate have been synthesized from 2α,4α-dibromo-5α-androstane-3α,17β- and 3β,17β-diol 17-acetate, respectively, by an intramolecular 1,3-elimination of bromide with zinc-copper couple in ethanol. Isomerization of the endo- cyclopropanol to the exo-cyclopropanol is shown to occur either with the couple or zinc acetate. The stereochemistry at C-3 of the epimers is established by the p.m.r. spectra of 2β,4β-cyclo-5α-androstane-3α,17β- and 3β,17β-diol diacetate, and further confirmed by solvolysis of 2β, 4β-cyclo-5α-androstane-3α,17β- and 3β,17β-diol 17-acetate 3-tosylate in sodium acetate/acetic acid.

Application of the above synthetic route to the 17β-hydroxy-5α-estran-3-one series gave 2β,4β-cyclo-5α-estrane-3β,17β-diol 17-acetate, isolated as the corresponding diacetate. The 3α-epimer was not obtained. It was shown in the 5α-androstane series that the proportion of cyclopropane to olefin formed by 1,3- as opposed to 1,2-elimination in the debromination step is dependent upon the stereochemical relationship between the C-2/C-4 bromine and the C-3 hydroxyl substituents of the 2α,4α-dibromoalcohols. To further investigate this reaction, di-

and tri- brominated derivatives of 5α-androstane and 5α-estrane were isolated and their structures determined by spectrometric analysis. The low yield of 2β,4β-cyclo-5α-estrane-3β,17β-diol 17-acetate and the lack of the 3α-epimer was attributed to the formation of 2,4-dibromo-alcohols possessing an axial-equatorial relationship between the bromine and alcohol substituents during the bromination and reduction steps.

2α,3-Cyclopropano-5α-androstane-3β,17β-diol has also been synthesized by addition of the Simmons-Smith reagent to 3(2-chloroethoxy)-5α-androst-2-ene-17β-yl acetate followed by cleavage of the resulting 3β(2-chloroethoxy)-2α,3-cyclopropano-5α-androstane-17β-yl acetate with n-butyl lithium. Addition of the Simmons-Smith reagent to several other steroid enol ethers were also investigated. The results are consistent with the greater reactivity of 5-membered over 6-membered ring olefins and the greater reactivity of enol ether over enol acetate towards the reagent.

The first examples of secondary and tertiary steroid cyclopropanol derivatives have been prepared and some biological tests carried out. The secondary cyclosteroid alcohols were prepared by means of a novel 1,3-elimination. Anabolic/androgenic and skin irritancy tests were carried out for the steroid cyclopropanols and their derivatives by standard assay methods. Among these compounds, 2β,4β-cyclo-5α-androstane-3α,17β-diol 17-acetate showed weak antiandrogenic activity. Irritancy tests to determine the potential for co-carcinogenic activity of the cyclopropanol moiety showed no significant effect.

The mass spectra of the epimeric 3-substituted hydroxy- and ethoxy-derivatives of 5,6α-cyclopropano-5α-cholestane have been measured. In

addition to the well-known electron-induced decomposition of substituted cholestane, particular fragment ions appear in all the spectra at m/e 247, 301, and 302; a strong signal at m/e 329 appears in the spectra of the 3β-alcohol and isomeric 3-ethers, whereas in the spectrum of the 3α-alcohol, this ion is much less abundant. The 3α-ether also has a prominent signal at m/e 384 not present in the other spectra. Deuterium labelled compounds were synthesized and mechanisms leading to these fragment ions are suggested and rationalized on the basis of mass spectral data of the unlabelled and deuterium-labelled derivatives. The formation of these characteristic fragment ions have been related to the presence of the cyclopropane ring in the 5,6α-cyclopropano-5α-cholestane derivatives.

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ABBREVIATIONS

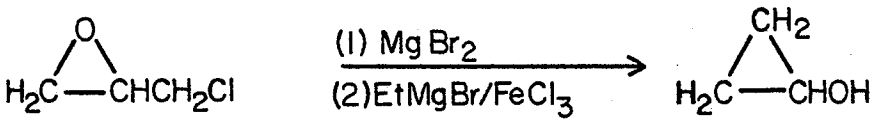
Ac	Acetyl
Bu	Butyl
Et	Ethyl
Me	Methyl
Pr	Propyl
Suc	Succinoyl
THP	Tetrahydropyranyl
TMS	Trimethylsilyl
Ts	p-Toluenesulfonyl
s	Singlet
d	Doublet
t	Triplet
q	Quartet
o	Octet
m	Multiplet
Anab.	Anabolic
Andro.	Androgenic

I. SYNTHESIS AND BIOLOGICAL PROPERTIES OF CYCLOPROPANOL CONTAINING STEROIDS

A. Introduction

The synthesis of cyclopropanol was first discovered accidentally by Magrene and Cottle [1] in 1942 from the reaction of epi-chlorohydrin (1) with magnesium bromide followed by treatment with ethylmagnesium bromide and ferric chloride. Cyclopropanol (2) was obtained in a maximum purity of 87% (Scheme 1). This was the first cyclopropanol isolated.

Scheme 1



1 2

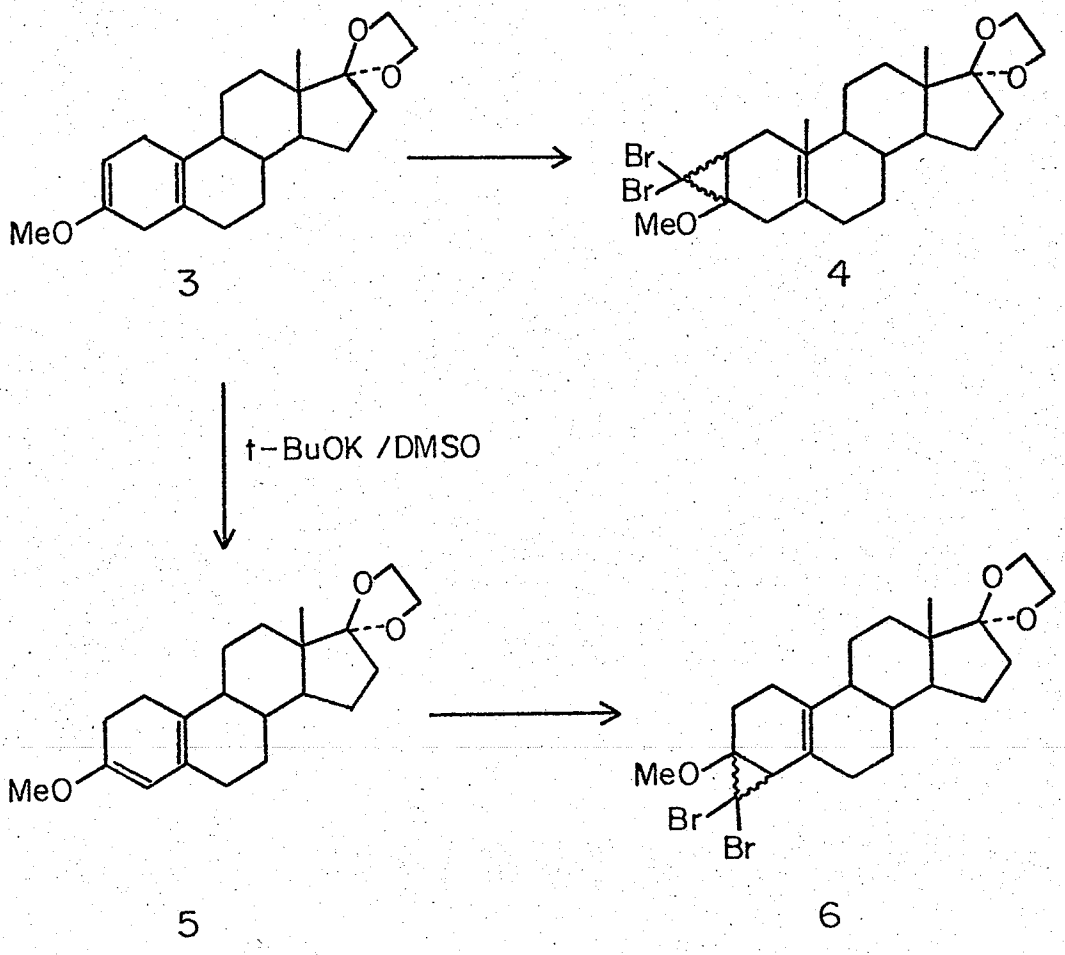
Since then little interest has been taken in this simple molecule and, until the last decade, the chemistry of cyclopropanols has not been extensively investigated. Cyclopropanol chemistry has been reviewed independently by DePuy [2] and Schöllkopf [3] in 1968 and more recently, a review written by Gibson and DePuy [4] has provided a summary of the methods of preparation of simple cyclopropanols. Although a wide variety of methods have been developed to synthesize cyclopropanols and their derivatives, few of them are readily applicable to the synthesis of steroid cyclopropanols. The following methods have been applied to the formation of steroid cyclopropanols and derivatives.

(a) Addition of dihalocarbene/carbene to enol ethers or acetates

Reaction of dihalocarbenes with the double bond carrying the methoxyl group in the 2,3- or 2,5-dihydroanisole derivatives has been

reported by Birch and Graves [5]. Birch, Graves, and Siddall [6] have successfully added dibromocarbene generated from bromoform and potassium tertiary butoxide to the enol ethers of 1,2- and 1,4-dihydroestrone 17-ethylene ketal (3 and 5). This reaction gave mainly the adducts 4 and 6, respectively, with a minor proportion of an adduct involving both double bonds (Scheme 2).

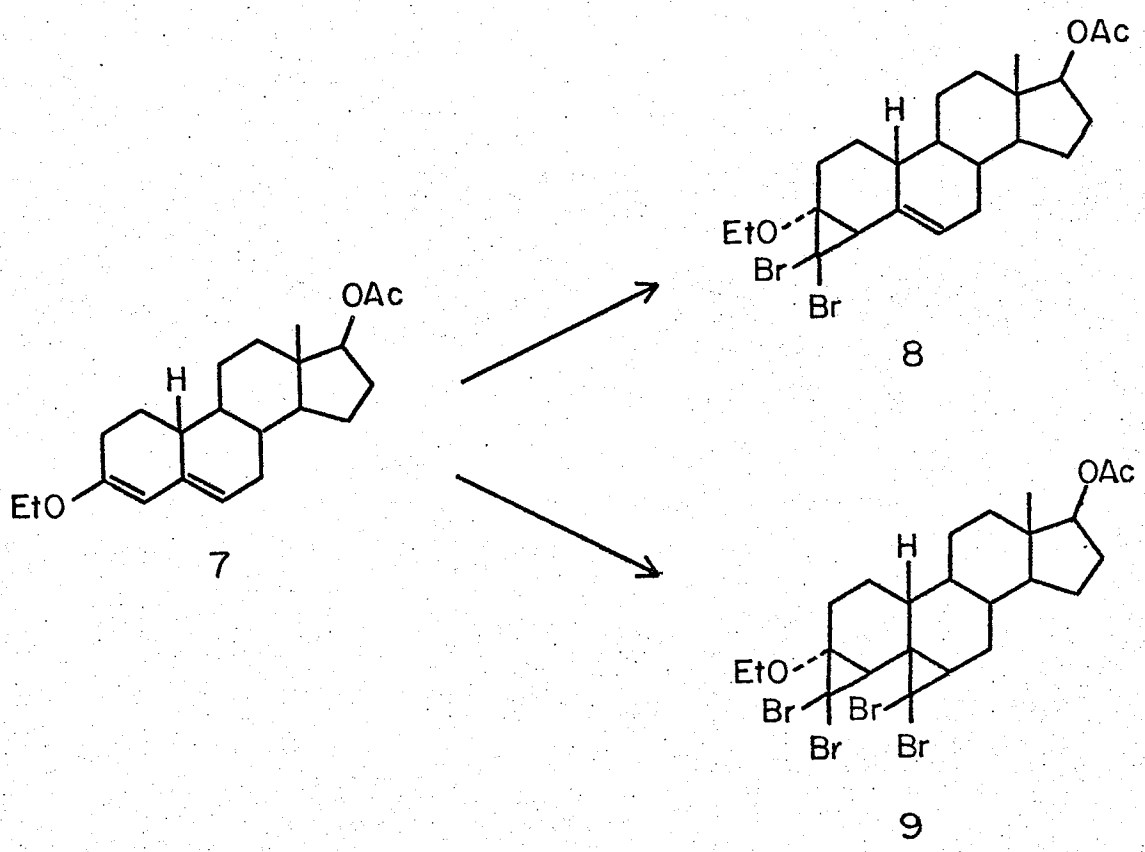
Scheme 2



3 4 5 6

Similarly, Font [7] has reported the reaction of dibromocarbene with 3-ethoxy-estra-3,5-diene-17β-yl acetate (7) to give the mono- and dicyclopropylethyl ether (8 and 9) (Scheme 3).

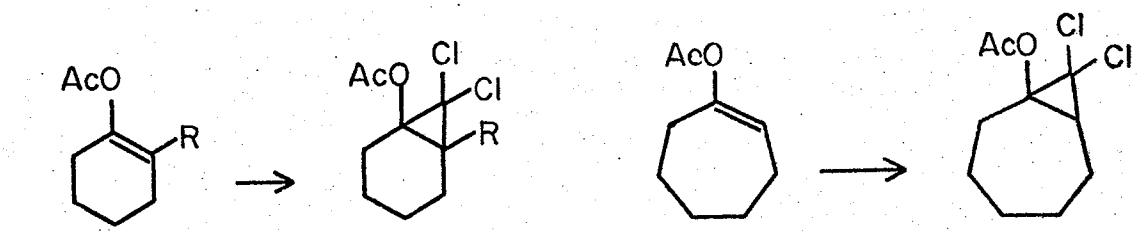
Scheme 3



Stork, Nussim, and August [8] have shown that the enol acetates of cyclohexanone (10a), cycloheptanone (12), and 2-methylcyclohexanone (10b) can be converted to the corresponding dichlorocarbene adducts 11a, 13, and 11b in good yields by heating with phenyl(bromodichloromethyl)mercury in benzene (Scheme 4). Under the same conditions, the 3-acetoxy-5α-cholest-2-ene (14) and the isomeric 3-ene (16) gave the dichlorocarbene adducts 15 and 17 in 83 and 72% yields, respectively (Scheme 5). These substances have not been converted into the corres-

ponding halogen free cyclopropanols.

Scheme 4



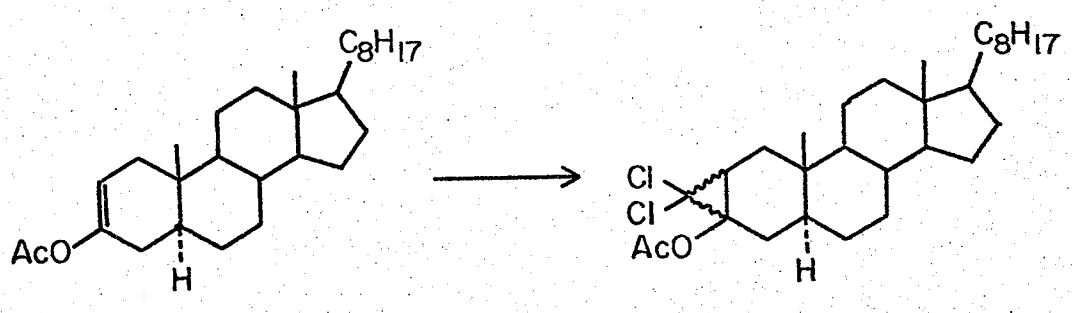
10a, R = H
b, R = CH_3

11a, R = H
b, R = CH_3

12

13

Scheme 5



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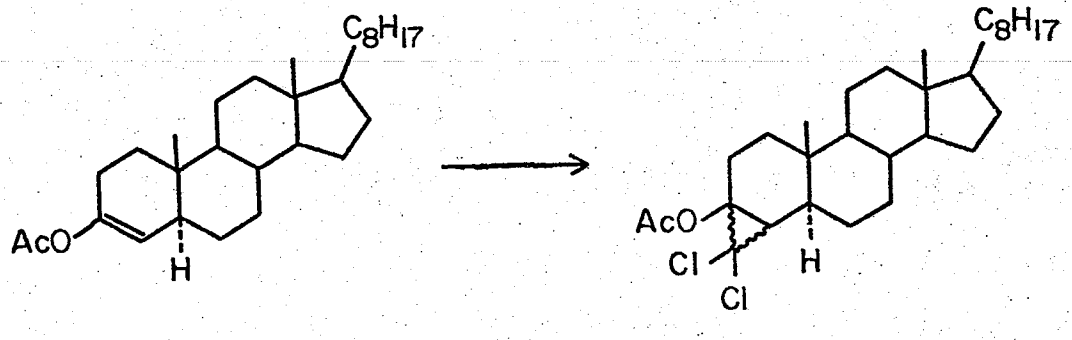
15

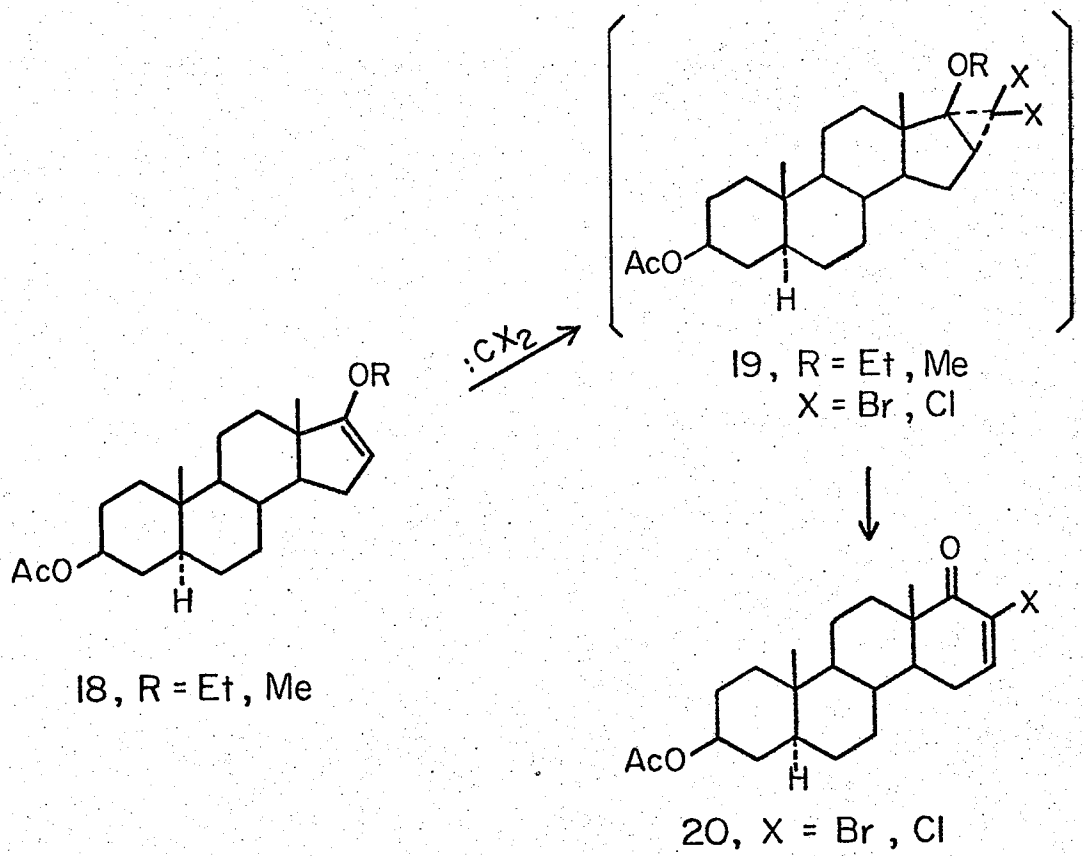


16

17

Enol ethers of 5α-androstan-17-one (18) add dichloro- and dibromocarbene, but the initially formed dihalocyclopropane derivatives (19) are too unstable for isolation, being rapidly rearranged to give the corresponding 17-halogeno-D-homoandrost-16-en-17a-one (20), as shown by Johns and Salamon [9] (Scheme 6).

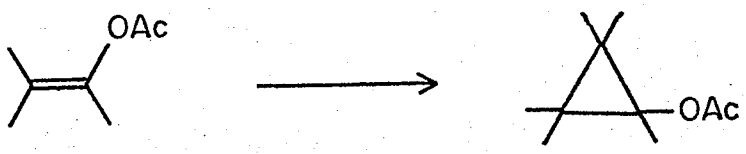
Scheme 6



(b) Methylene addition to enol acetates or ethers

Simmons and Smith [10] who developed the methylenation of olefins with a mixture of zinc-copper couple and diiodomethane have synthesized cyclopropyl acetate (21) by addition of methylene to various vinyl acetates (22) (Scheme 7), but the yields were poor.

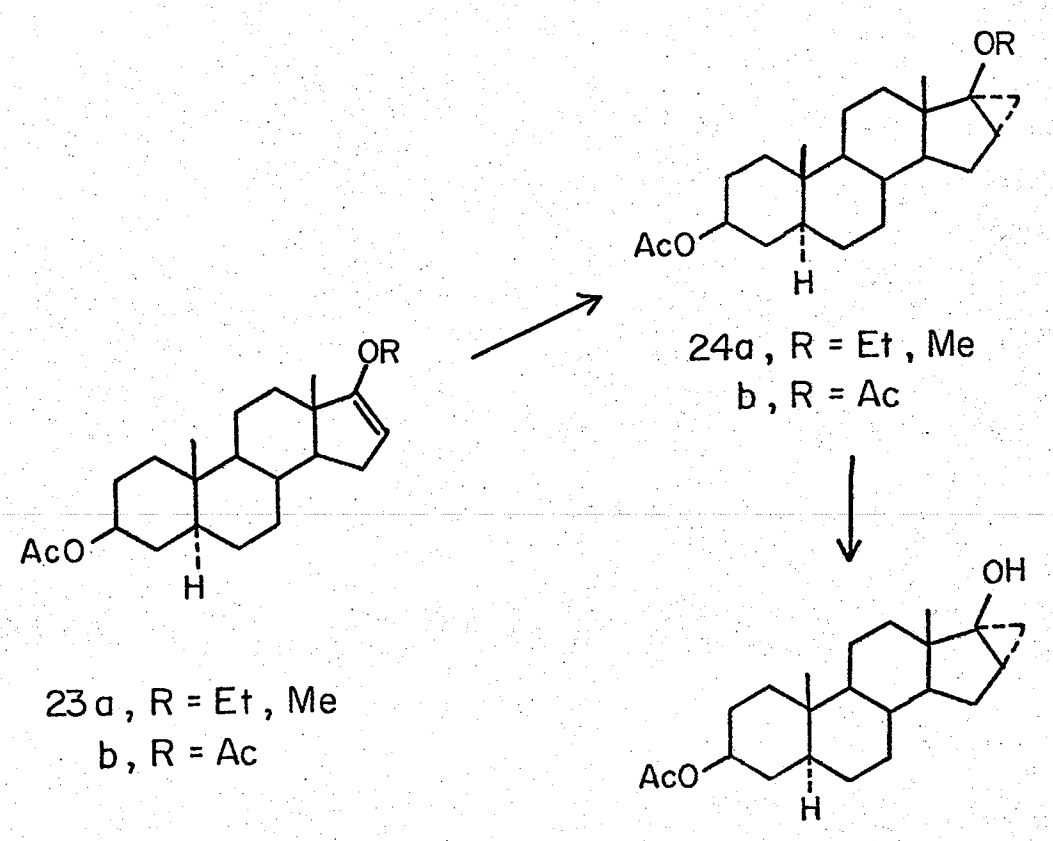
Scheme 7



21 22

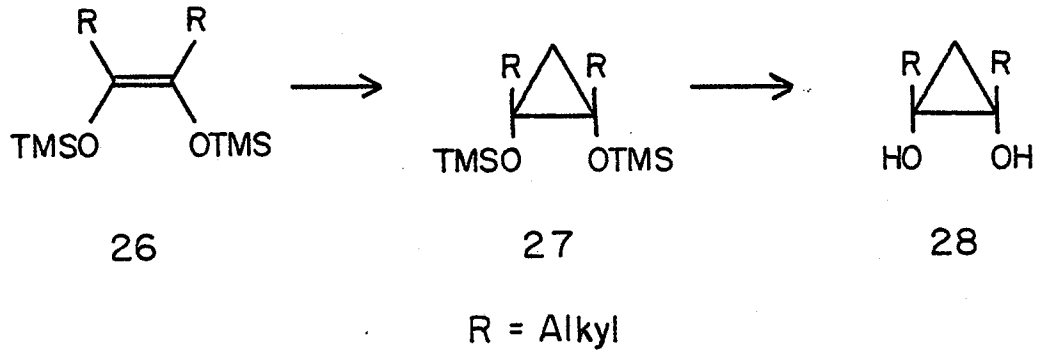
Addition of carbene, using diethylzinc and diiodomethane, to the enol ether (23a) and enol acetate (23b) derivatives of several 17-oxo steroids have been reported by Johns and Salamon [9]. The cyclopropyl acetate (24b) afforded the cyclopropanol (25) with lithium aluminum hydride (Scheme 8).

Scheme 8



This reaction has been applied by Audibrand, LeGoaller, and Arnaud [11] to several trimethylsilylalkenyl ethers (26) to prepare the cyclopropyl ethers (27), which were then converted to the corresponding alcohols (28) with methyllithium (Scheme 9).

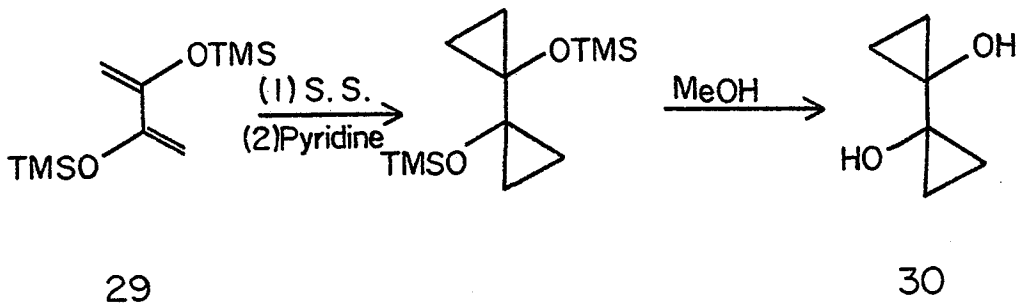
Scheme 9



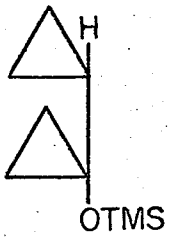
This method has later been used by LeGoaller and Pierre [12], Rubottom and Lopez [13], and Murai, Aya, and Sonoda [14] for the synthesis of different types of silyl cyclopropyl ethers and cyclopropanols.

Denis and Conia [15] have introduced a modified Simmons-Smith reaction in which a zinc-silver couple was used instead of the usual zinc-copper couple. They prepared a 1,1'-bicyclopropanol (30) from the bis(trimethyl-siloxy)-2,3-butadiene (29) (Scheme 10).

Scheme 10



Fitjer and Conia [16] have obtained the 1-hydroxy-bicyclopropyl-silyl ether (31) by a similar route.



31

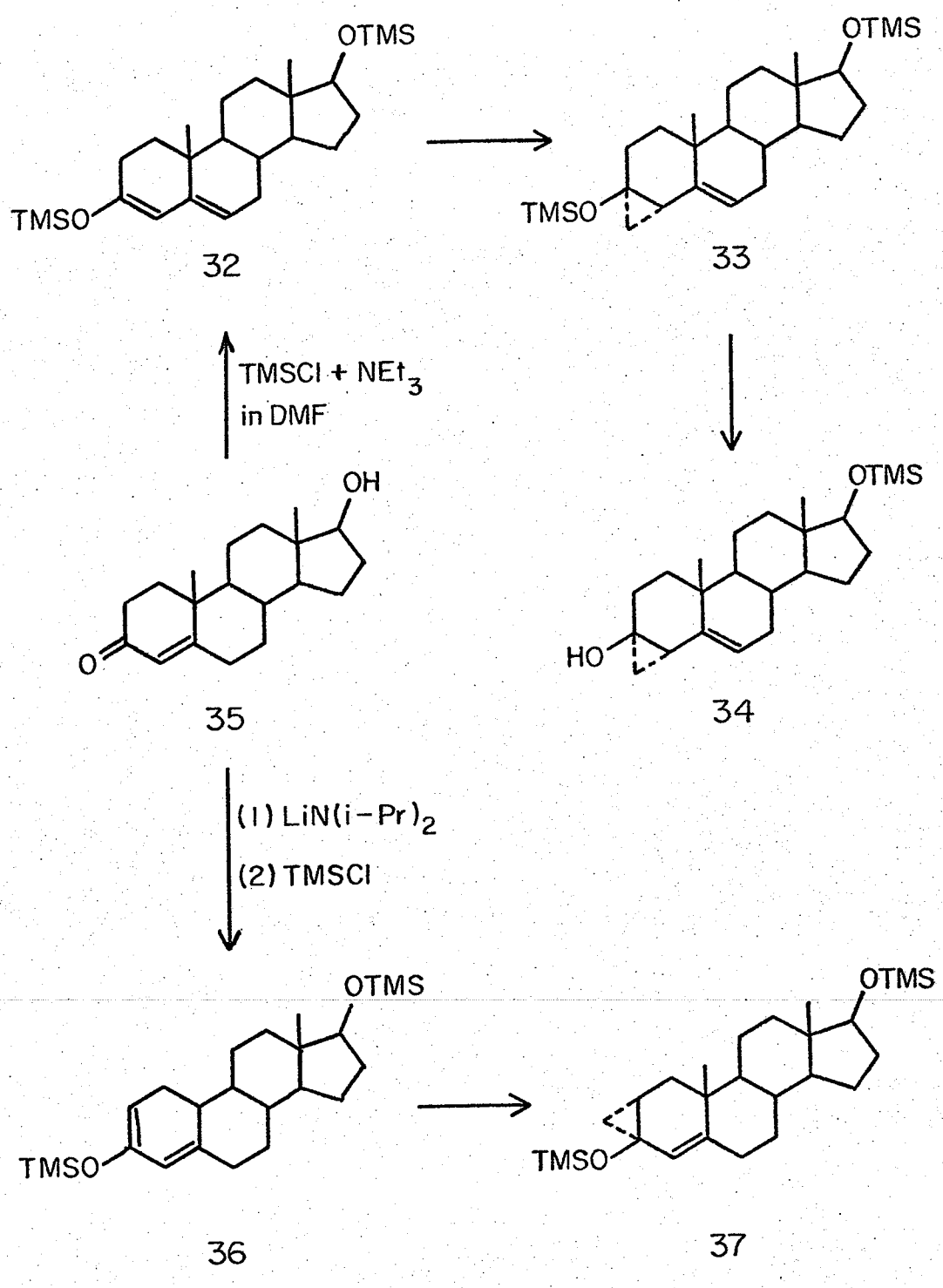
Conia and Girard [17,18] have also employed this method to synthesize the steroid cyclopropanol (34) from the trimethyl silyl enol ethers (32 and 36) of 17β-hydroxy-androst-4-en-3-one (35) (Scheme 11).

(c) Photolysis

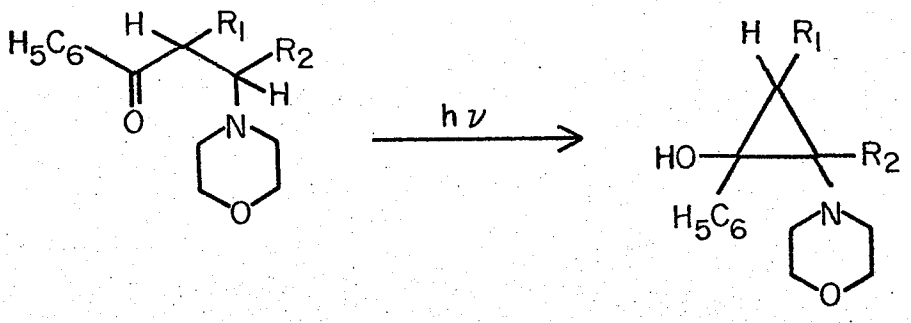
High yields of cyclopropanols (39) have been obtained by Roth and ElRaie [19] from the photolysis of 3-morpholinopropiophenones (38) in dioxane (Scheme 12). A similar photochemical reaction has been shown by Gull, Wehrli, and Jeger [20] in the formation of a tertiary steroid cyclopropanol (41) (Scheme 13).

The three methods mentioned above lead to tertiary cyclopropanols. The following reactions which have not been applied to the steroid system may also be useful synthetic methods.

Scheme 11


OTMS
TMSO
32
OTMS
TMSO
33
TMSCl + NEt3
in DMF
OH
O
35
OTMS
HO
34
(1) LiN(i-Pr)2
(2) TMSCl
OTMS
TMSO
36
OTMS
TMSO
37

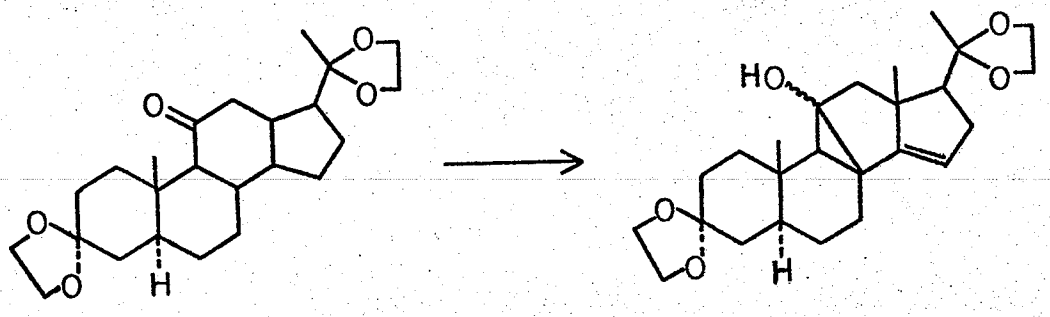
Scheme 12



38 39

R_1	R_2
Me	H
C_6H_5	H
H	C_6H_5

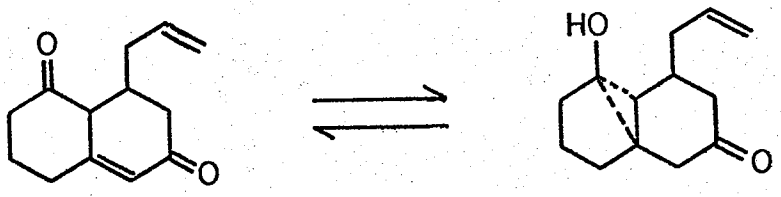
Scheme 13



40 41

A reaction which involves reduction of an unsaturated diketone (42) by a solution of lithium in ammonia and ether yielding a tertiary cyclopropanol (43) has been carried out by Venkataramani and Reusch [21] (Scheme 14). This reaction may be applicable to certain steroid diketones having a properly sited double bond.

Scheme 14

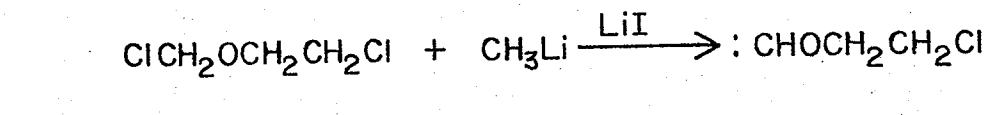


42 43

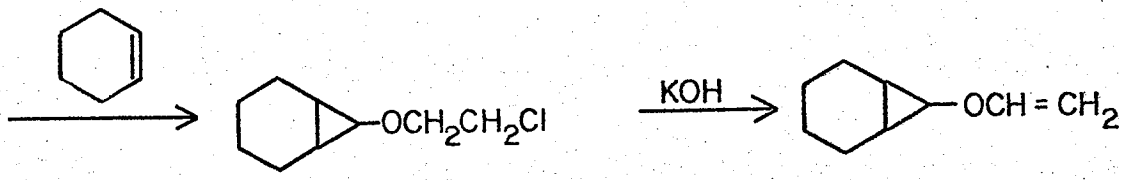
Schöllkopf et al.[22] who developed the 2-chloroethyl ether as a means of preparing cyclopropanols have reported a method in which a carbene (45) generated from chloromethyl-2-chloroethyl ether (44) is added to a double bond, forming a 2-chloroethyl ether (46) of a cyclopropanol. Dehydrochlorination to the vinyl ether (47) followed by mild acid hydrolysis gives the cyclopropanol (48) (Scheme 15). The method has been used to prepare secondary cyclopropanols.

Another method for the preparation of secondary cyclopropanols (52), reported by Longone and Wright [23], involves the oxygenation of cyclopropyl lithiums prepared from cyclopropyl bromide (51) by reaction with the metal. The cyclopropyl bromides (50) were synthesized by dibromocarbene insertion into alkenes (49) followed by tri-n-butyltin hydride reduction (Scheme 16).

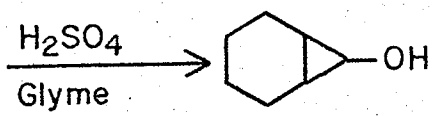
Scheme 15



44 45

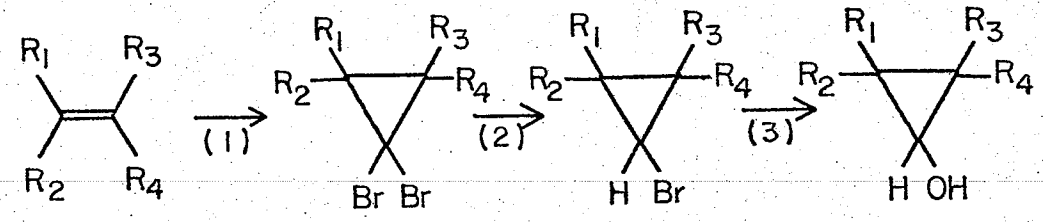


46 47



48

Scheme 16



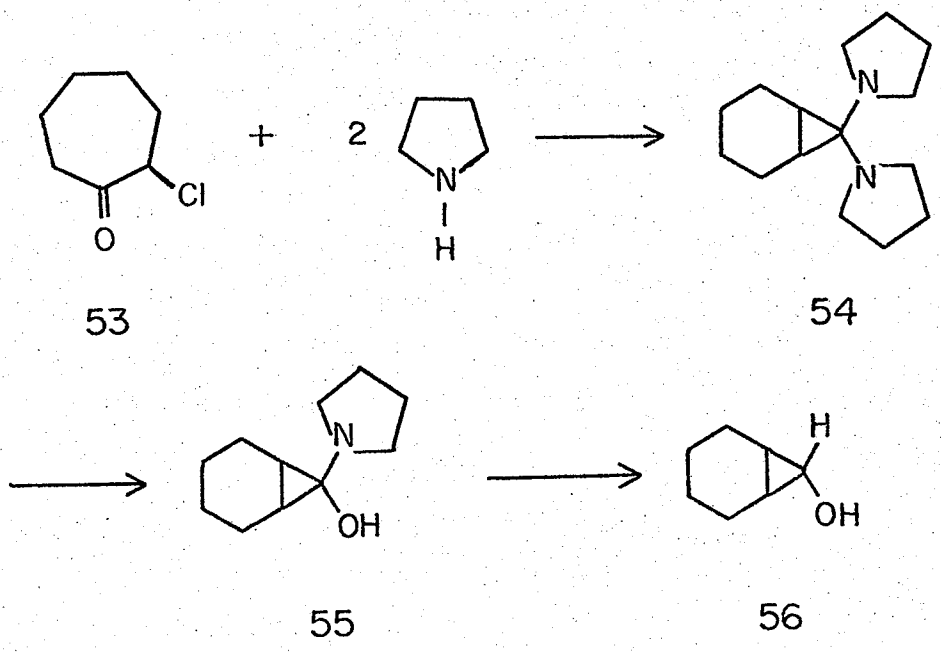
49 50 51 52

R_1, R_2, R_3, R_4 = H, AlKyl
(1) t-BuOK/$CHBr_3$
(2) $(n\text{-}Bu)_3SnH$
(3) Li/Et_2O, O_2, NH_4^+

These synthetic routes should, in principle, lead to the corresponding cyclopropanols of olefinic steroids.

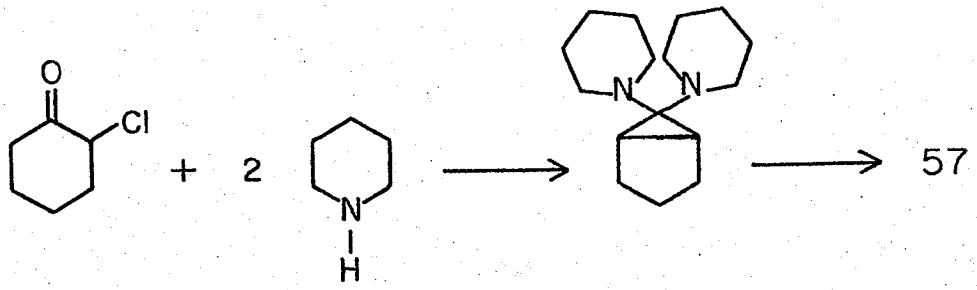
A Favorskii type of reaction has been applied by Szmuszkovicz et al.[24] to the synthesis of a secondary cyclopropanol —— the endo-7-norcaranol (56) (Scheme 17). The α-chlorocycloheptanone (53) reacts with pyrrolidine to afford 7,7-dipyrrolidino-bicyclo|4.1.0|-heptane (54) which undergoes acid hydrolysis to 7-pyrrolidino-7-norcaranol (55). The latter upon reduction with sodium borohydride, yields endo-7-norcaranol (56).

Scheme 17



However, the 6,6-dipiperidino-bicyclo |3.1.0|-hexane (58) obtained from α-chlorocyclohexanone (57) and excess piperidine reverts to α-chlorocyclohexanone (57) with aqueous hydrochloric acid [25] (Scheme 18). This method may be useful for preparing secondary cyclopropanols from the α-haloketones of some homosteroids.

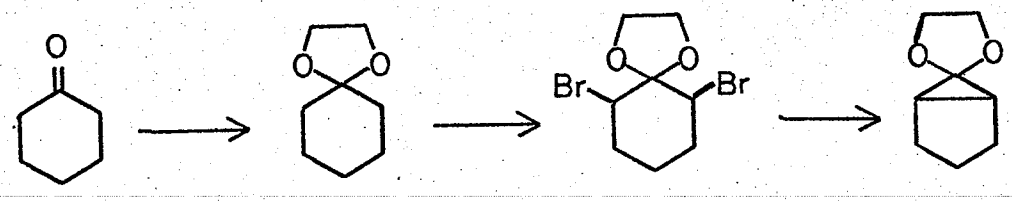
Scheme 18



57 58

Intramolecular 1,3-elimination of bromide with either magnesium in tetrahydrofuran (THF) or zinc in hexamethylphosphoric triamide (HMPT) has been shown to occur by Giusti, Morales, and Feugeas [26] in the preparation of several cyclopropanone ketals, e.g., a bicyclic cyclopropanone ketal (62), from the corresponding α,α'-dibromoketal (61) which was obtained from the ketone (59) via ketalization followed by bromination of the resulting ketal (60) (Scheme 19).

Scheme 19



59 60 61 62

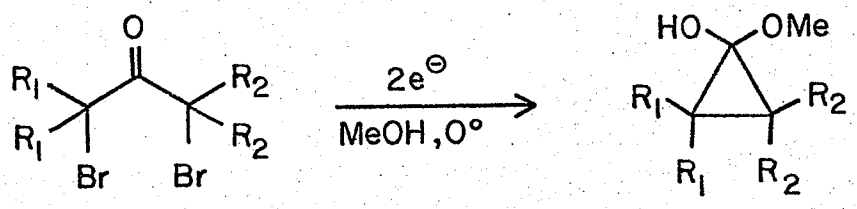
In principle, this synthetic scheme could be applied to the 3-oxo steroid system to yield the corresponding cyclopropanone ketal. However, it has not been possible to brominate a steroid-3-cycloethy-

leneketal at the α,α'-positions, as shown by Marquet et al.[27]. It has also been reported earlier by Dauben, Löken and Ringold[28] that the 2, 4-dibromo-3-ketones of steroids are inert to cycloethyleneketal formation.

Although some methyl hemiketals (64) have been prepared by Fry and Scoggins[29] by an electrochemical reduction of α,α'-dibromoketones (63) in the presence of methanol (Scheme 20); reduction of α,α'-dibromoketone (65) with a zinc-copper couple in methanol yields the parent ketone (67) and the α-methoxyketones (66) in varying amounts, as shown by Hoffmann[30] (Scheme 21).

However, electrochemical reduction of the 1,3-dibromo-2-propanol (68) provides cyclopropanol (69) in good yield (Scheme 22), as reported by Gerdil[31].

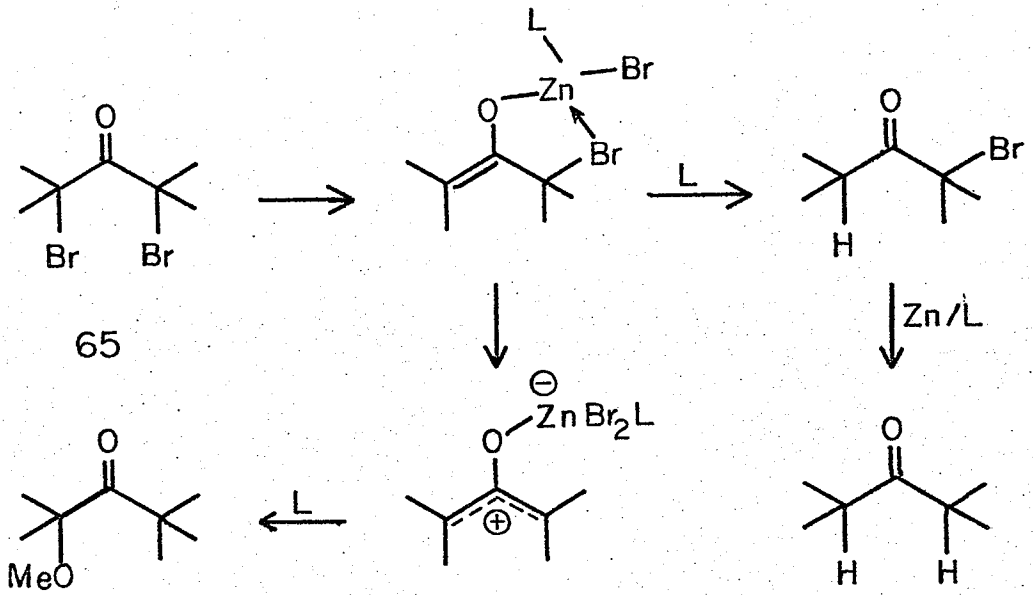
Scheme 20



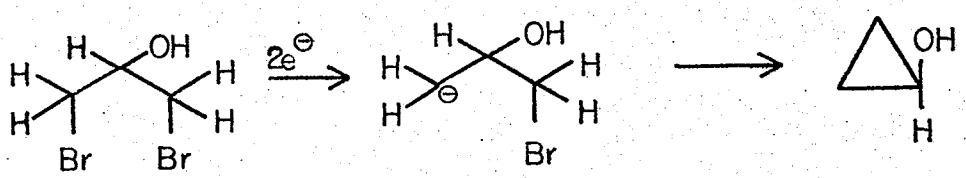
63 64

R_1 = H , Me ; R_2 = Me

Scheme 21



Scheme 22



68

69

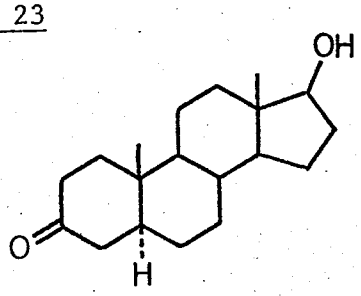
B. Results and discussion

a. 5α-Androstane series

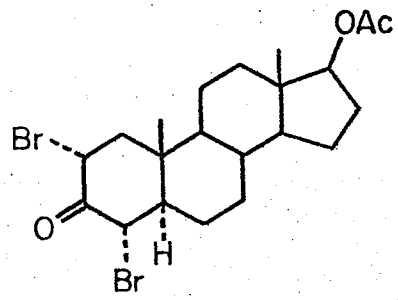
Treatment of 17β-hydroxy-5α-androstan-3-one (70) with two moles of bromine in acetic acid afforded the known 2α,4α-dibromo-17β-acetoxy-5α-androstan-3-one (71) [32]. Reduction of this sterically hindered ketone with sodium borohydride in ethanol proceeds rapidly to give a mixture of the 2α,4α-dibromo-5α-androstane-3α,17β- and 3β,17β-diol 17-acetate (72a and 73a). Chromatography on neutral alumina or silica fails to separate the dibromoalcohols (72a and 73a) satisfactorily. Fractional crystallization of the epimers gave in low yield the pure dibromoalcohols which were further characterized by the formation of their corresponding acetates (72b and 73b). The major product from the reduction is the 3α-epimer (72a) determined by the proton magnetic resonance (p.m.r.) spectra of the crude dibromoalcohols (72a and 73a) and their acetates (72b and 73b) (see page 44). The dibromoalcohols show the presence of a triplet at 4.27 δ ($J_{2\beta,3\beta} = J_{3\beta,4\beta} = 2.5$ Hz) and 3.80 δ ($J_{2\beta,3\alpha} = J_{3\alpha,4\beta} = 10.0$ Hz) consistent with axial-equatorial coupling of an equatorial proton at C-3 and diaxial coupling of an axial proton at C-3, respectively [33a]. Similarly, the p.m.r. spectra of the acetate (72b) contain a triplet at 5.70 δ ($J_{2\beta,3\beta} = J_{3\beta,4\beta} = 2.5$ Hz) and acetate (73b) a triplet at 5.27 δ ($J_{2\beta,3\alpha} = J_{3\alpha,4\beta} = 10.0$ Hz), in agreement with the assignment of 72 as the 3α- and 73 as the 3β-derivatives.

Because of the difficulty encountered in purifying the epimeric alcohols (72a and 73a), a mixture of the dibromoalcohols was then used without further purification. Treatment of the dibromoalcohols with

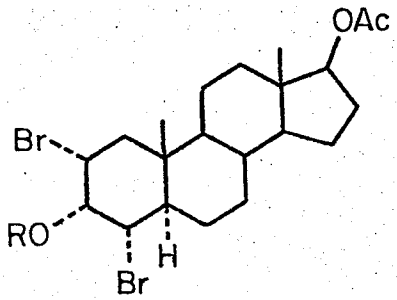
Scheme 23



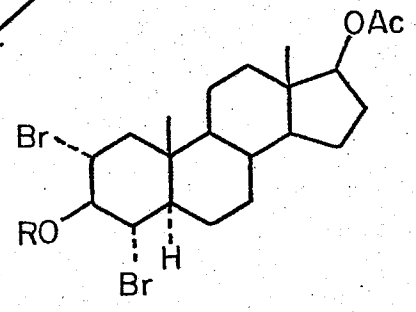
70



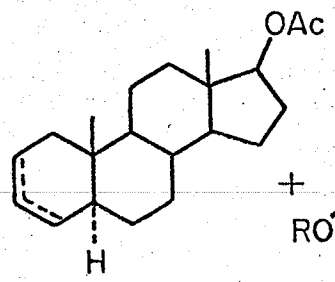
71



72 a , R = H
b , R = Ac

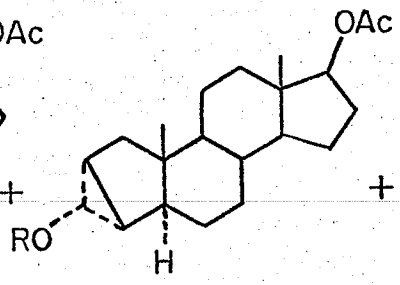


73 a , R = H
b , R = Ac

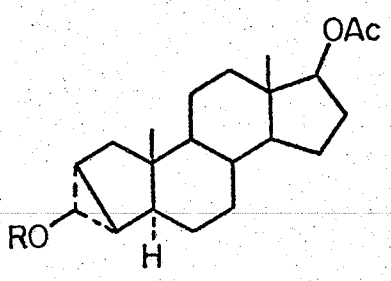


74

2-ene and 3-ene



75 a , R = H
b , R = Ac
c , R = Ts
d , R = Suc

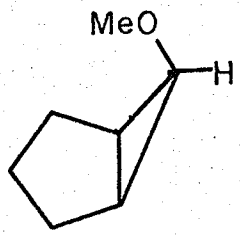


76 a , R = H
b , R = Ac
c , R = Ts
d , R = Suc

a freshly prepared 2% zinc-copper couple in ethanol at room temperature showed by thin-layer chromatography (t.l.c.) and gas-liquid chromatography (g.l.c.) analysis the formation of three major products and no starting material after thirty minutes. The less polar material (by t.l.c.) was largely dissolved by petroleum ether and extraction of the crude reaction mixture gave a crystalline compound from the petroleum ether soluble portion which showed vinylic C-H stretching vibration at 3040 cm^{-1} and alkene C=C stretching vibration at 1653 cm^{-1} in the infared (i.r.) spectrum. The p.m.r. spectrum [34] showed a multiplet between 5.14 to 5.62 δ which integrated for two protons and the mass spectrum [35] contained the parent ion at m/e 316 indicating an isomeric mixture of 17β-acetoxy-5α-androst-2 and 3-ene (74) which was not further purified. The petroleum ether mother liquor gave a further crystalline substance as a minor product which was recrystallized from methanol and identified as 2β,4β-cyclo-5α-androstane-3β,17β-diol 17-acetate (76a). The petroleum ether insoluble residue on recrystallization from ether gave as the major product —— 2β,4β-cyclo-5α-androstane-3α, 17β-diol 17-acetate (75a). The cyclosteroid alcohols (75a and 76a) were identified on the basis of the following evidence.

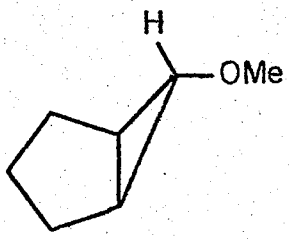
Carbon and hydrogen analysis and the presence of an ion at m/e 332 in the mass spectrum was consistent with the molecular formula of 75a and 76a. The i.r. spectrum of 75a showed the presence of hydroxyl absorption at 3509 cm^{-1} and cyclopropyl C-H stretching vibration at 3030 and 3049 cm^{-1}. Similarly 76a showed absorption at 3549 cm^{-1} (hydroxyl) and 3040 cm^{-1} (cyclopropyl C-H). Acetylation of 75a and 76a led to the 2β,4β-cyclo-5α-androstane-3α,17β- and 3β,17β-diol diacetate (75b and

76b). Incorporation of a second acetyl group indicates the presence of a single hydroxyl function in 75a and 76a. The p.m.r. spectra of the diacetates (75b and 76b) showed no olefinic protons in region between 5.20 to 5.70 δ, however, the presence of a triplet at 4.25 δ J = 6.0 Hz, and an unresolved triplet at 3.63 δ, by comparison to the endo/exo isomeric methoxycyclopropanes (77 and 78) [36], are consistent with a cyclopropyl acetate having an acetoxy function at C-3 with endo (75b) and exo (76b) configurations, respectively.



3.05 δ (J = 7.0 Hz)

77



2.78 δ (J = 3.0 Hz)

78

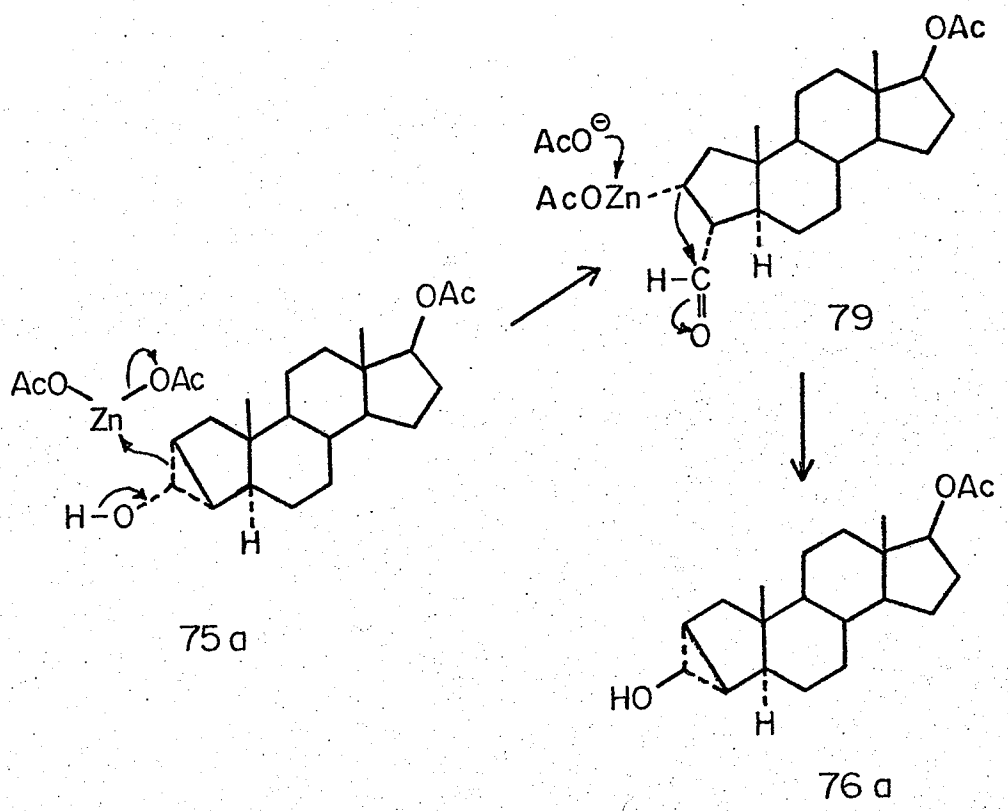
Compound 75a and 76a on treatment with alcoholic potassium hydroxide, were converted into a non-crystalline product which showed the presence of aldehyde carbonyl absorption at 1720 cm^{-1} and C-H stretching vibration at 2700 and 2800 cm^{-1} in the i.r. spectrum. The p.m.r. spectra also contained an aldehyde proton at 9.62 δ (multiplet) and loss of the signals associated with the cyclopropanol ring. This result would be expected from opening of the cyclopropanol ring in 75a and 76a to give the A-nor derivative (85) containing an aldehyde function in either the C-2 or C-3 position.

In an attempt to determine which of the dibromoalcohols, 72a or 73a, is the major precusor of the isomeric 17β-acetoxy-5α-androstan-2- and -3-ene (74), the analytically pure 3α-dibromoalcohol (72a) and 3β-dibromoalcohol (73a) were treated with zinc-copper couple separately under the same reaction conditions. However, an unexpected result was observed; the 3β-dibromoalcohol (73a) gave the corresponding 3β-cyclopropanol (76a) as the only major product, whereas the 3α-dibromoalcohol (72a) led to three products in which the corresponding 3α-cyclopropanol (75a) was the major, and in addition to the olefinic compounds (74), the 3β-cyclopropanol (76a) was also formed in the reaction as a minor product.

The greater formation of the olefinic products (74) from the 3α- rather than the 3β-dibromoalcohol is consistent with the greater ease of elimination of the 3α-(axial) hydroxyl substituent and the lesser stability of the endo-alcohol (75a). The formation of the 3β-cyclopropanol (76a) from the 3α-epimer (75a) was then shown to occur under the reaction conditions with the zinc-copper couple. Also the 3α-cyclopropanol (75a) in ethanol containing zinc acetate was converted slowly into the 3β-epimer. From both reactions, the 3β-epimer (76a) (>50%) was isolated as the corresponding acetate (76b) after acetylation and chromatographic separation. This conversion most probably involved the opening and reclosing of the cyclopropane ring. A mechanism is proposed for the conversion (Scheme 24), which involves addition of zinc acetate at either C-2 or C-4 of the 3α-cyclopropanol (75a) to give the A-nor intermediates (79) which then undergoes ring closure. Sterically the endo-alcohol (75a) will be the thermodynamically less stable pro-

duct and ring closure then yields the more stable exo-alcohol (76a).

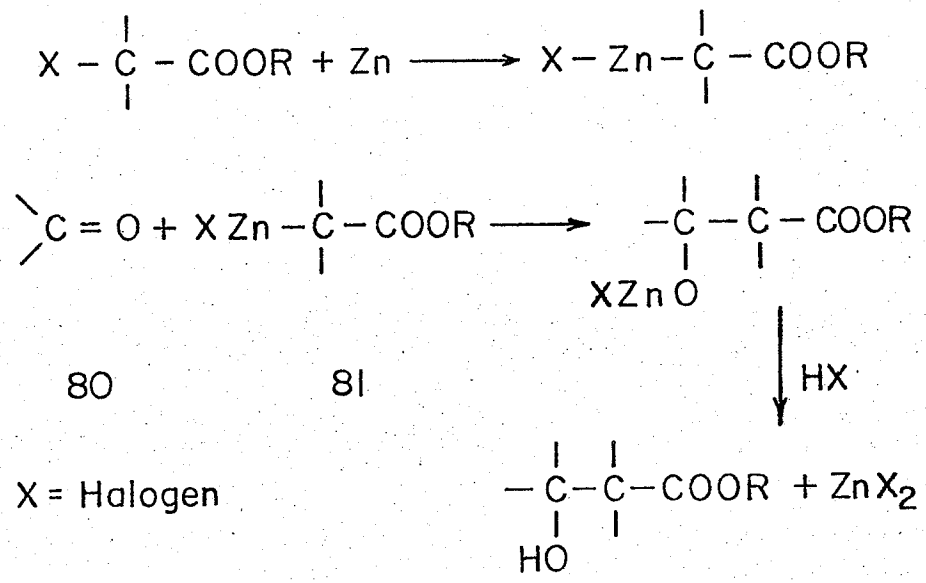
Scheme 24



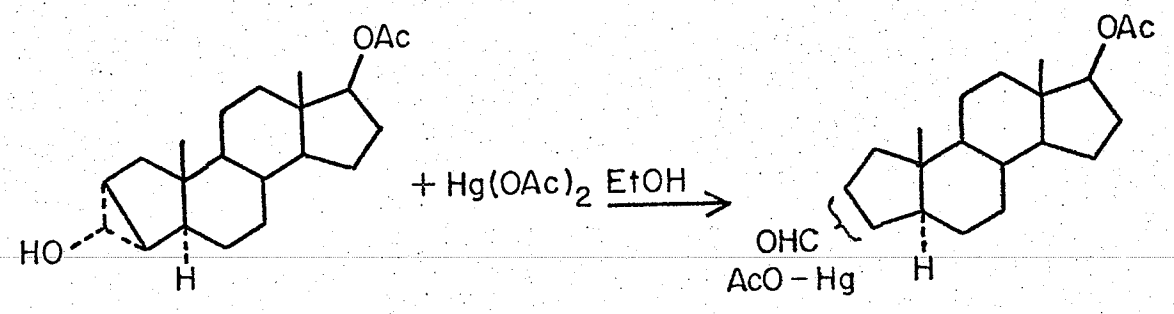
The mechanism for ring closure is comparable to that of the Reformatsky reaction [37] in which an organozinc compound (81) adds to the carbonyl group of the aldehyde or ketone (80) (Scheme 25).

With mercuric acetate in ethanol, the 3α-cyclopropanol (75a) was converted into an ethanol insoluble substance which was not identified, but is believed to be the organomercury compound (82) (Scheme 26). An analogous ring opening has been shown [38] to occur by reaction of a cyclopropanol (83) with mercuric acetate and the organomercury compound (84) isolated (Scheme 27).

Scheme 25

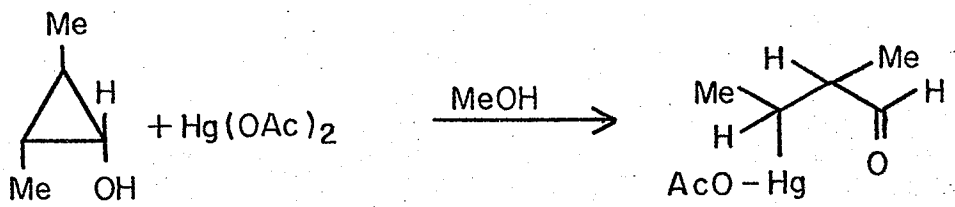


Scheme 26



75a 82

Scheme 27

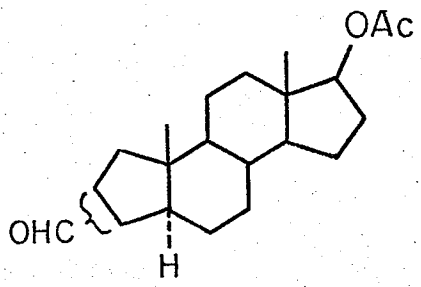


83 84

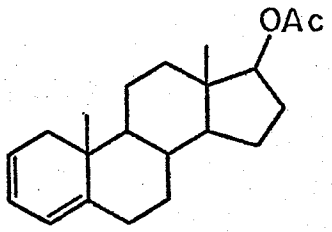
The endo and exo assignments of the cyclosteroid alcohols 75a and 76a are in agreement with the assignments of the configuration for the dibromoalcohols 72a and 73a, respectively.

When the 2α,4α-dibromo-5α-androstane-3α,17β- and 3β,17β-diol diacetate (72b and 73b) were similarly treated with zinc-copper couple, only a mixture of the olefinic products (74) was detected in the reaction as determined by t.l.c. This may be due to the fact that acetoxy is a better leaving group than a hydroxyl.

Further evidence for the stereochemical assignment of the epimeric cyclopropanols (75a and 76a) was provided by solvolysis of 2β,4β-cyclo-5α-androstane-3α,17β- and 3β,17β-diol 17-acetate 3-tosylate (75c and 76c) with sodium acetate in acetic acid. The tosylates were prepared by reaction of 75a and 76a with a solution of p-toluenesulfonyl chloride in pyridine. In the case of the isomers, endo-bicyclo|3.1.0|-hexyl 6-p-toluenesulfonate (91) and exo-bicyclo|3.1.0|-hexyl 6-p-toluenesulfonate (92), a solvolysis rate of 25,000 and <<0.01, respectively, relative to cyclopropyl p-toluenesulfonate was found [39]. The endo-isomer gave cis-2-cyclohexen-1-yl acetate (93) as the isolated product



85



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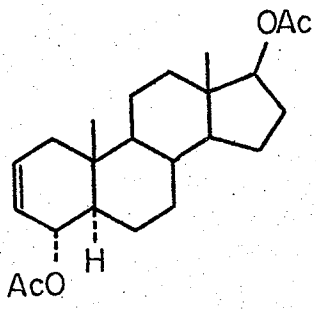

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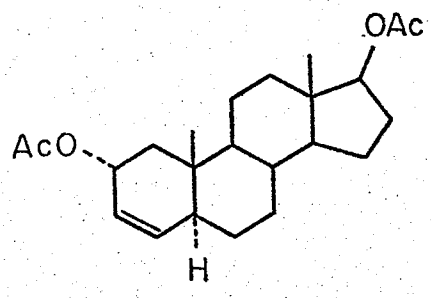

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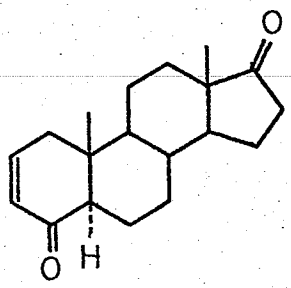

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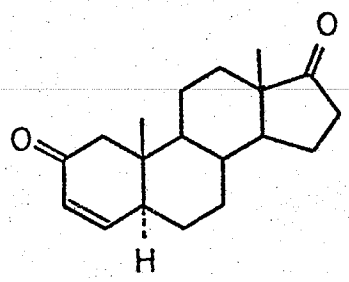
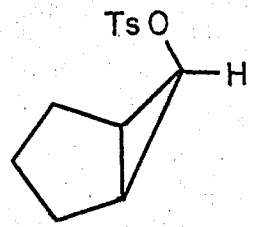
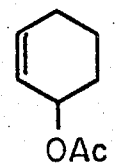
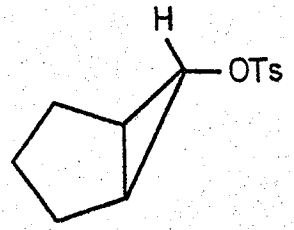


90

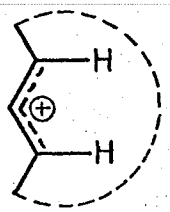
Table I. Acetolysis constants of the endo- and exo-bicyclo|3.1.0|-hexyl tosylate [39]

Tosylate	K_{rel}* (100°)	Product isolated
endo  91	25,000	 93
exo  92	<<0.01	-

* Relative to cyclopropyl tosylate, k = 3.89 x 10^{-8} sec^{-1}.

(Table I).

2β,4β-Cyclo-5α-androstane-3β,17β-diol 17-acetate 3-tosylate (76c) was recovered unchanged after refluxing for twenty hours with sodium acetate in acetic acid. Similar treatment of 2β,4β-cyclo-5α-androstane-3α,17β-diol 17-acetate 3-tosylate (75c) gave no starting material and a mixture of products after fifteen minutes as shown by t.l.c. This result is in agreement with the Woodward-Hoffmann prediction [40] that a cyclopropyl tosylate [41] rearranges in a disrotatory fashion so as to generate the required symmetry of the lowest molecular orbital of the allyl cation. In a 2,3-disubstituted cyclopropyl tosylate, two different disrotatory transformations are allowed on the symmetry grounds, however, the mode of rotation is dependent upon the stereochemistry of the leaving group, i.e., substituents cis to the leaving group rotate inwardly and substituents trans to the leaving group rotate outwardly. The lack of reactivity of the exo-bicyclo|3.1.0|-hexyl 6-p-toluenesulfonate (92) [39] and 2β,4β-cyclo-5α-androstane-3β,17β-diol 17-acetate 3-tosylate (76c) is due to the formation of a trans-trans allyl cation (94) which can not be accommodated in a six-membered ring, whereas a cis-cis cation (95) can.



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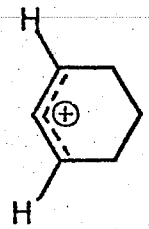


95

The products from the solvolysis of 2β,4β-cyclo-5α-androstane-3α, 17β-diol 17-acetate 3-tosylate (75c) were isolated by chromatography on alumina. The p.m.r. spectrum of the least polar fraction (by t.l. c.) showed the presence of olefinic protons at 5.75-5.32 δ (multiplet) and a single acetoxy function. Recrystallization of the fraction from methanol gave a substance showing the u.v. absorption of a conjugated diene. Literature data [42] indicates that it is principally the homoannular 17β-acetoxy-androsta-2,4-diene (86) (Table II).

The two more polar fractions were recrystallized from methanol to give the analytically pure compounds identified as 5α-androst-2-ene-4α, 17β-diol diacetate (87) and 5α-androst-3-ene-2α,17β-diol diacetate (88) by i.r., p.m.r., and mass spectrometry analysis. Both 87 and 88 showed vinylic C-H stretching vibration at 3030 cm^{-1}, ester C=O absorption at 1728 cm^{-1}, and C=C absorption at 1650 cm^{-1}. P.m.r. indicated the presence of two acetoxy groups in 87 and 88, and olefinic protons at 5.73, 5.86, and 5.59 δ, respectively. Mass spectra provided the molecular ion at m/e 374 for 87 and 88. The structures of 87 and 88 were further confirmed by hydrolysis with methanolic potassium hydroxide, followed by oxidation with Jones' reagent [43] to give 5α-androst-2-ene-4,17-dione (89) and 5α-androst-3-ene-2,17-dione (90), respectively. Both 89 and 90 showed vinylic C-H stretching vibration at 3040 and 3030 cm^{-1}; carbonyl absorption corresponding to a five-membered ring at 1730 and 1745 cm^{-1}; and a carbonyl of an α,β-unsaturated six-membered ring at 1675 and 1667 cm^{-1}, respectively, in the i.r. spectra. The position of the double bond in 89 and 90 was established by comparison of the olefinic proton signal in the p.m.r. spectra with that of the known [44]

Table II. Melting point and u.v. of 17β-acetoxy-androsta-2,4-diene (86)

	Experimental	Literature [42]
Melting point	98-99°	102-104°
u.v. λ_{max} (EtOH) nm	266, 272-3	266, 272-3
log ε	3.69, 3.65	3.78, 3.74

17β-acetoxy-5α-androst-2-en-4-one (96) and 17β-acetoxy-5α-androst-3-en-2-one (97) (Table III). The stereochemistry of the acetoxy group in 87 and 88 was not determined directly, however, addition from the least hindered side of the intermediate cation would yield the 2α- and 4α-acetates.

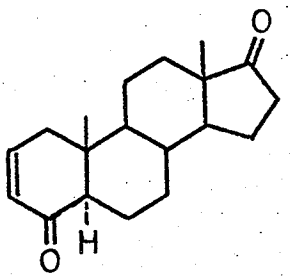
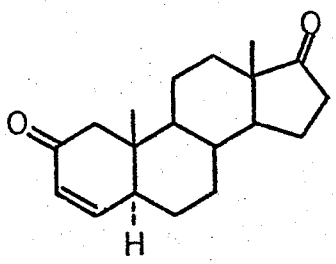
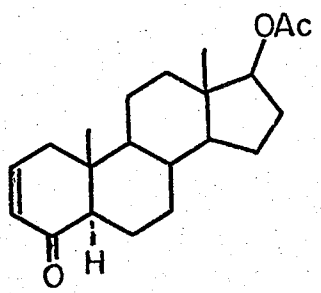
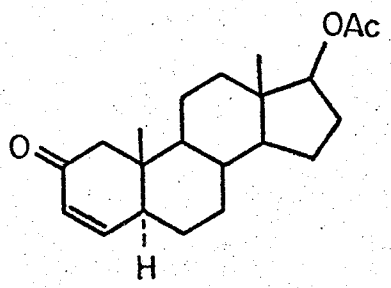
Besides the formation of the cyclosteroid acetates (75b and 76b) and tosylates (75c and 76c), the hemisuccinates (75d and 76d) were also prepared by treating the alcohols (75a and 76a) with a solution of succinic andydride in pyridine. Both 75d and 76d showed free and bonded O-H stretching vibration at 3515, 3510, and 3300-2500 cm^{-1}; cyclopropyl C-H at 3030, and 3040 cm^{-1}, respectively; and carbonyl absorption of ester and carboxylic acid at 1736 and 1712 cm^{-1} in the i.r. spectra. The p.m.r. spectra of 75d and 76d contained carboxylic proton signal at 10.96 and 10.98 δ; and succinoyl methylene protons at 2.70 and 2.60 δ, respectively. The 3β- proton in 75d appeared as a triplet at 4.32 δ (J = 6.0 Hz), whereas the 3α- proton in 76d as an unresolved triplet at 3.77 δ, comparable to those of the corresponding acetates (75b and 76b).

Since the 3α-cyclopropanol (75a) showed some indication of antiandrogenic properties (see Table VIII), we decided to prepare the corresponding 19-norderivatives. Removal of the 19-methyl group is associated with a decrease in androgenicity [45].

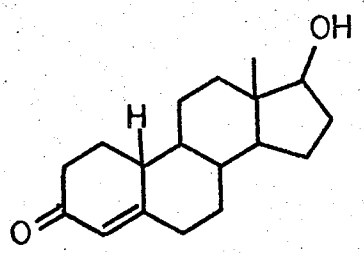
b. 5α-Estrane series

Reduction of 17β-hydroxy-estr-4-en-3-one (98) with lithium in liquid ammonia gave the known 17β-hydroxy-5α-estran-3-one (99) [46]. The exclusive formation of the A/B trans isomer has been explained by con-

Table III. Chemical shifts and coupling constants of olefinic proton in the p.m.r. spectra

Specific proton	 89	 90
C-2	6.81 δ (o, J = 10.0, 6.0, and 2.5 Hz)	-
C-3	6.01 δ (o, J = 10.0, 3.0, and 1.0 Hz)	5.99 δ (o, J = 10.0, 3.0, and 1.0 Hz)
C-4	-	6.59 δ (q, J = 10.0 and 2.0 Hz)
Specific proton	 96	 97
C-2	6.80 δ (o, J = 10.0, 5.0, and 3.0 Hz)	-
C-3	6.03 δ (o, J = 10.0, 2.3, and 1.0 Hz)	5.97 δ (o, J = 10.0, 3.0, and 1.0 Hz)
C-4	-	6.55 δ (q, J = 10.0 and 2.0 Hz)

sideration of the reaction mechanism. The fact that most steroid-4-en-3-ones and similar compounds give products with the trans-fused all-chair structure of mimimum energy led Barton and Robinson [47] to the generalization that the more stable product is produced by protonation of an intermediate carbanion (100) in its thermodynamically preferred conformation, i.e., 101, in which the orbitals concerned maintain the maximum overlap.



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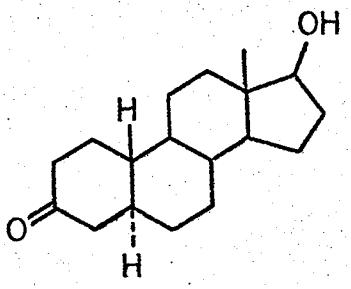

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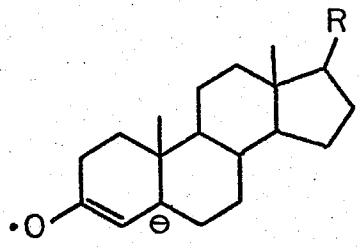

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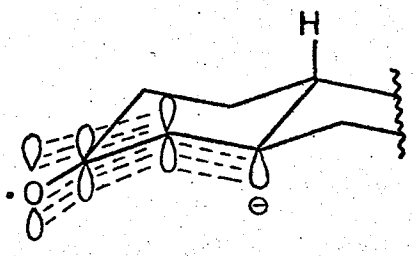
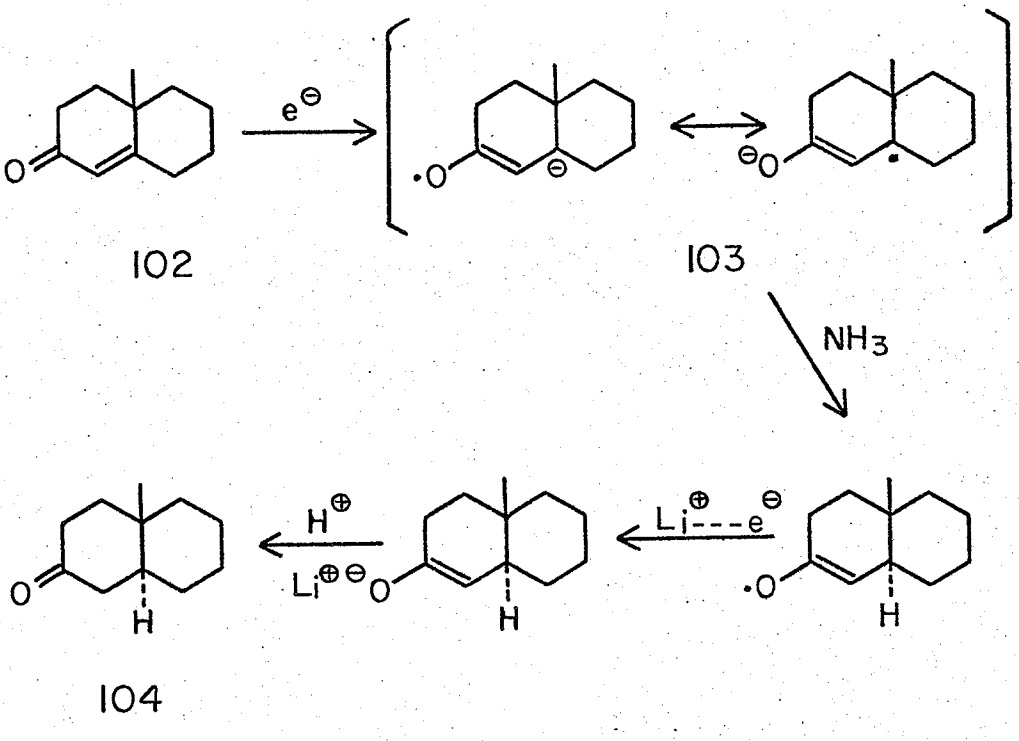


101

Further studies by Stork et al.[48] including the reduction of substituted octalones indicates that the initial stage in the reduction is probably the addition of one electron to the conjugated system (102) to give a mesomeric radical-anion (103) which has nucleophilic properties at C-5 and is able to abstract a proton from the solvent ammonia. The stereochemistry of the final product (104) is decided at this stage

in which the stable conformation of the carbanion produced is the one with an axial lone-pair (Scheme 28).

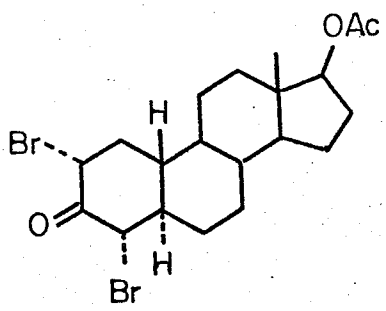
Scheme 28



102 103 104

Treatment of the 17β-hydroxy-5α-estran-3-one (99) with two molar equivalents of phenyltrimethylammonium perbromide in acetic acid gave a mixture of the dibromoketones (105, 106, and 107). Incorporation of at least two bromine into the steroid molecule was supported by the C, H, and Br analysis (Table IV); the presence of low field proton resonance (4.30-4.50 δ) in the p.m.r. spectrum of the crude product indicated the formation of the α,α'-dibromoketones although the α,α- or α',α'-dibromoketones (108 and 109) can not be excluded. Formation of a 2β,4β-dibromoketone (110) is very unlikely due to a strong 1,3-diaxial interactions similar to the substituted cyclohexanone system [49].

Attempts to isolate the unstable individual dibromoketones were



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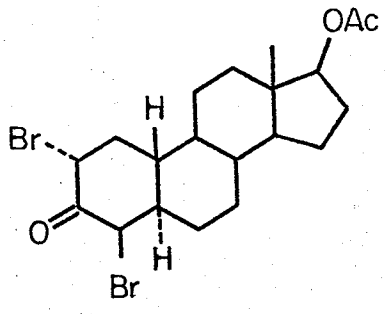

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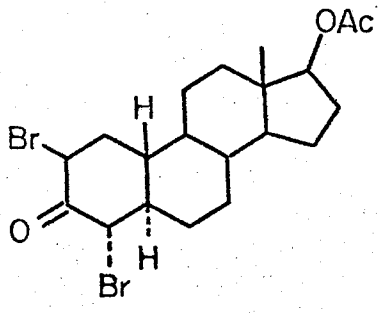

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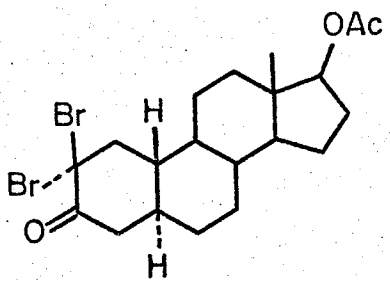

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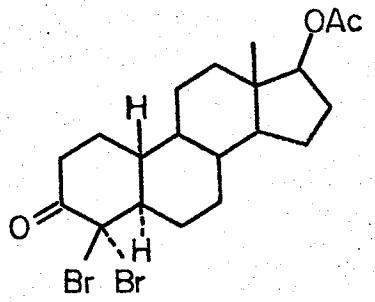

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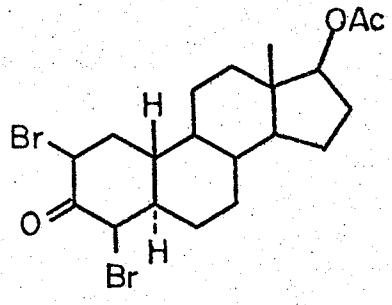

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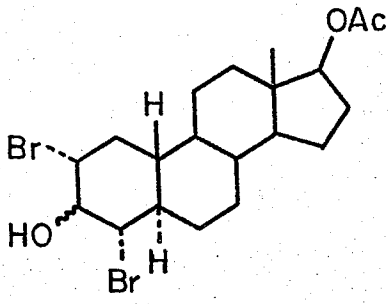

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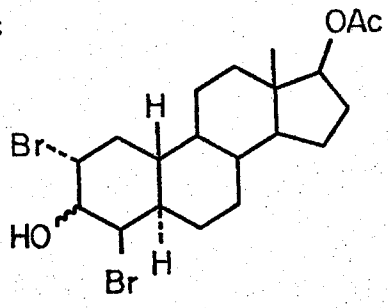

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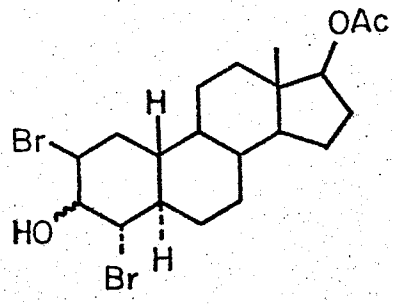

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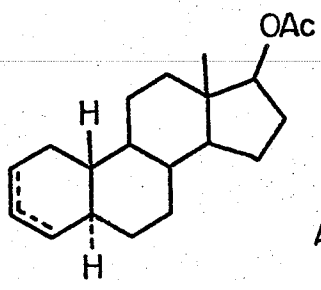

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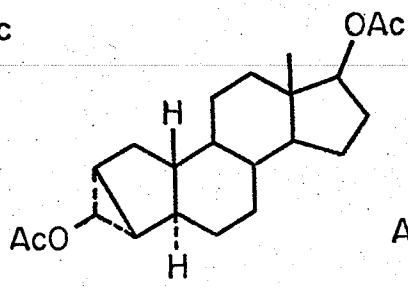

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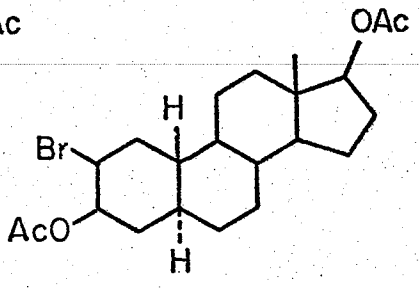


116

2-ene and 3-ene

Table IV. Elemental content of the bromo-derivatives of 17β-acetoxy-5α-estran-3-one

Derivative	C	H	Br
Monobromo-	60.45	7.38	20.11
Dibromo-	50.44	5.93	33.56
Tribromo-	43.27	4.90	43.18
Found	48.75	6.03	37.23

unsuccessful and the total crude product was then reduced with sodium borohydride in ethanol to afford a mixture of the epimeric hydroxyl derivatives (111, 112, and 113).

The dibromoalcohols (111, 112, and 113) did not afford any crystalline material from commonly used organic solvents, and we were unable to obtain the pure epimeric compounds. A mixture of the dibromoalcohols was treated with zinc-copper couple in ethanol to give three major products as shown by t.l.c. Extraction of the crude reaction product with petroleum ether which was successful with the androstane derivatives failed to separate these substances. The total crude mixture was then treated with acetic anhydride in pyridine to prepare the more stable acetates for chromatographic separation.

The least polar fraction (by t.l.c.) showed vinylic C-H stretching vibration at 3021 cm^{-1} in the i.r. spectrum and olefinic protons at 5.67-5.24 δ (multiplet) in the p.m.r. spectrum. The mass spectrum provided an ion at m/e 302 indicating the formation of 17β-acetoxy-5α-estr-2- and 3-ene (114) which was not further purified. One of the more polar fractions was recrystallized from methanol to afford an analytically pure compound identified as 2β,4β-cyclo-5α-estrane-3β,17β-diol diacetate (115). The spectroscopic properties of 115 are comparable to those of 2β,4β-cyclo-5α-androstane-3β,17β-diol diacetate (76b). The other fraction, upon crystallization from ethanol, gave 2β-bromo-5α-estrane-3β,17β-diol diacetate (116). The assignment of the structure is consistent with the presence of a broad multiplet ($W_{1/2}$ = 35.0 Hz) [50] at 4.64 δ for the axial proton at C-3 and an unresolved multiplet ($W_{1/2}$ = 6.0 Hz) [50] at 4.52 δ for the equatorial proton at C-2.

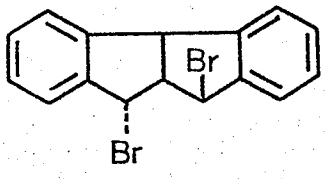
C, H, and Br analysis and mass spectrum showed that 116 is a monobromo derivative.

These results showed that application of the synthetic scheme (Scheme 23) to the 19-norsteroid involved several complicating factors. Debromination of the mixture of the dibromoalcohols (111, 112, and 113) gave a substantial amount of the olefinic products (114) and a low yield of the 3β-cyclopropanol isolated as the corresponding acetate (115). The epimeric 3α-cyclopropanol was not obtained, rather, a monobromoacetate (116) which was not found in the reaction of the corresponding androstane series, was isolated. The proportion of cyclopropane to olefin formed by 1,3- as opposed to 1,2-elimination in the debromination step is dependent upon the stereochemical relationship between the bromine and the hydroxyl substituents. Furthermore, the epimer ratio of the alcohols (111, 112, and 113) depends upon the stereochemistry of the sodium borohydride reduction of the bromoketones (105, 106, and 107).

In a recent report, Cristol, Dahl, and Lim [51] have shown that the trans-1,3-dibromide (117) which has an "exo-sickle" conformation (118) was converted quantitatively to the cyclopropane (119) upon treatment with zinc in 95% ethanol, and the product was the same when the zinc was washed with aqueous copper sulfate to prepare various zinc-copper couple compositions. On the other hand, the cis-1,3-dibromide (120) which has a relatively rigid " W " conformation (121) gave no cyclopropane (119) on treatment with zinc in ethanol and the product was a mixture of the syn and anti epimers of the monoethyl ether (122). However, a couple containing approximately 1 g-atom of copper/6 g-atom of

zinc gave about 60% cyclopropane (119) and 40% ethers (122) from 120. Intermediate copper contents gave less of 119 and more 122. The results are rationalized by the need for "exo-sickle" conformation (118) for ready 1,3-elimination.

The coupling of copper to zinc has long been known [10,52] to serve to activate the zinc metal surface, however, Sorm et al.[53] found that a 0.5% copper content of the couple is optimal in the formation of 5, 7β-cyclo-5β-cholestane-3β-yl acetate by Simmons-Smith reaction. Cristol, Dahl, and Lim [51] have suggested the possibility of diverting the formation of organozinc intermediates to that of organocopper intermediates by addition to the zinc of substantial amounts of copper. The organocopper intermediates may differ substantially in reactivity toward protonation, epimerization, and cyclopropane formation from the organozinc analogs.



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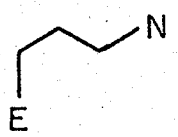

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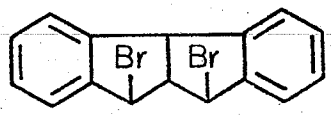

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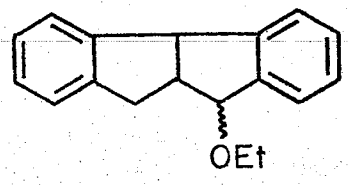


122

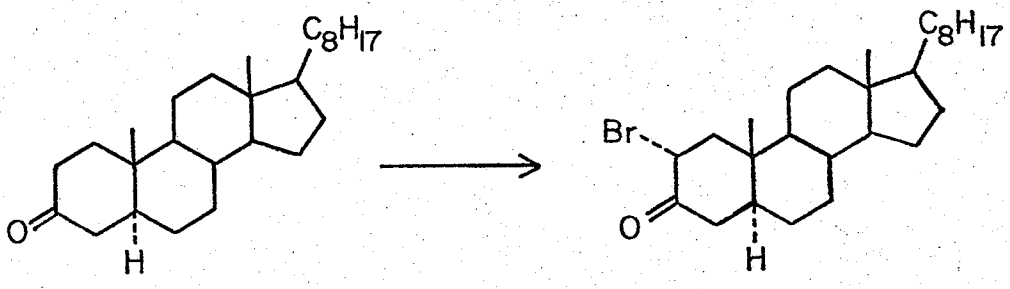
Although the 2α,4α-dibromo-5α-androstane-3α,17β- and 3β,17β-diol 17-acetate (72a and 73a) which have a " W " conformation similar to 121 gave no cyclosteroid alcohols (75a and 76a) (by t.l.c.) with zinc in ethanol and since we do not have the corresponding isomers with an "exo-sickle" conformation (118), the requirement of an "exo-sickle" geometry in the formation of the cyclosteroids (75a and 76a) with zinc in ethanol has to be left as an open question. In order to investigate this reaction further in the estrane derivatives it became necessary to determine the structure of the bromination and reduction products.

c. Brominated derivatives of 17β-hydroxy-5α-androstan-3-one and 17β-hydroxy-5α-estran-3-one

In the bromination of ketones [54a], the first, and rate-determining step, is the conversion of the ketone into the enolate anion if the catalyst is a strong base, or into the neutral enol if the catalyst is an acid; in either case bromination occurs by a fast electrophilic attack of bromine on the enolic intermediate. In the presence of added or generated hydrogen bromide, the bromination results in the thermodynamically more stable product. Kinetically controlled bromination may be effected by carrying out the reaction in the presence of an agent capable of removing hydrogen bromide as it is formed, i.e., sodium acetate. The kinetic product may be the same as the thermodynamic one or different from it depending upon steric factors.

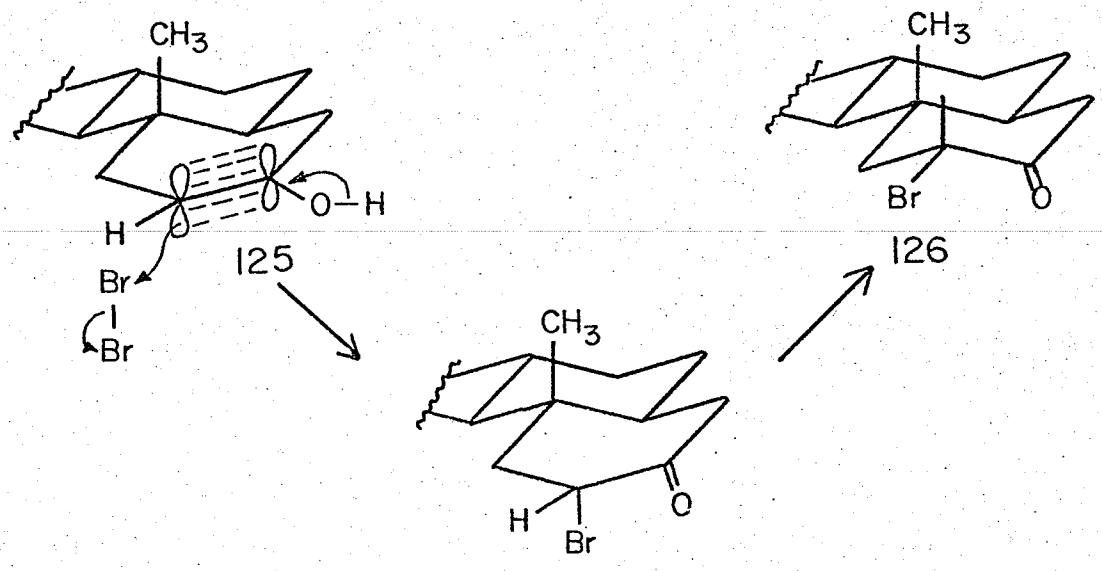
The product from monobromination of 5α-cholestan-3-one (123) is the 2α-bromoketone (124) [55]. This appeared to contravene the principle [56] that stereoelectronic control favors the formation of the axial

bromoketone. However, the stereoelectronic preference for axial attack is fulfilled by the molecule adopting a boat or flexible conformation (125) of ring A during attack by bromine, and changing later into the chair conformation with equatorial bromine (126) (Scheme 29). Initial α-bromination is a consequence of steric hindrance from the 19-methyl group to β-face attack upon the enol.



123 124

Scheme 29



Dibromination of 5α-cholestan-3-one (123) [57] gave the 2,2-dibromo-derivative (127) under kinetic control, which rearranges under acidic conditions into the more stable isomeric 2α,4α-dibromoketone (128) which is the thermodynamically controlled product (Scheme 30), due to the 1,3-diaxial interaction between the 2β-bromo and the 19-methyl groups. Formation of the 2,2-dibromoketone is due to the strong preference for a Δ^2-double bond in the 5α-steroid-3-ketones (Table V) [58].

Scheme 30

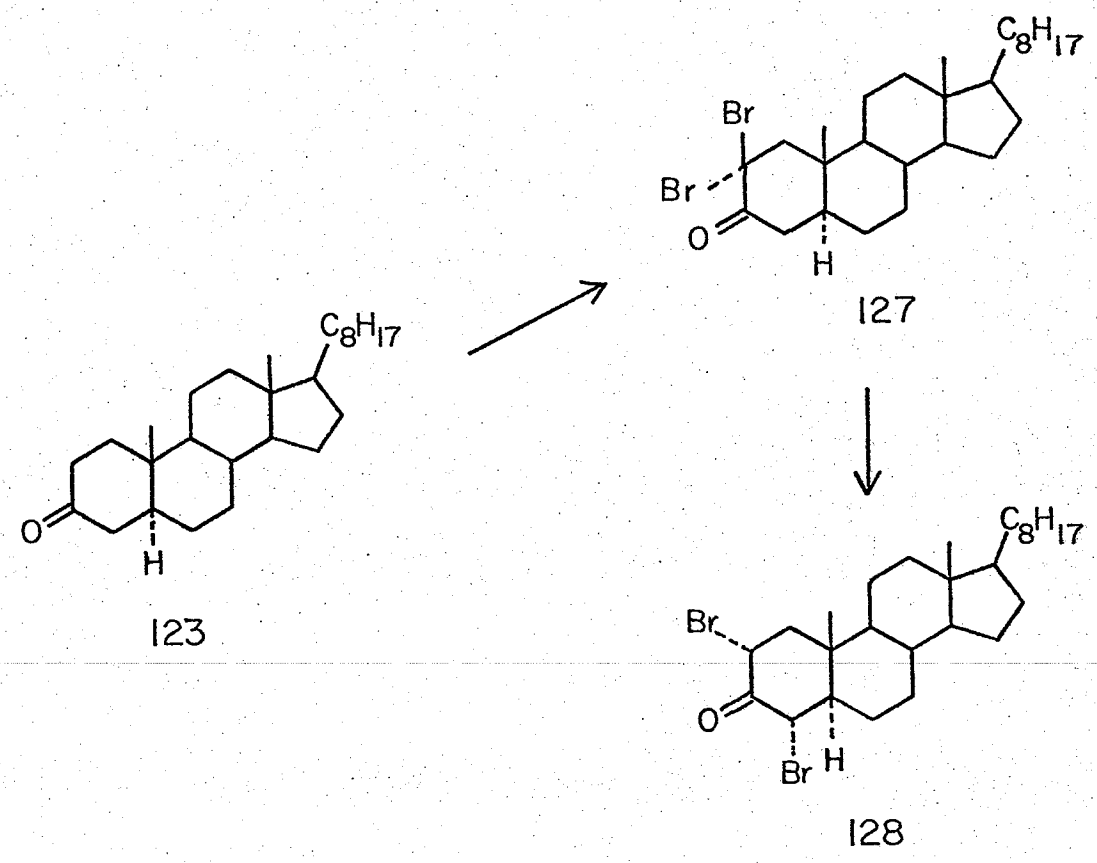


Table V. Enol acetylation of 5α-steroid-3-ketones [54e]

	% Conversion to enol		Free-energy difference favoring Δ^2-enol
	Δ^2	Δ^3	
Unsubstituted 5α-steroid-3-ketone	100	0	2.1 Kcal/mole*
19-Nor-5α-steroid-3-ketone	67	33	0.4 Kcal/mole

* The enthalpy difference between cholest-2-ene and 3-ene.

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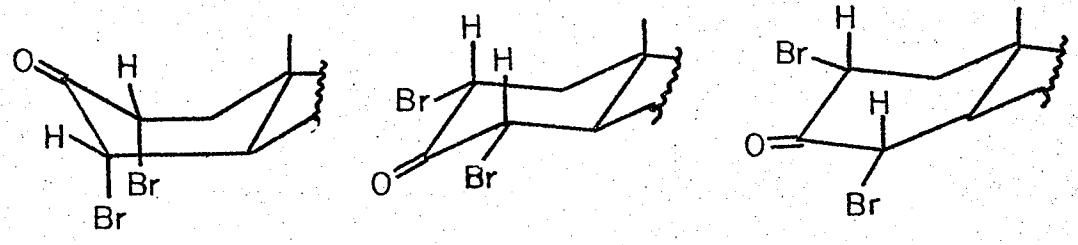
Therefore, dibromination of 17β-hydroxy-5α-androstan-3-one (70) led to the 2α,4α-dibromo-17β-acetoxy-5α-androstan-3-one (71) [32]. The conformation of this molecule has not been determined. The boat conformation (129) which involves 1,3-diaxial interaction of the 2α and 4α bromine atoms is unlikely, although the p.m.r. spectrum of 71 showed a doublet at 4.60 δ (J = 12.0 Hz) consistent with a diaxial vicinal coupling of the 4β-proton with the 5α-proton and a quartet at 4.76 δ (J = 13.0 and 6.0 Hz) consistent with a diaxial and an axial-equatorial vicinal coupling of the 2β-proton with the 1α- and 1β-protons, respectively; this result is inadequate to determine whether the molecule adopts a chair (130) or a half-chair conformation (131). However, the strong electrostatic interactions between the 2α,4α-bromine atoms and the C-3 carbonyl function which are coplanar in the chair conformation (Dreiding model), would favor the half-chair in which the three groups are no longer coplanar.



129 130 131

Compound 71 was reduced with sodium borohydride in ethanol in five minutes at room temperature to give a mixture of the epimeric dibromoalcohols (72a and 73a). It has been shown by Mateos [59] that 2α-bromo-5α-cholestan-3-one is reduced by sodium borohydride about ten

times more rapidly than the unbrominated ketone and he suggested that interactions between the dipoles of the ketone and the bromine destabilize the bromoketone.

The mixture of dibromoalcohols was acetylated to the more stable dibromoacetates (72b and 73b) which were subjected to chromatographic separation on neutral alumina. Elution with 15-25% benzene/petroleum ether gave the 2α,4α-dibromo-5α-androstane-3α,17β-diol diacetate (72b) and with benzene gave the 2α,4α-dibromo-5α-androstane-3β,17β-diol diacetate (73b). The isomeric dibromoacetates were obtained in an approximate ratio of 3:1 (3α : 3β), i.e., sodium borohydride reduction of the dibromoketone (71) led predominately to the axial alcohol (72a). Since the conformation of the 2α,4α-dibromo-5α-androstan-3-one (71) is uncertain, we were unable to rationalize the result by comparison with the literature [54b,60].

The structures of 72b and 73b were determined by their p.m.r. spectra (Figs. I and II), which showed a triplet at 5.70 δ ($J_{2\beta,3\beta} = J_{3\beta,4\beta}$ = 2.5 Hz) and 5.27 δ ($J_{2\beta,3\alpha} = J_{3\alpha,4\beta}$ = 10.0 Hz) for the C-3 equatorial and the C-3 axial protons, respectively. The multiplet centered at 4.33 δ ($J_{1\alpha,2\beta}$ = 13.0; $J_{1\beta,2\beta}$ = 4.5; $J_{2\beta,3\beta}$ = 2.5 Hz) and 4.28 δ ($J_{1\alpha,2\beta}$ =13.0; $J_{2\beta,3\alpha}$ = 10.0; $J_{1\beta,2\beta}$ = 4.4 Hz) is due to the C-2 axial proton and the quartet at 4.16 δ ($J_{4\beta,5\alpha}$ =11.0; $J_{3\beta,4\beta}$ = 2.5 Hz) and 3.92 δ ($J_{4\beta,5\alpha}$ = 11.7; $J_{3\alpha,4\beta}$ = 10.0 Hz) to the C-4 axial protons of 72b and 73b, respectively.

Bromination of 17β-hydroxy-5α-androstan-3-one (70) with three molar equivalents of phenyltrimethylammonium perbromide in acetic acid led to a mixture of bromoketones which without further purification,

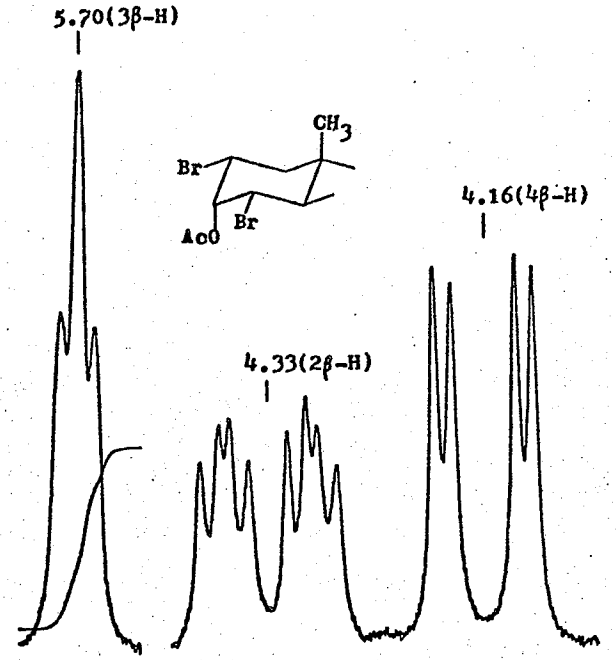


Figure I. 220 MHz p.m.r. spectrum of 2α,4α-dibromo-5α-androstane-3α,17β-diol diacetate (72b).

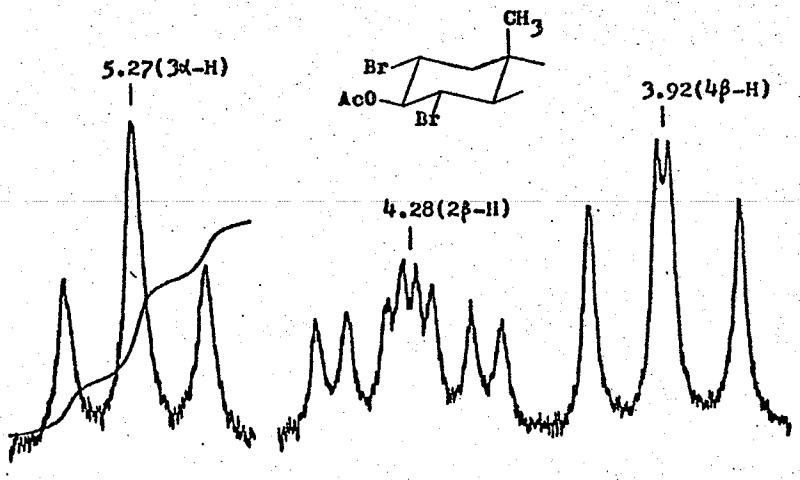


Figure II. 220 MHz p.m.r. spectrum of 2α,4α-dibromo-5α-androstane-3β,17β-diol diacetate (73b).

was reduced with sodium borohydride to the hydroxyl derivatives. Acetylation of the bromoalcohols followed by chromatography on neutral alumina gave on elution with 5% benzene/petroleum ether a tribromo-acetate which showed (Fig. III) a doublet at 5.39 δ ($J_{3\alpha,4\beta}$ = 10.0 Hz) due to the C-3 axial proton; a quartet at 4.14 δ ($J_{4\beta,5\alpha}$ = 12.0; $J_{3\alpha,4\beta}$= 10.0 Hz) due to the C-4 axial proton; no proton signal of the 2β-H type in Fig. II was observed. The presence of two doublets at 3.15 δ (J_{gem} = 15.0 Hz) and 2.60 δ (J_{gem} = 15.0 Hz) corresponded to the C-1 equatorial and C-1 axial protons, respectively, further supported the formation of 2,2,4α-tribromo-5α-androstane-3β,17β-diol diacetate (132).

With 5-10% benzene/petroleum ether, a substantial amount of the 2α,4α-dibromo-5α-androstane-3α,17β-diol diacetate (72b) was eluted. It was identical to the sample obtained previously by mixed melting point and i.r. spectrum

Elution with 25-50% benzene/petroleum ether gave an unsaturated tribromoacetate (133). The p.m.r. spectrum (Fig. IV) showed a doublet at 5.87 δ ($J_{2\beta,3\alpha}$ = 9.0 Hz) due to the C-3 axial proton; a multiplet similar in pattern to the 2β-H in Fig. II at 4.37 δ ($J_{1\alpha,2\beta}$ = 13.8; $J_{2\beta,3\alpha}$ = 9.0; $J_{1\beta,2\beta}$ = 3.5 Hz) assigned to the C-2 axial proton. A quartet at 5.59 δ (J = 4.4 and 2.1 Hz) was assigned to the 6α-proton. The configuration of the bromine atom at C-6 was further confirmed by comparison of the calculated[33a,61] chemical shifts of the 19-methyl protons (Table VI) of the 6α-bromo- and 6β-bromo- derivatives with the experimental value. The result establishes the structure of 2α,4, 6β-tribromo-androst-4-ene-3β,17β-diol diacetate (133).

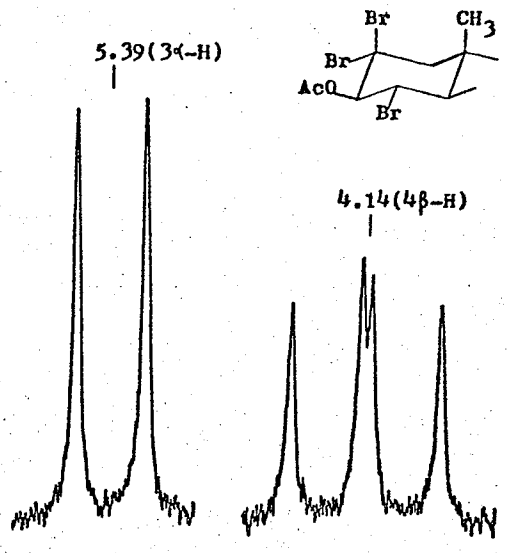


Figure III. 220 MHz p.m.r. spectrum of 2,2,4α-tribromo-5α-androstane-3β,17β-diol diacetate (132).

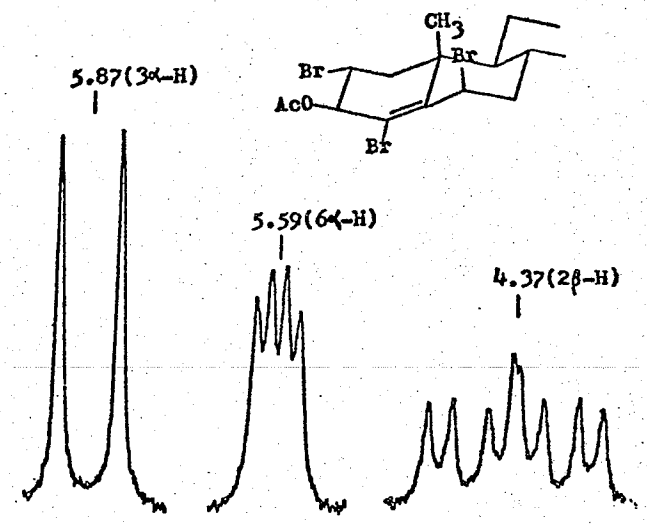
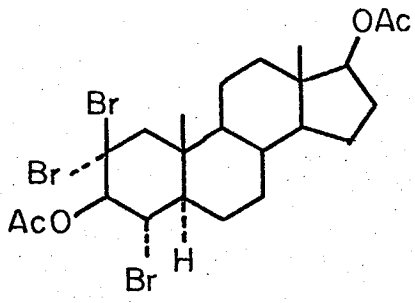
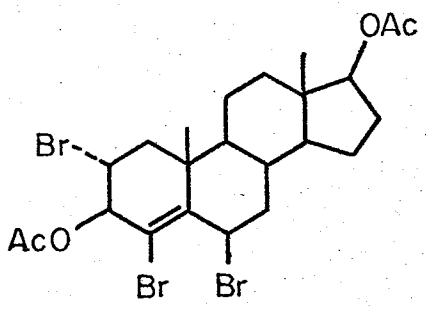


Figure IV. 220 MHz p.m.r. spectrum of 2α,4,6β-tribromo-androst-4-ene-3β,17β-diol diacetate (133).



132



133

The formation of dibromo, tribromo and unsaturated tribromo derivatives may be rationalized as follows (Scheme 31). Tribromination of 70 had led to a mixture of the 2α,4α-dibromoketone (71) and the 2,2,4α-tribromoketone (134) similar to the tribromination of 139 (Scheme 32) [62].

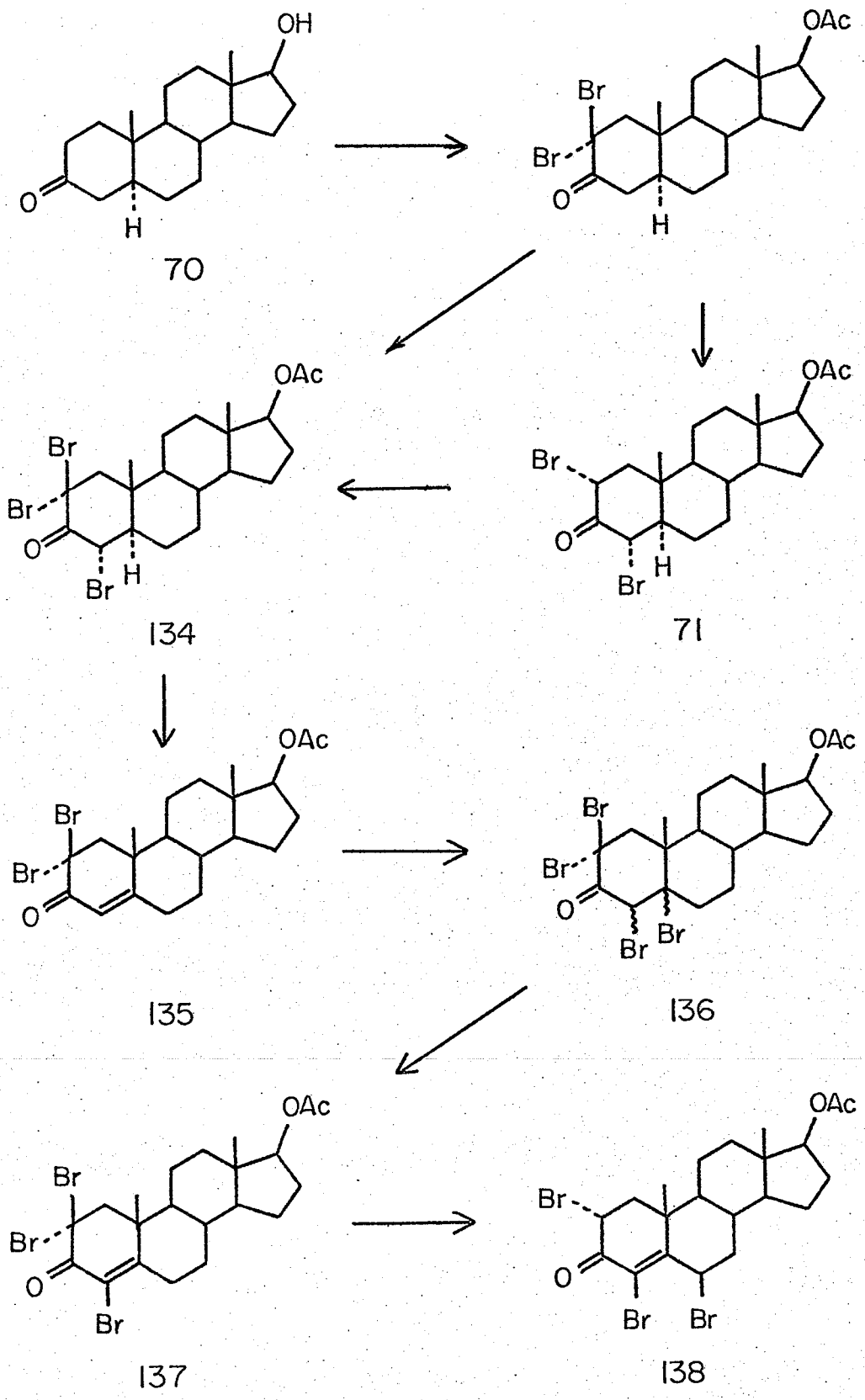
The conformation of the 2,2,4α-tribromoketone (134) has not been determined, however, a boat conformation (140) has been suggested [63] for the 2,2,4α-tribromo-5α-cholestan-3-one which was proposed as an intermediate in the bromination of either 2,2-dibromo- or 2α,4α-dibromo-5α-cholestan-3-one (127 and 128) (Scheme 33).

The unstable tribromoketone (134) may eliminate hydrogen bromide to give the 2,2-dibromo-4-en-3-one (135) which, by further addition of bromine across the Δ^4-double bond, gave rise to a tetrabromoketone (136) similar to the 2,2,4ξ,5ξ-tetrabromo-5α-cholestan-3-one (141) isolated from dibromination of either 2,2-dibromo- or 2α,4α-dibromo-5α-cholestan-3-one (127 and 128) (Scheme 33) [63].

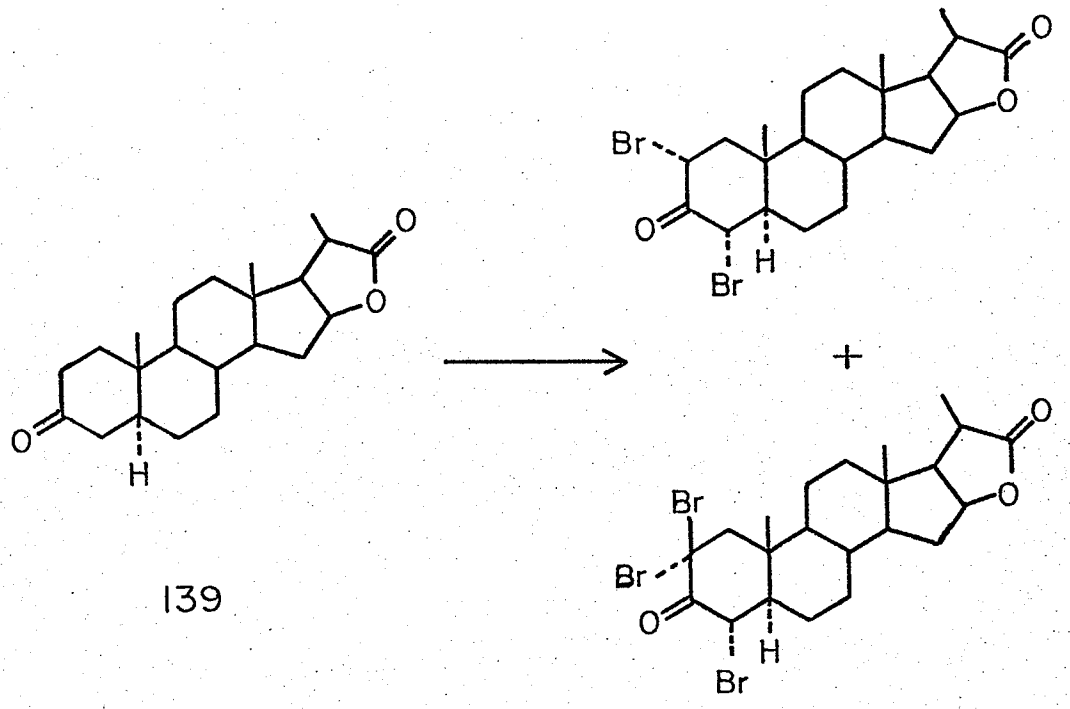
Table VI. Substituent increments (ppm) for the chemical shift of the C-19 protons [33a,61]

Substituents	C-19 Protons	
	6α-Bromo-	6β-Bromo-
6α-Bromo-5α-androstane	0.810	-
6β-Bromo-5α-androstane-3β-yl acetate	-	1.100
2α-Br	0.075	0.075
3β-OAc	0.054	-
4-Br (at Δ^4)	0.022	0.022
Δ^4	0.250	0.250
17β-OAc	0.020	0.020
Calculated	1.231	1.467
Experimental		1.520

Scheme 31


OH
O
H
70
OAc
Br
Br
O
H
OAc
Br
Br
O
Br
H
134
OAc
Br
Br
O
Br
H
71
OAc
Br
Br
O
Br
Br
135
OAc
Br
Br
O
Br
Br
136
OAc
Br
Br
O
Br
137
OAc
Br
Br
O
Br
Br
138

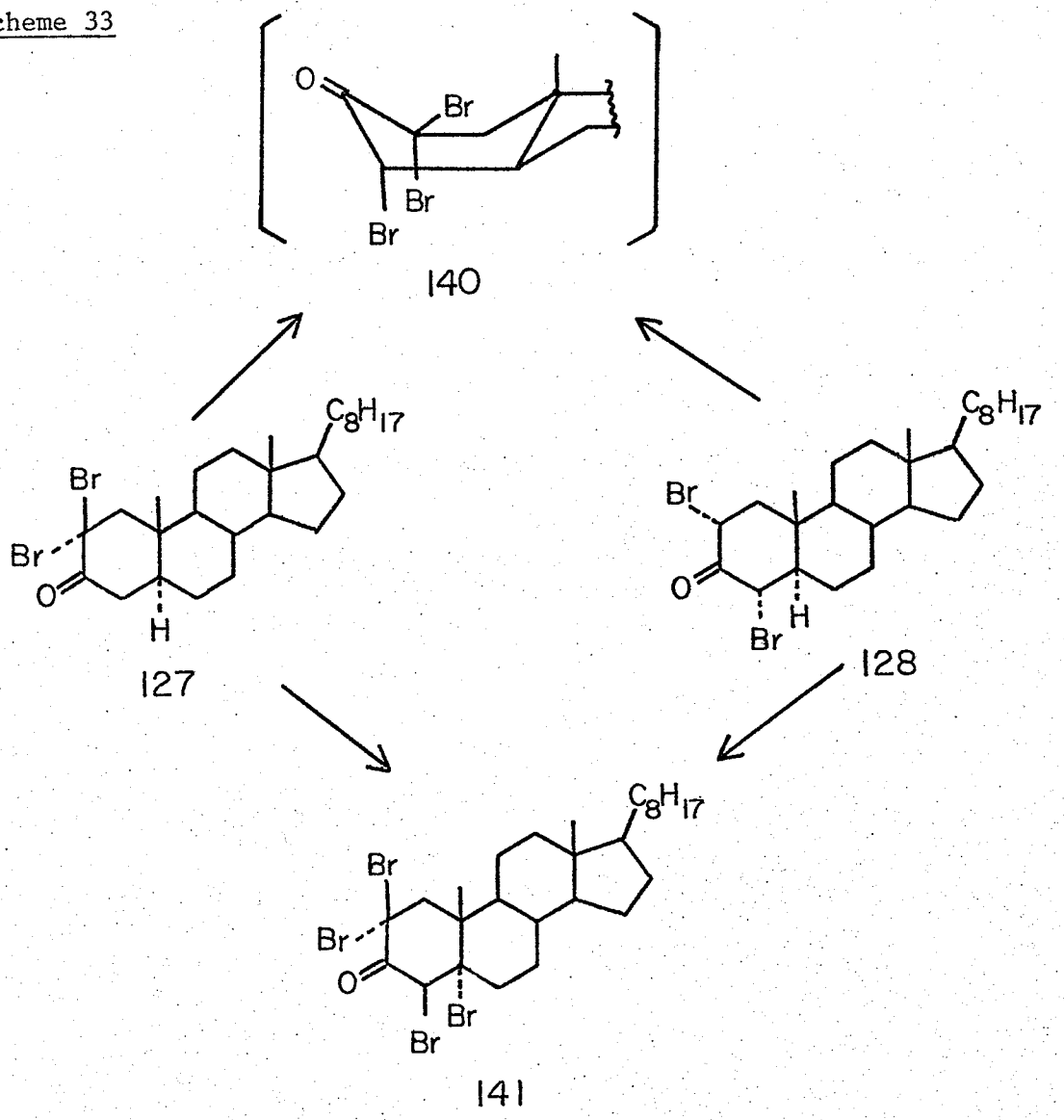
Scheme 32



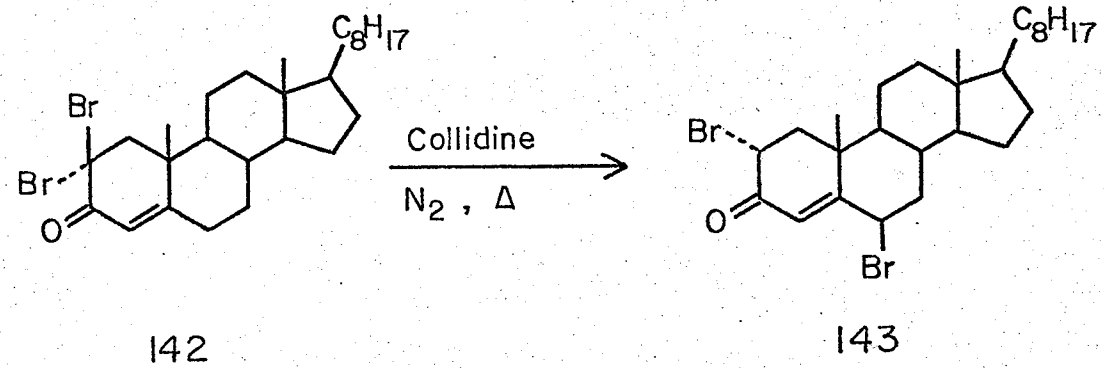
Elimination of hydrogen bromide from the tetrabromoketone (136) led to 2,2,4-tribromo-4-en-3-one (137) from which the C-2 axial bromine atom rearranged to the allylic C-6 position to give 2α,4,6β-tribromo-4-en-3-one (138). Rearrangement of C-2 axial bromine atom (142) to the allylic C-6 (143) position has been shown to occur (Scheme 34) [64].

The numbers of bromines atom present in the bromoacetates (72b, 73b, 132 and 133) were further confirmed by C, H, Br, and mass spectrometric analysis. The isomeric 2α,4α-dibromoacetates (72b and 73b) showed a weak molecular ion at m/e 532, 534, 536 and prominant peaks at m/e 472, 474, 476; 393, 395; and 333, 335 corresponded to the elimination of acetic acid and bromine from the molecular ion (Scheme 35).

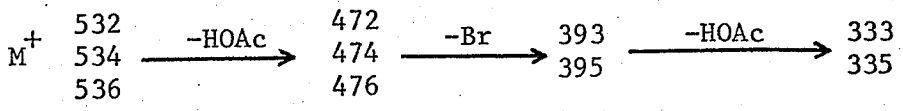
Scheme 33



Scheme 34

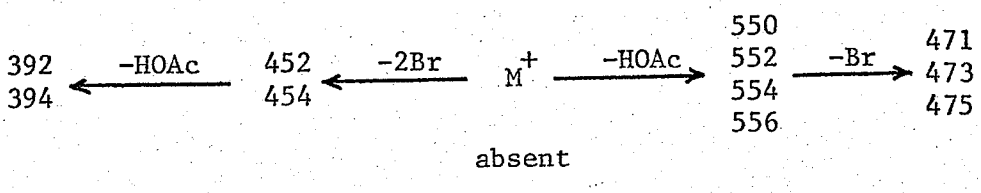


Scheme 35



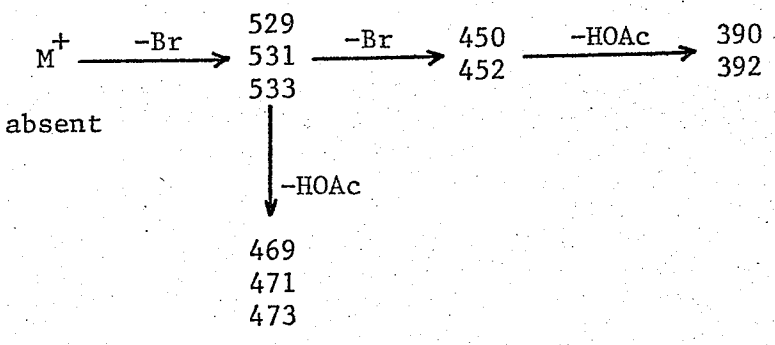
The mass spectrum of the 2,2,4α-tribromo-5α-androstane-3β,17β-diol diacetate (132) contained no molecular ion, the ion of highest mass arose from elimination of acetic acid from the molecular ion. It was noted that elimination of bromine from the molecular ion was also a primary process in this molecule, other major fragments are shown in the following scheme.

Scheme 36



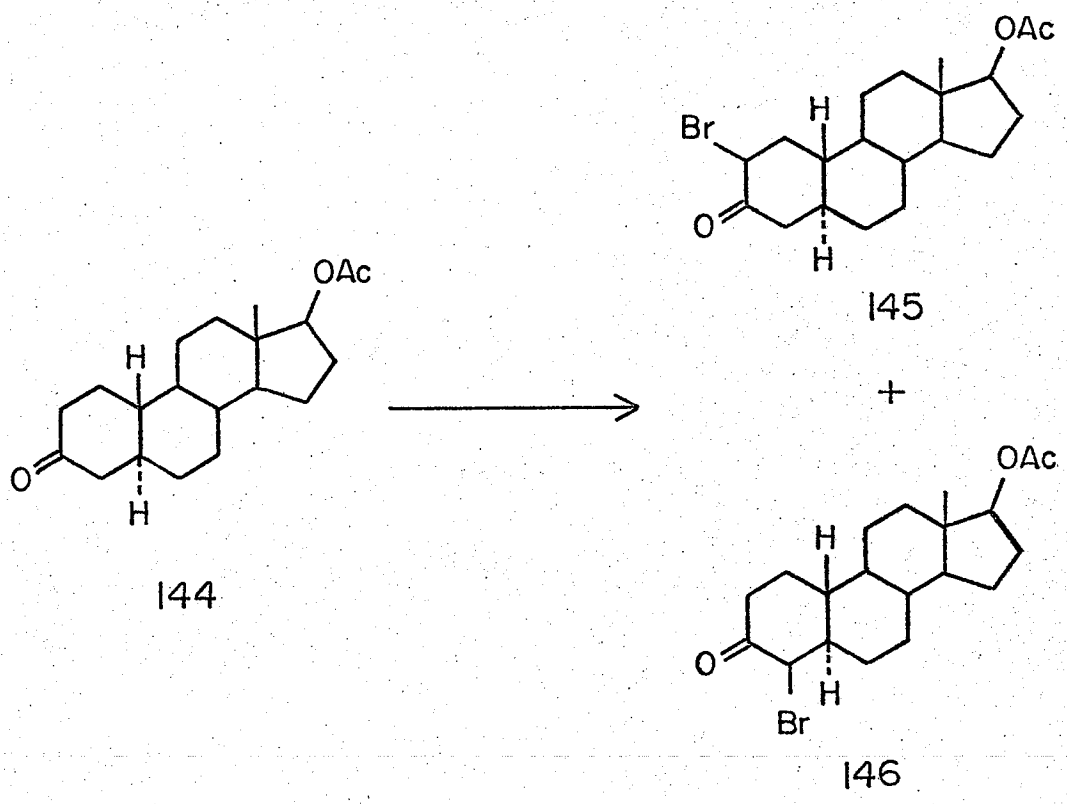
The molecular ion of the unsaturated tribromoacetate (133) was not observed in its mass spectrum and elimination of bromine became the sole primary fragmentation process which is consistent with the presence of an allylic bromine atom in the molecule (Scheme 37).

Scheme 37



In the 19-norsteroid-3-ketone, the stereochemical outcome of the bromination was complicated by the removal of the 19-methyl group which led to partial formation of the Δ^3-enol [58] (Table V). It has been shown [65] that kinetic controlled monobromination of 17β-acetoxy-5α-estran-3-one (144) led to a mixture of products which was composed of 63% 2β-bromo- and 37% 4β-bromo-3-ketone (145 and 146) derived from rotatary dispersion measurements (Scheme 38).

Scheme 38



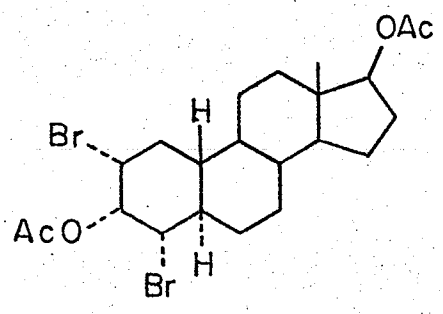
Since the steric balance on the α- and β-faces is restored in the 19-norsteroid, the predicted preference for 2β-attack is seen.

Dibromination of 17β-hydroxy-5α-estran-3-one (99) led to a mixture of dibromoketones (105, 106, and 107) (see page 33) which was reduced with sodium borohydride followed by acetylation. The resulting dibromoacetates were chromatographed on neutral alumina, elution with 10% benzene/petroleum ether gave a mixture of two dibromoacetates which were recrystallized from ether to afford two analytically pure compounds. P.m.r. spectrum of compound 147 (Fig. V) showed a triplet at 5.71 δ ($J_{2\beta,3\beta} = J_{3\beta,4\beta} = 2.5$ Hz) due to the C-3 equatorial proton; an octet at 4.10 δ ($J_{1\alpha,2\beta} = 12.5$; $J_{1\beta,2\beta} = 4.5$; $J_{2\beta,3\beta} = 2.5$ Hz) due to the C-2 axial proton, and a quartet at 3.87 δ ($J_{4\beta,5\alpha} = 11.5$; $J_{3\beta,4\beta} = 2.5$ Hz) due to the C-4 axial proton. These proton signals were similar in pattern to those in Fig. I and was consistent with the structure of 2α,4α-dibromo-5α-estrane-3α,17β-diol diacetate (147). Compound 148 showed a quartet centered at 4.78 δ ($J_{2\beta,3\alpha} = 10.8$; $J_{3\alpha,4\alpha} = 3.3$ Hz) for the C-3 axial proton, another quartet at 4.47 δ ($J_{3\alpha,4\alpha} = 3.5$; $J_{4\alpha,5\alpha} = 1.9$ Hz) for the C-4 equatorial proton (Fig. VI). The multiplet centered at 4.39 δ ($J_{1\alpha,2\beta} = 11.6$; $J_{2\beta,3\alpha} = 10.4$; $J_{1\beta,2\beta} = 5.0$ Hz) which partly overlaps with the 4α-proton signal was similar in pattern to the 2β-proton in Fig. II and was consistent with the C-2 axial proton in 2α,4β-dibromo-5α-estrane-3β,17β-diol diacetate (148).

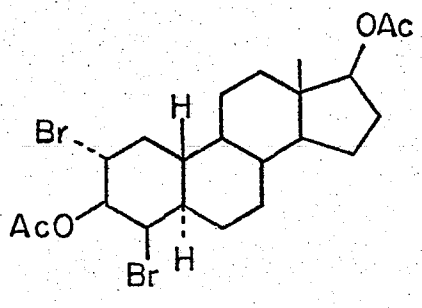
Elution with 50% benzene/petroleum ether gave 2β,4α-dibromo-5α-estrane-3β,17β-diol diacetate (149) which showed (Fig. VII) a quartet at 4.81 δ ($J_{3\alpha,4\beta} = 10.0$; $J_{2\alpha,3\alpha} = 3.8$ Hz) as the 3α-proton in Fig. VI, due to the C-3 axial proton; an unresolved multiplet at 4.66 δ ($W_{\frac{1}{2}} = 10.0$ Hz) [50] which overlaps with the 17α-proton signal, due to the C-2 equatorial proton. A triplet at 4.09 δ ($J_{3\alpha,4\beta} = J_{4\beta,5\alpha} = 10.5$ Hz) cor-

responded to the C-4 axial proton. The isolation of these dibromoacetates (147, 148, and 149) confirmed the previous suggestion (see page 33) that dibromination of 17β-hydroxy-5α-estran-3-one (99) led to the 2α,4α-dibromo, 2α,4β-dibromo, and 2β,4α-dibromoketones (105, 106, and 107, respectively).

17β-Hydroxy-5α-estran-3-one (99) was treated with three molar equivalents of phenyltrimethylammonium perbromide, the brominated product was reduced with sodium borohydride. Acetylation of the tribromoalcohol followed by chromatography on neutral alumina gave only one tribromoacetate, no other bromoacetates were detected in the mother liquor by p.m.r. determination. The tribromoacetate (150) showed a doublet at 5.30 δ ($J_{3\alpha,4\beta}$ = 10.0 Hz) due to the C-3 axial proton and a triplet at 3.91 δ ($J_{3\alpha,4\beta} = J_{4\beta,5\alpha}$ = 10.5 Hz) due to the C-4 axial proton, in the p.m.r. spectrum (Fig. VIII). This was consistent with the formation of 2,2,4α-tribromo-5α-estrane-3β,17β-diol diacetate (150) as compared to the p.m.r. spectrum of 132 (Fig. III).



147



148

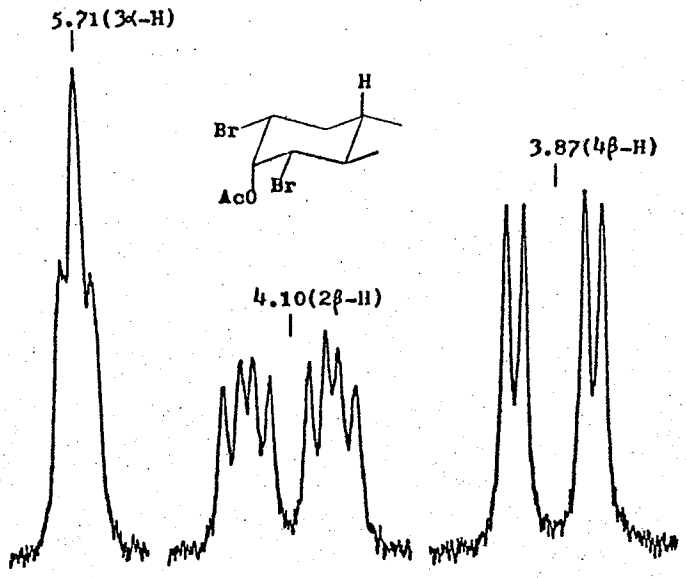


Figure V. 220 MHz p.m.r. spectrum of 2α,4α-dibromo-5α-estrane-3α,17β-diol diacetate (147).

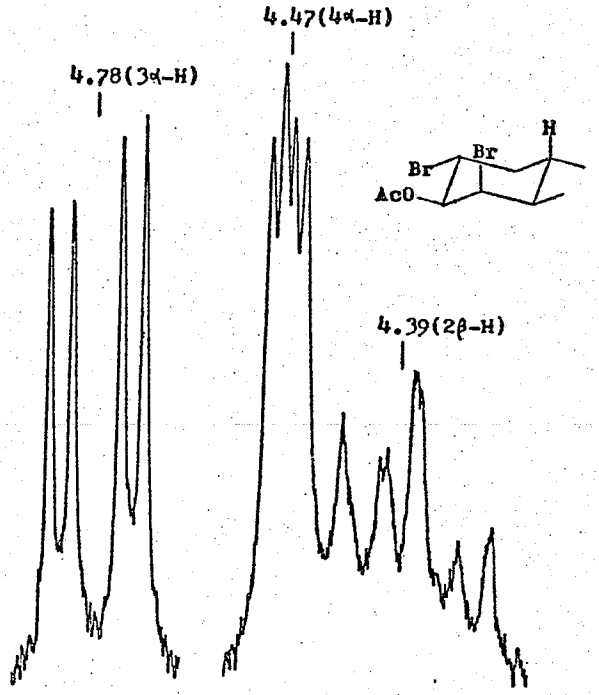


Figure VI. 220 MHz p.m.r. spectrum of 2α,4β-dibromo-5α-estrane-3β,17β-diol diacetate (148).

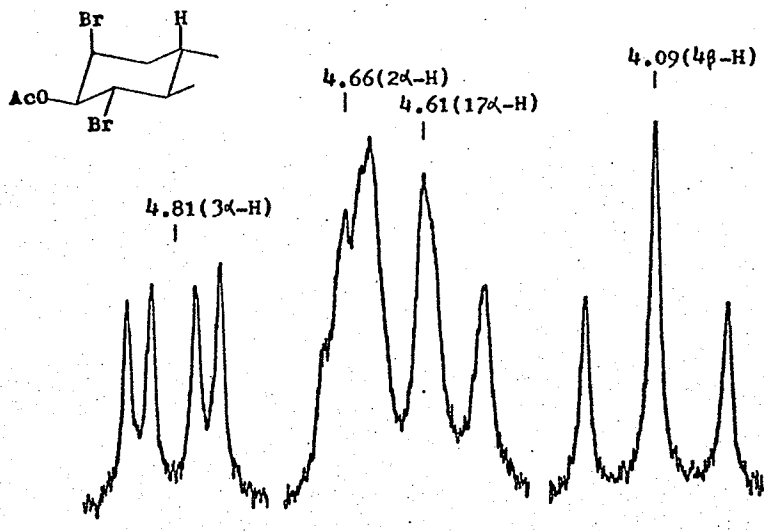


Figure VII. 220 MHz p.m.r. spectrum of 2β,4α-dibromo-5α-estrane-3β,17β-diol diacetate (149).

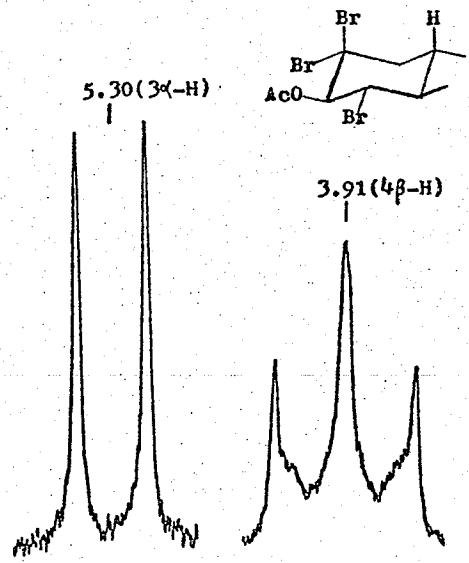
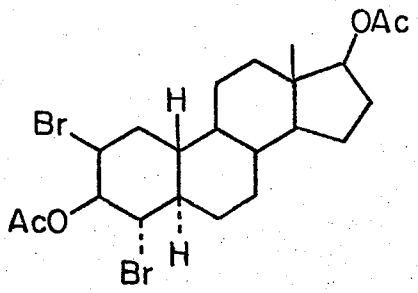
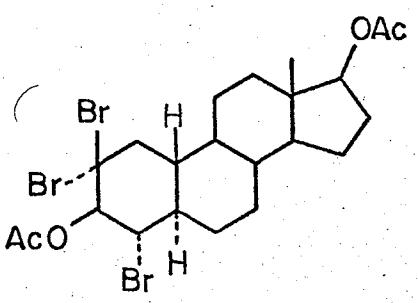


Figure VIII. 220 MHz p.m.r. spectrum of 2,2,4α-tribromo-5α-estrane-3β,17β-diol diacetate (150).



149



150

The molecular composition of the dibromo and tribromoacetates (147, 148, 149, and 150) was also determined by C, H, and Br as well as mass spectrometric analysis. The mass spectra of the dibromoacetates (147, 148, and 149) contained no molecular ion, elimination of acetic acid from the molecular ion was the primary fragmentation process (Scheme 39).

Scheme 39

$$M^{+} \xrightarrow{-HOAc} \begin{matrix}458\\460\\462\end{matrix} \xrightarrow{-Br} \begin{matrix}379\\381\end{matrix} \xrightarrow{-HOAc} \begin{matrix}319\\321\end{matrix} \xrightarrow{-Br} 240$$

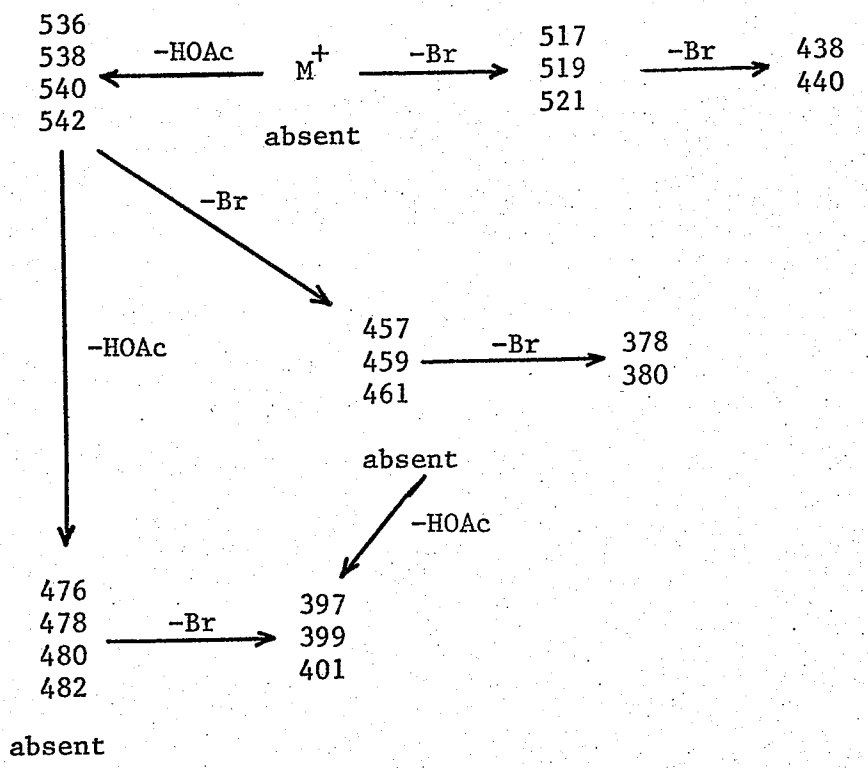
absent

In the mass spectrum of the tribromoacetate (150), the fragment ion of highest mass arose from elimination of acetic acid from the molecular ion; elimination of bromine also occurred (Scheme 40).

It is apparent that in the 19-norsteroid the C-2 or C-4 axial bromination is possible since the 1,3-diaxial interaction between the bromine and 19-methyl substituent has been removed. The three 19-nor dibromoacetates (147, 148, and 149) possess the axial-equatorial rela-

tionship between the bromine and C-3 oxygen function similar to the 2α,4α-dibromo-5α-androstane-3α,17β-diol diacetate (72b), this would be expected to increase olefins formation and accounts for the absence of the 3α-cyclopropanol.

Scheme 40

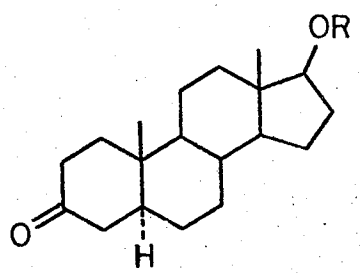


d. Addition of Simmons-Smith reagent to enol derivatives

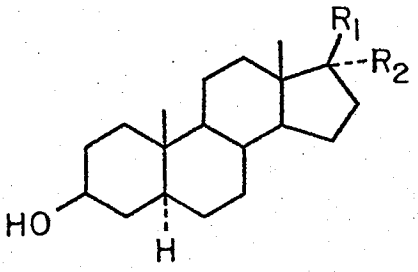
Addition of methylene to an oxygen substituted enolic double bond is a direct way of forming tertiary cyclopropanol derivatives (see page 5) as well as a method of preparation, possibly stereoselectively, of α-methyl substituted carbonyl compounds [66]. However, because of the acid and base sensitivity of cyclopropanols, it is not always possible to obtain cyclopropanol from the corresponding enol derivative. The ease of removal of the derivative is therefore of critical importance.

5α-Androst-2-ene-3,17β-diol diacetate (153a) [67] and 5α-androst-16-ene-3β,17-diol diacetate (23b) [68] were prepared by enol acetylation of 17β-hydroxy-5α-androstan-3-one (151a) and 3β-hydroxy-5α-androstan-17-one (152a), respectively. Addition of methylene to the Δ^2-enol acetate (153a) was not observed under the usual conditions of refluxing ether, starting material being recovered in high yield. At higher temperatures a complex mixture of unidentified products was obtained. However, as has been reported [9], addition of methylene to the Δ^{16}-enol acetate (23b) does occur to give 16α,17-cyclopropano-5α-androstane-3β,17β-diol diacetate (24b). These results are consistent with the greater reactivity of 5-membered over 6-membered ring olefin reported by Rickborn and Chan [69].

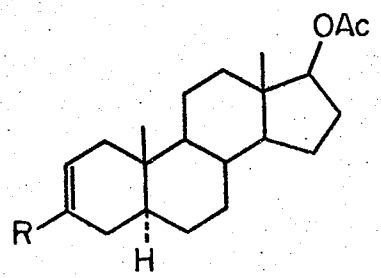
It is known that the Simmons-Smith reagent reacts with enol ethers more readily than with the analogous double bond [70], but to be less reactive towards enol acetates [10,71], due to the electrophilic nature of the reagent. Ketalization of 17β-acetoxy-5α-androstan-3-one (151b) in dry methanol catalyzed by p-toluenesulfonic acid gave the known 3,3-dimethoxy-5α-androstane-17β-yl acetate (154a) [72], which, by pyrolysis at reduced pressure, led to a high yield of the 3-methoxy-5α-androst-2-ene-17β-yl acetate (153b) [73]. Addition of methylene occurred to the double bond of 153b on treatment with the Simmons-Smith reagent as shown by the presence of high field proton resonance (0.08 δ) characteristic of cyclopropyl hydrogens in the p.m.r. spectrum; the absence of the enolic double bond in the i.r. spectrum and an increase in the molecular ion of 14 a.m.u. in the mass spectrum. The iodomethylzinc iodide reagent has been shown to be sensitive to steric effects [74], therefore, as in the analogous addition of methylene to Δ^2 steroid olefins



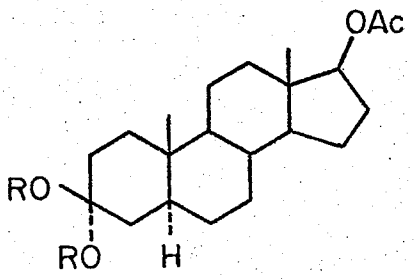
151a, R = H
b, R = Ac



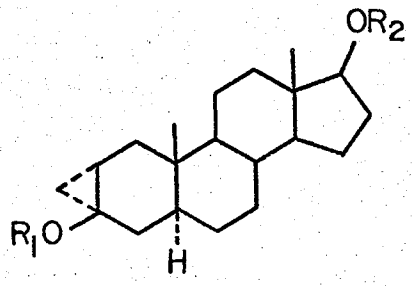
152a, R_1R_2 = O
b, R_1 = OAc, R_2 = H



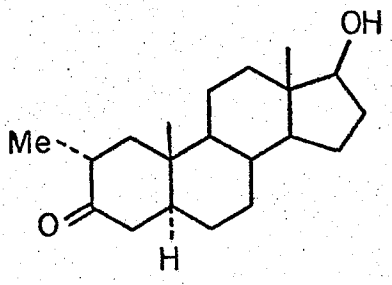
153a, R = OAc
b, R = OMe
c, R = CH_2OAc
d, R = OCH_2CH_2Cl



154a, R = Me
b, R = CH_2CH_2Cl



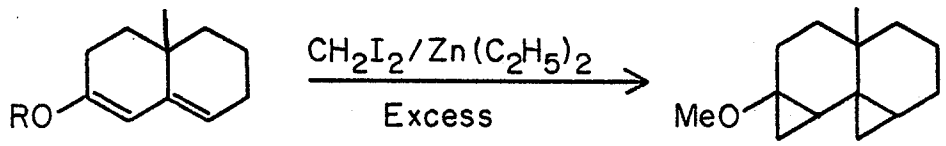
155a, R_1 = Me, R_2 = Ac
b, R_1 = Me, R_2 = H
c, $R_1 = R_2$ = Ac
d, $R_1 = CH_2CH_2Cl$, R_2 = Ac
e, $R_1 = R_2$ = H



156

[75], the presence of the 19-methyl substituent would be expected to hinder addition from the β-face [54c] of the steroid molecule to give 3β-methoxy-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155a). This result is similar to the addition of methylene to 2-methoxy-3,4,4a,5,6,7-hexahydro-4a-methylnaphthalene (]57a) (Scheme 4]) [76]. 2-Acetoxy-3,4,4a,5,6,7-hexahydro-4a-methylnaphthalene (]57b) was recovered unchanged under the same reaction conditions in which the methyl dienol ether derivative (157a) gave the corresponding cyclopropano-derivative (158).

Scheme 41



157a , R = Me
b , R = Ac

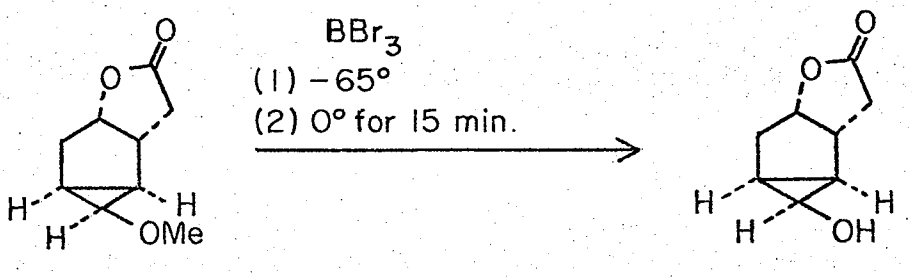
158

Acidic hydrolysis of the cyclopropyl ether (155a) at room temperature led to 3β-methoxy-2α,3-cyclopropano-5α-androstan-17β-ol (155b) which showed free and bonded O-H stretching vibration at 3623 and 3480 cm^{-1}, respectively, and cyclopropyl C-H stretching vibration at 3067 cm^{-1} in the i.r. spectrum. High field proton resonance (-0.02 δ) was also observed in the p.m.r. spectrum indicating that the 3-membered ring remained intact. Treatment of 155a with dilute acid in refluxing methanol gave 17β-hydroxy-2α-methyl-5α-androstan-3-one (156) [77] which was identical with an authentic sample by mixed melting point and i.r. spectrum. Molecular rotation differences have been shown to be simi-

lar for corresponding epoxide and cyclopropane derivatives [78a], however, the difference between 17β-hydroxy-2α,3α-epoxy-5α-androstane ($|\alpha|_D$ +40°) and the 2β,3β-isomer ($|\alpha|_D$ +51°) [78b] is not sufficient to distinguish the stereochemistry of the three-membered ring at C-2/C-3.

Attempts to prepare the alcohol by oxidation of the methoxy group in 155a with chromic anhydride in acetic acid [79] gave either unidentified water soluble acidic substances or starting material depending on the reaction conditions. No evidence of formate formation was observed. Although a cyclopropylmethyl ether (159) has been successfully converted to the cyclopropanol (160) by low temperature reaction with a solution of boron tribromide (3 equivalents) in dichloromethane (Scheme 42) [80], with boron trifluoride etherate in acetic anhydride [81], 155a was converted into 3-acetoxymethyl-5α-androst-2-ene-17β-yl acetate (153c). The expected 2α,3-cyclopropano-5α-androstane-3β,17β-diol diacetate (155c) was not obtained, although the mass spectra of both 153c and 155c provided the isomeric molecular ion at m/e 388. Compound 153c showed ester carbonyl stretching vibration at 1745 and 1738 cm^{-1} in the i.r. spectrum. The p.m.r. spectrum showed the presence of a vinylic proton at 5.50 δ and a singlet at 4.35 δ due to the methylene protons of the C-3 substituent consistent with the structure of 153c.

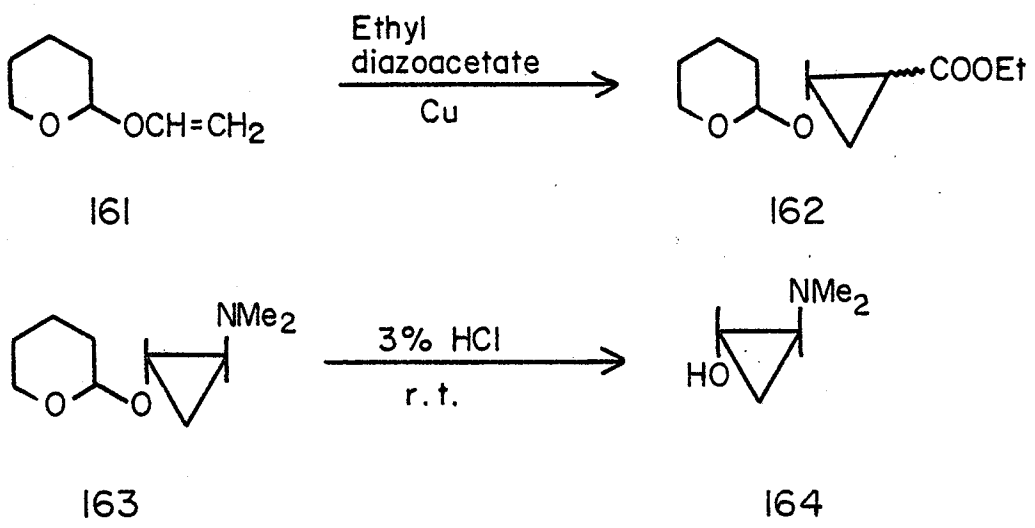
Scheme 42



159 160

The 2-tetrahydropyranyl moiety which is stable in neutral and basic media, and is easily cleaved under very mild acidic conditions [82], has been used in the cyclopropanation of 2-vinyloxytetrahydropyran (161) (Scheme 43) [83]. The tetrahydropyranyl group was removed from 163 under mild conditions to afford a quantitative yield of the trans-2-dimethylaminocyclopropanol (164) (Scheme 43) [83].

Scheme 43



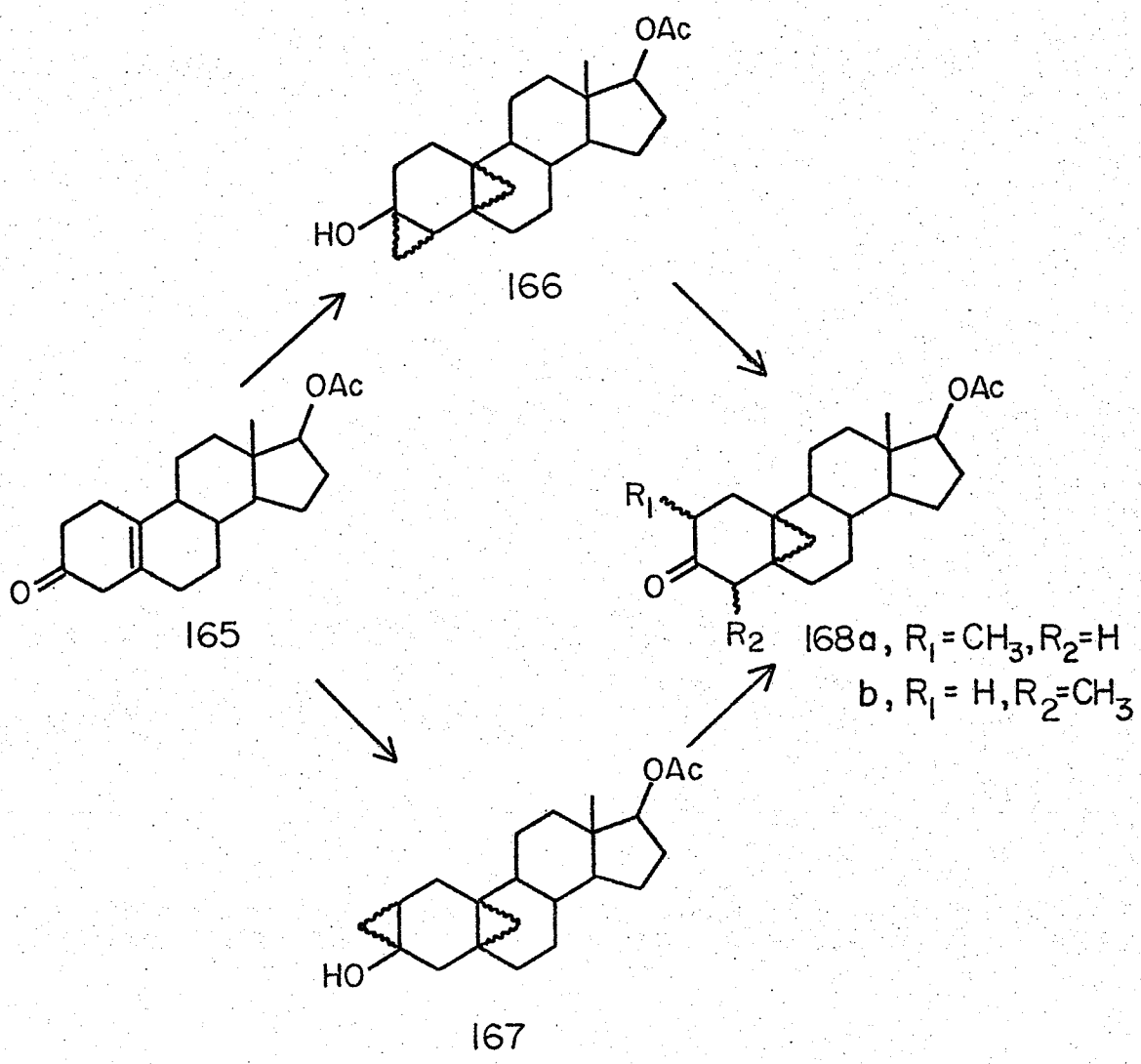
161 162 163 164

However, attempts to obtain the 3-pyranylenol ether of 17β-hydroxy-5α-androstan-3-one (151a) under various reaction conditions were unsuccessful.

It has been shown by Harrison et al.[84] that treatment of the non-hydroxylic ketone (165) with the Simmons-Smith reagent gave 17β-acetoxy-2ξ-methyl-5,19ξ-cycloandrostan-3-one (168a) and the isomer 168b (Scheme 44). These α-methyl ketones were thought to originate from the unstable cyclopropanol intermediates (166 and 167) formed by methylenation of enol intermediates in the reaction. Treatment of 17β-ace-

toxy-5α-androstan-3-one (151b) with sodium hydride in ether under a nitrogen atmosphere followed by the Simmons-Smith reagent gave no cyclopropanol or methyl ketone formation. 5α-Androstane-3β,17β-diol 17-acetate (152b) was isolated from the product and further oxidized with Jones' reagent [43] to the 17β-acetoxy-5α-androstan-3-one (151b) identified by mixed melting point and i.r. spectrum with an authentic sample.

Scheme 44



The 2-chloroethyl group which was introduced by Schöllkopf [22] in the preparation of a secondary cyclopropanol (see page 11) was found useful in the formation of a tertiary cyclopropanol in the steroid. 3,3-Bis(2-chloroethoxy)-5α-androstane-17β-yl acetate (154b) was prepared by azeotropic removal of water from 17β-acetoxy-5α-androstan-3-one (151b) in an acidic benzene solution [54d]. A multiplet centered at 3.54 δ integrated for eight protons in the p.m.r. spectrum of 154b and was assigned to the ethylene hydrogens of the two 2-chloroethoxy substituents. The mass spectrum which contained the molecular ion at m/e 474, 476, and 478, and the C, H, and Cl analysis indicated the presence of two chlorine atoms in 154b. Pyrolysis [73] of 154b led to 3(2-chloroethoxy)-5α-androst-2-ene-17β-yl acetate (153d) which showed enolic C=C stretching vibration at 1675 cm^{-1} in the i.r. spectrum and vinylic proton at 4.44 δ overlapped with the 17α-proton signal in the p.m.r. spectrum. A multiplet at 3.68 δ integrated for four protons corresponded to the ethylene hydrogens of the remaining 2-chloroethoxy substituent. Treatment of the 3(2-chloroethoxy)enol ether (153d) repeatedly with an excess of Simmons-Smith reagent (three times with ten molar excess) gave 3β(2-chloroethoxy)-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155d) after three days of reflux in ether. The reaction product was chromatographed over neutral alumina. Elution with petroleum ether gave a major fraction which on crystallization from methanol afforded an analytically pure sample. The i.r. spectrum showed cyclopropyl C-H stretching vibration at 3075 cm^{-1} and no enolic C=C stretching band was observed; the p.m.r. spectrum showed characteristic high field cyclopropyl proton resonance at 0.06 δ, consistent with

the structure of 155d.

The 3β(2-chloroethoxy)-cyclopropyl ether (155d) was converted into the 2α,3-cyclopropano-5α-androstane-3β,17β-diol (155e) with n-butyllithium using the method of Schöllkopf [22]. On crystallization from chloroform retention of one-half mole of solvent per mole of 155e was shown by elemental analysis. Similarly from methanol, a product containing one-half mole of water per mole of 155e was obtained. These solvates could not be decomposed by drying at room temperature under reduced pressure. The analytical sample of 155e showed only one substance on t.l.c. when developed in four different solvent systems, i.e., 25% ethyl acetate/petroleum ether; 50% ether/benzene; 5% acetone/chloroform; and 5% methanol/carbon tetrachloride. This compound was heat labile, as shown by t.l.c. The melt gave two spots corresponding to the starting material and 17β-hydroxy-2α-methyl-5α-androstan-3-one (156). Similarly, only one peak corresponding to 17β-hydroxy-2α-methyl-5α-androstan-3-one (156) was observed in the gas-liquid chromatogram. Compound 155e showed strong hydroxylic absorption at 3400 cm^{-1} and cyclopropyl C-H stretching vibration at 3090 cm^{-1} in the i.r. spectrum and no carbonyl absorption, heating the disc at 180° gave a diminished 3400 cm^{-1} band and a new absorption at 1725 cm^{-1} due to carbonyl stretching vibration. The p.m.r. spectrum showed high field cyclopropyl protons at 0.32 δ. The mass spectrum of a sample crystallized from methanol contained the ion of highest mass at m/e 304 consistent with the molecular formula of 155e; the sample when crystallized from chloroform showed the same major peaks, and in addition, three strong signals at m/e 82, 84, and 86 corresponded to

CCl_2^+ arisen from chloroform.

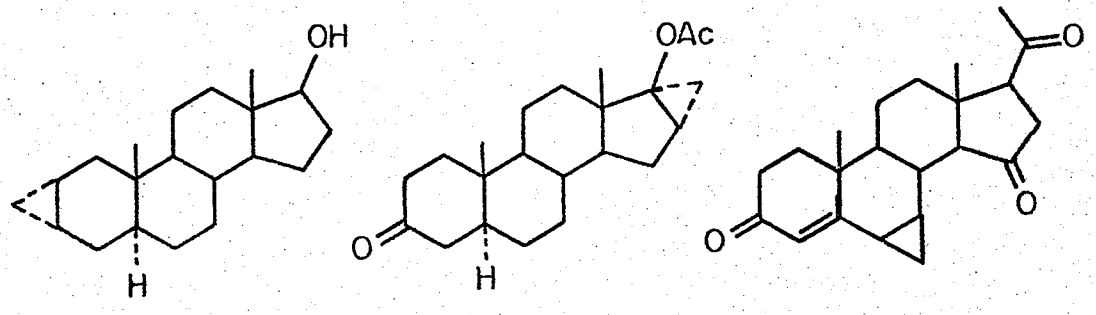
Treatment of the hemihydrate of 155e with either dilute acid or base gave 17β-hydroxy-2α-methyl-5α-androstan-3-one (156) as shown by comparison of the i.r., p.m.r., and mass spectrum and mixed melting point with an authentic sample. Acetylation of 155e with acetic anhydride in pyridine yielded 2α,3-cyclopropano-5α-androstane-3β,17β-diol diacetate (155c) which showed cyclopropyl C-H and ester C=O stretching vibration at 3090 and 1735 cm^{-1}, respectively, in the i.r. spectrum. The p.m.r. spectrum showed two acetoxy methyl protons signals at 2.02 and 1.95 δ, and cyclopropyl protons at 0.50 δ. The molecular composition of 155c was further confirmed by mass spectrometric and elemental analysis.

e. Biological activity

It is well established that the attachment of the steroid to specific cytoplasmic receptors is a prerequisite for subsequent biological effects [85a,86,87]. The interaction between the steroid and the molecules of the biological object is determined by the physicochemical properties of the steroid which in turn closely relate to the structure of the steroid [85b,88]. In the past, many structurally different steroids have been synthesized and structure-activity relationships have been extensively investigated [89,90,91] in order to draw insight into basic structural requirements for certain types of activity. Some preliminary generalizations concerning the relationship of chemical structure to biological activity of steroids have been made [89-91].

Derivatives of cyclopropane have been found in natural products and synthetic compounds of biological interest [92]. Steroids contain-

ing the cyclopropane structure have been synthesized in attempts to alter their biological properties, For example, 2α,3α-methano-5α-androstan-17β-ol (169) [75] is as active as testosterone propionate in the anabolic test and about one-third as active in the androgenic test; 17β-acetoxy-3'H-cycloprop|16α,17|-5α-androstan-3-one (170) [9,93] has been shown to have a high level of oral androgenic activity; and 15-keto-6β,7β-methyleneprogesterone (171) [94] has been found to possess antimineralocorticoid activity when given subcutaneously, but was inactive orally. These examples show that introduction of a cyclopropane ring into the steroid structure can have a considerable effect on biological activity.



169 170 171

In physical and chemical properties cyclopropane bonds differ markedly from sp^3 σ-bond and are more similar to sp^2 π-bond (Table VII) [95]. Therefore, they may be expected to interact through van der Waals forces with a protein receptor in an analogous way to π-bond.

Burger et al.[96], in studies of the effect of structure on a series of 2-substituted cyclopropylamines upon monoamine oxidase (MAO)-inhibitory activity, have shown that the cyclopropane ring is an essential structural requirement for potent and prolonged MAO-inhibition

Table VII. Some C-H bond characeteristics [95]

C-H Property	C-H(sp^2)	C-H(Δ)	C-H(sp^3)
H-C-H bond angle (degrees)	120	118	109.5
C-H bond length (Angstron)	1.086	1.093	1.102
C-H force constant ($x10^5$ dynes/cm)	5.1	5.5	4.79
i.r. C-H frequency (cm^{-1})	3040	3009	2853
% s character	33	33	25
protonation	yes	yes	no

in vivo and they suggested that the cyclopropane ring may serve as a point of attachment to the enzyme, its high electron density possibly providing a focus for bonding. Belleau and Moran [97] have also postulated that the electronic and steric properties of the cyclopropane system play a highly specific role in the binding of *trans*-2-phenylcyclopropylamine, which is a potent MAO-inhibitor and a clinically useful antidepressant agent. Wolff *et al.*[75] have similarly proposed that the 2α,3α-cyclopropane substituent functions by the formation of a π-complex with the receptor site to account for the androgenic activity of the 2α,3α-methano-5α-androstane derivatives. This proposal has recently been used as a rationalization for the synthesis of some 6β,7β-methyleneprogesterone derivatives [94].

The cyclosteroid derivatives (75a, 75b, 75d, 76a, 76b, and 76d) were assayed [98] in the immature castrate male rat (21 days of age) by daily injection (subcutaneous) for 7 days. Autopsy was performed on the day following the last day of administration. Androgenic and anabolic activities of the steroids were calculated on the basis of the weights of the seminal vesicles, ventral prostate and levator ani muscles [45] relative to testosterone as the standard. The preliminary biological data was summarized in Table VIII. Among these compounds, the 3α-cyclopropanol (75a) appears to inhibit the growth of the seminal vesicles and ventral prostate suggesting an antiandrogenic activity.

Natural steroid hormones and most biologically active synthetic derivatives possess an oxygen function at C-3 of the A-ring. The possibility of *in vivo* conversion of steroids without a C-3 oxygen into the C-3 oxygenated compounds [99] led to the synthesis (see above) of

Table VIII. Anabolic/androgenic assays of 2β,4β-cyclo-5α-androstane-17β-yl acetate derivatives

Treatment (Total dose)	mg	Ventral prostate mg	Seminal vesicles mg	Levator-ani mg	Ventral prostate %	Seminal vesicles %	Levator-ani %	Anab./Andro.
Controls (CMC Solution)		24.0 ±0.65	15.1 ±0.06	31.1 ±1.30	-	-	-	-
Standard (Testosterone)	3.0	189.6 ±1.77	111.7 ±4.72	79.9 ±3.57	100.0	100.0	100.0	-
3α-OH (75a)	3.0	18.8 ±1.55	12.9 ±1.50	30.0 ±1.27	Antiandrogenic			
3β-OH (76a)	3.0	29.7 ±1.46	15.4 ±0.48	38.5 ±2.95	3.4	0.3	15.2	8.0
3α-OAc (75b)	3.0	29.9 ±2.46	16.6 ±0.77	35.4 ±1.61	3.5	1.5	8.8	3.5
3β-OAc (76b)	3.0	35.5 ±2.41	18.4 ±0.84	37.4 ±1.99	6.9	3.4	12.9	2.5
3α-Suc (75d)	3.0	23.4 ±1.57	16.2 ±0.71	27.4 ±1.53	-	1.1	-	-
3β-Suc (76d)	3.0	19.0 ±1.27	16.1 ±0.51	29.3 ±1.34	-	-	-	-

CMC = carboxymethylcellulose

Weight: Initial 60-62 g

Final 90-101 g

2α,3-cyclopropano-5α-androstane-3β,17β-diol (155e) which was thought to be a potential metabolic product of the anabolically and androgenically active 2α,3α-methano-5α-androstan-17β-ol (169) [75]. However, 155e was inactive in both the anabolic and androgenic tests (Table IX). It has also been shown in this laboratory [100,101] that no C-3 oxygenated product could be detected among the neutral urinary metabolites of 169 in the rabbit.

A search for compounds with antiandrogenic properties has been prompted by clinical considerations. The necessity for treatment of endocrinological disorders such as hormone-dependent carcinomas, hirsutism in women and male pattern baldness has long been appreciated. The most extensively studied antiandrogens are 17α-acetoxy-6-chloro-1α,2α-methylene-4,6-pregnadiene-3,20-dione (cyproterone acetate) (172) [102] and 6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one (BOMT) (173) [103]. Some other related structures of testosterone and progesterone showing antiandrogenic activity include 17α-methyl-B-nortestosterone (SK & F7690) (174) [104]; A-nortestosterone (175) [105]; C-nor-D-homo-androst-4-ene-17α-yl acetate (176) [105]; A-norprogesterone (177) [106]; and 17α-acetoxy-6-chloro-4,6-pregnadiene-3,20-dione (chlormadinone acetate) (CMA) (178) [107]. 2-(1-Ethinyl-1-hydroxyethyl)-7-oxa-dodecahydrophenanthrene (RO 2-7239) (179) [108] and 4'-nitro-3'-trifluoromethyl-isobutyrlanilide (SCH 13521) (180) [109] are nonsteroid antiandrogens.

Wiechert et al.[110] have investigated the structure-antiandrogenic activity of a series of steroids structurally related to cyproterone acetate (172) and showed that the most potent antiandrogens belong to

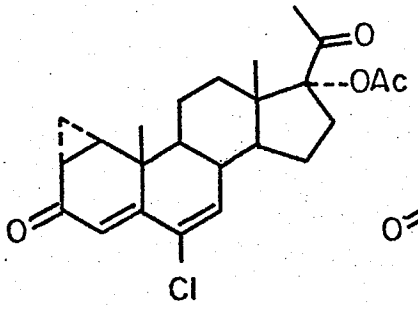
Table IX. Anabolic/androgenic assays of 2α,3-cyclopropano-5α-androstane-3β,17β-diol (155e)

Treatment (Total dose)	mg	Ventral prostate mg	Seminal vesicles mg	Levator-ani mg	Ventral prostate %	Seminal vesicles %	Levator-ani %	Anab./Andro.
Controls (CMC Solution)		15.6 ±0.74	10.2 ±0.69	25.1 ±1.70	-	-	-	-
Standard (Testosterone)	3.0	107.2 ±6.75	80.8 ±2.31	67.9 ±1.49	100.0	100.0	100.0	-
Test (155e)	3.0	27.6 ±0.79	15.4 ±0.61	33.8 ±2.64	13.1	7.4	20.3	2.0

CMC = carboxymethylcellulose

Wieght: Initial 54-55 g

Final 92-100g



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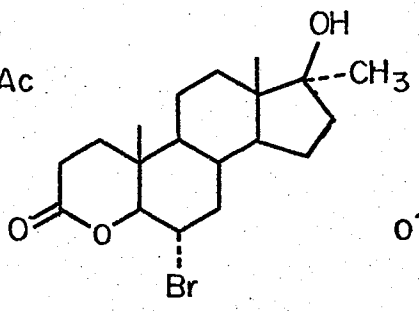

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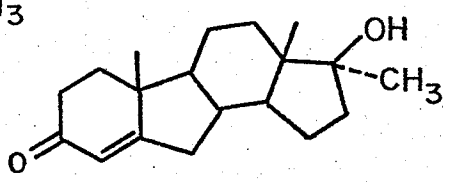

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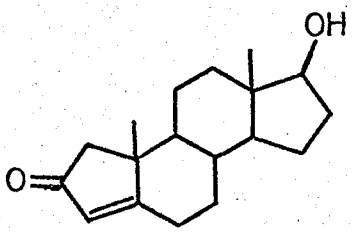

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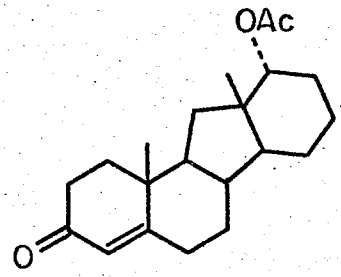

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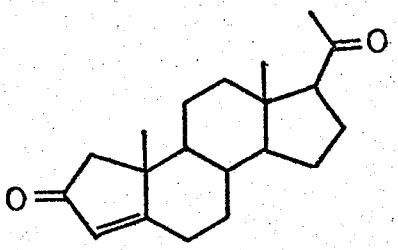

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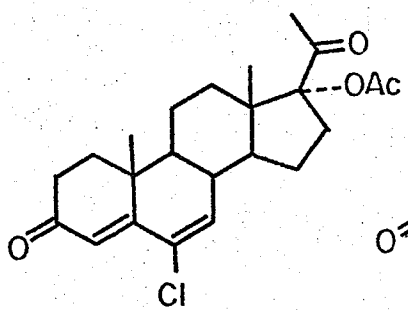

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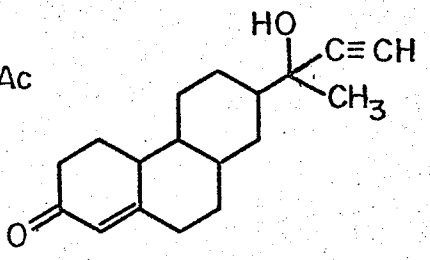

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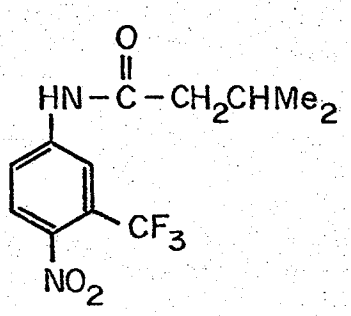
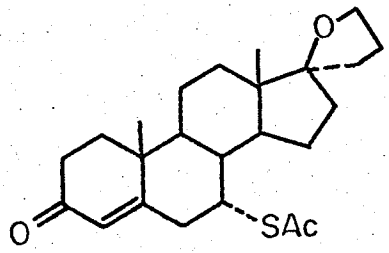


180

the group of 1α,2α-methylene steroids. More recently, Rasmusson et al.[111] showed that the antiandrogenic activity associated with the tetrahydrofuran-2'-spiro-17-androstenones (181 and 182) has been increased by introduction of a 1α,2α-methylene function and by incorporation of either a 6-chloro-Δ^6 or 6α,7α-difluoromethylene group to the basic steroid skeleton. However, the diversity of the structures of the currently available antiandrogenic steroids provide no sound theoretical background for generalization to be made for the structure-antiandrogenic activity relationship.



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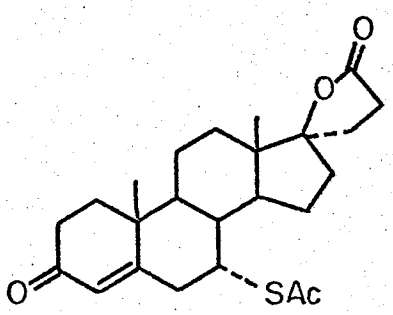
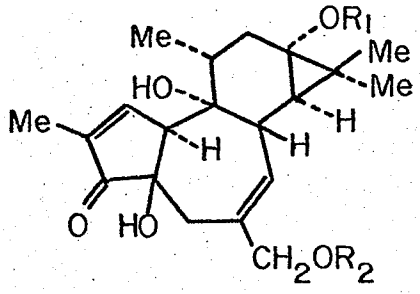


182

The tetracyclic terpene, phorbol (183), and its esters, is unique in that it is the only naturally occurring compound to contain a cyclopropanol ring system. Phorbol, a constituent of croton oil, has been shown to possess co-carcinogenic properties and some studies have been carried out to determine structure-activity relationships[112]. The gross similarity of the phorbol structure to the steroid molecule and the presence of the cyclopropanol ester prompted us to carry out skin irritancy tests on the cyclosteroid derivatives which we have prepared. (75 and 76). These substances were assayed[113] by the mouse ear test and showed no significant irritancy effect in doses up to 100 μg. As

a comparison, resiniferatoxin was found to produce a 50% response in mice at a dose level of 0.0009 µg. Intradermal injections on the shaved backs of anaesthetised guinea pigs did not produce vasodilation at a 100 µg dose level; histamine produced vasodilation on this model at doses of less than 1 µg.



183, R_1 = Fatty acid chain
R_2 = H, Ac

It is concluded that the co-carcinogenic properties of phorbol and its esters is not a direct reflection of the presence of the cyclopropanol moiety and that co-carcinogenicity is not a general property of the oxygenated three-membered ring. This is of significance considering the increasing presence of the cyclopropane ring system in synthetic potential drug molecules (see above for steroid examples) and the recent evidence for <u>in vivo</u> hydroxylation of the cyclopropane ring [100].

II. MASS SPECTRA OF SOME 5,6α-CYCLOPROPANO-5α-CHOLESTANE DERIVATIVES

A. Introduction

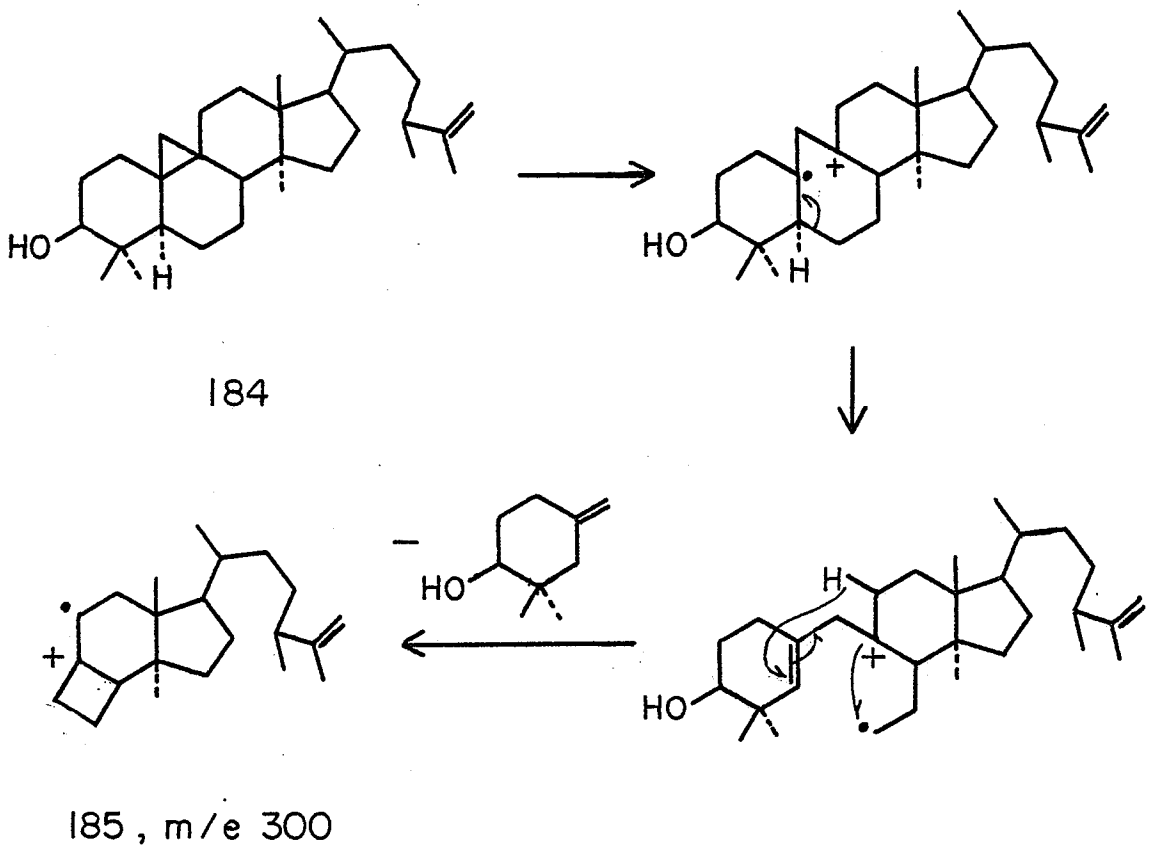
In the initial phase of this work 5,6α-cyclopropano-5α-cholestane derivatives were prepared as model substances for the synthesis of the corresponding estrane, androstane, and pregnane derivatives in order to determine the effect of replacement of the 5,6-unsaturated steroid double bond by a cyclopropane ring on the biological properties of these derivatives [75,114]. However, after preparation of the cholestane derivatives a series of reports appeared by Kohout, Fajkos, and Sorm [115] containing a thorough study of the chemistry of these substances.

The electron impact mass spectrometry of steroids has been extensively investigated [116,117]. However, only a few studies have been concerned with the mass spectra of cyclopropano-steroids [118]. The mass spectra of simple aliphatic [119] and functionalized cyclopropanes [120] have been reported, and recently, generalizations for interpreting the mass spectra of cyclopropanes have been made by Dias and Djerassi [121] based on the examination of many deuterium labelled aliphatic cyclopropanes. They have rationalized the decomposition paths of aliphatic cyclopropanes after electron impact by invoking preferential ionization of the most highly substituted cyclopropane bond in which the positive charge is located on the most substituted carbon atom.

The fragmentation induced by a cyclopropane ring has been observed in some naturally occurring triterpenes. The mass spectrum of cycloaudenol (184) [122] contains an intense fragment at m/e 300 (185) which has been shown by high resolution measurements to have the composition $C_{22}H_{36}$ and corresponds to the loss of ring A plus one hydrogen atom.

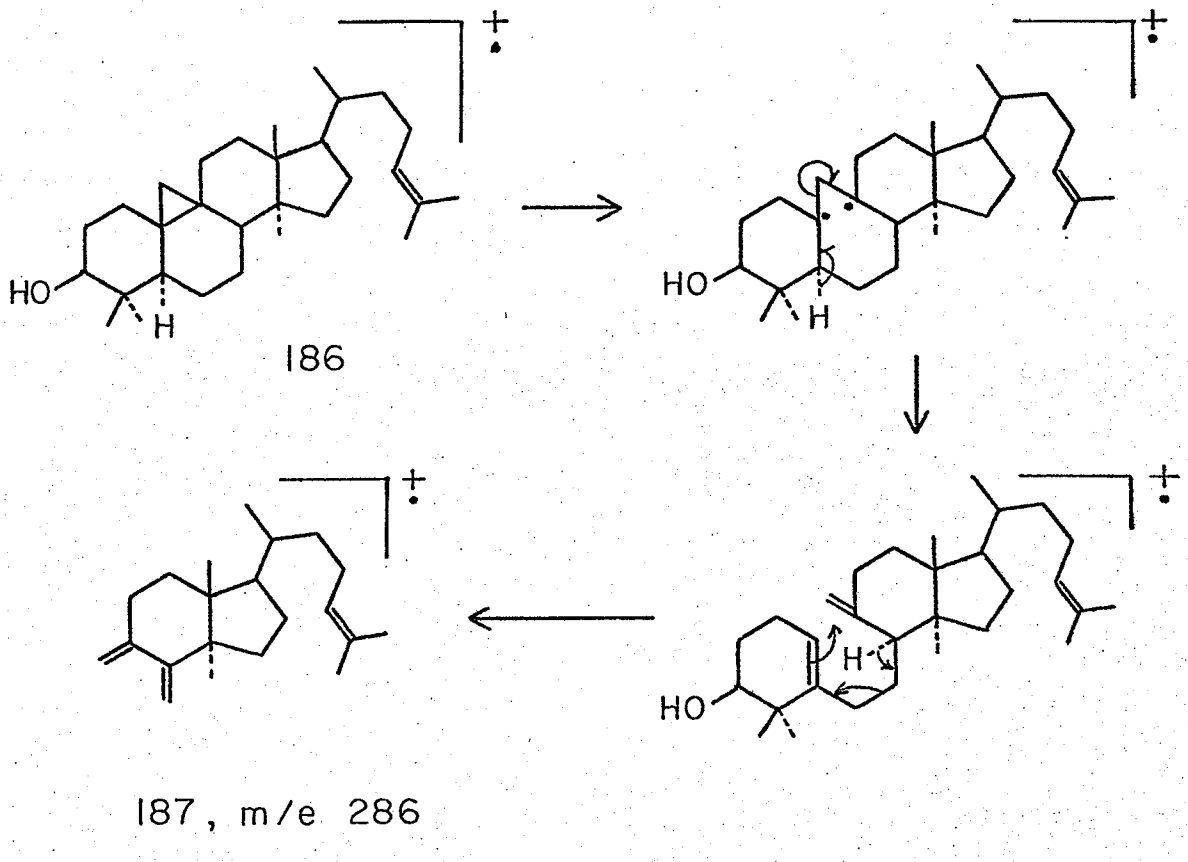
The appearance of an intense metastable peak at m/e 204.6 indicates that the fragment ion (185) is formed from the molecular ion m/e 440 (184) by a one step process which involves opening of the 9,10-bond followed by cleavage of the 5,6-bond and McLafferty rearrangement (Scheme 45).

Scheme 45



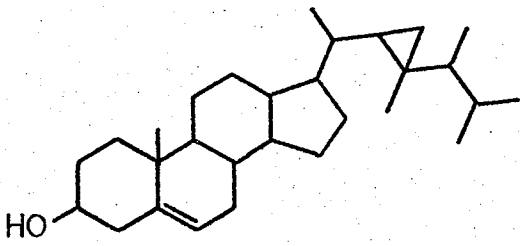
From cycloartenol (186) [123], the characteristic ion fragment at m/e 286 (187) has been proposed to originate from a rupture of the cyclopropane ring and its formation requires the loss of ring A along with C-6 or C-19 (Scheme 46). However, no labelling experiment has been done to determine which of these two carbons is lost.

Scheme 46



A cyclopropane ring in the C-17 side chain [124], e.g., gorgosterol (188) [124a], leads to abundant fragments from cleavage across the ring with no apparent hydrogen rearrangement. However, no systematic study is available.

Synthetic steroids containing a 1,2-methylene group, e.g., 1,2ξ-methylene-5ξ-androstane-17β-yl acetate (189) [125], induces loss of C_3H_6, probably comprising the cyclopropane ring, but nothing is known about the origin of the two additional hydrogens. In contrast, 2-spirocyclopropano-5α-cholestan-3-one (190) [126] does not undergo cyclopropyl fission upon electron impact.



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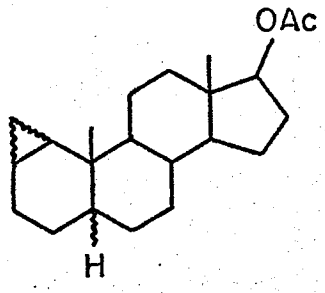

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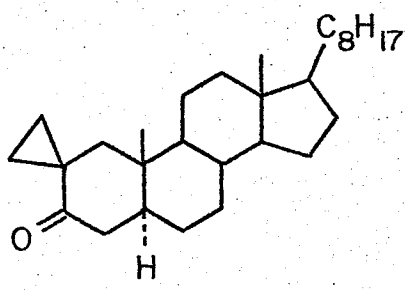

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In earlier work a series of 5,6α-cyclopropano-5α-cholestane derivatives having a C-3 oxygen function have been prepared [115,127]. In general, the mass spectra of the epimeric 3α- and 3β-hydroxy and the corresponding 3-ethoxy compounds (Figs. IX-XII) show the expected fragmentations of saturated steroid derivatives [128,129]. In most cases, the basic fragmentations are similar, including the loss from the molecular ion of methyl, the side chain (C_8H_{17}), the side chain + 42, the C-3 oxygen function (R'OH), and combination of these (Table X). These fragmentations are well-known electron impact decompositions of substituted cholestane [128,129]. Three other fragments of moderate intensity appear in all the spectra at m/e 247 (10-45%), 301 (15-65%), and

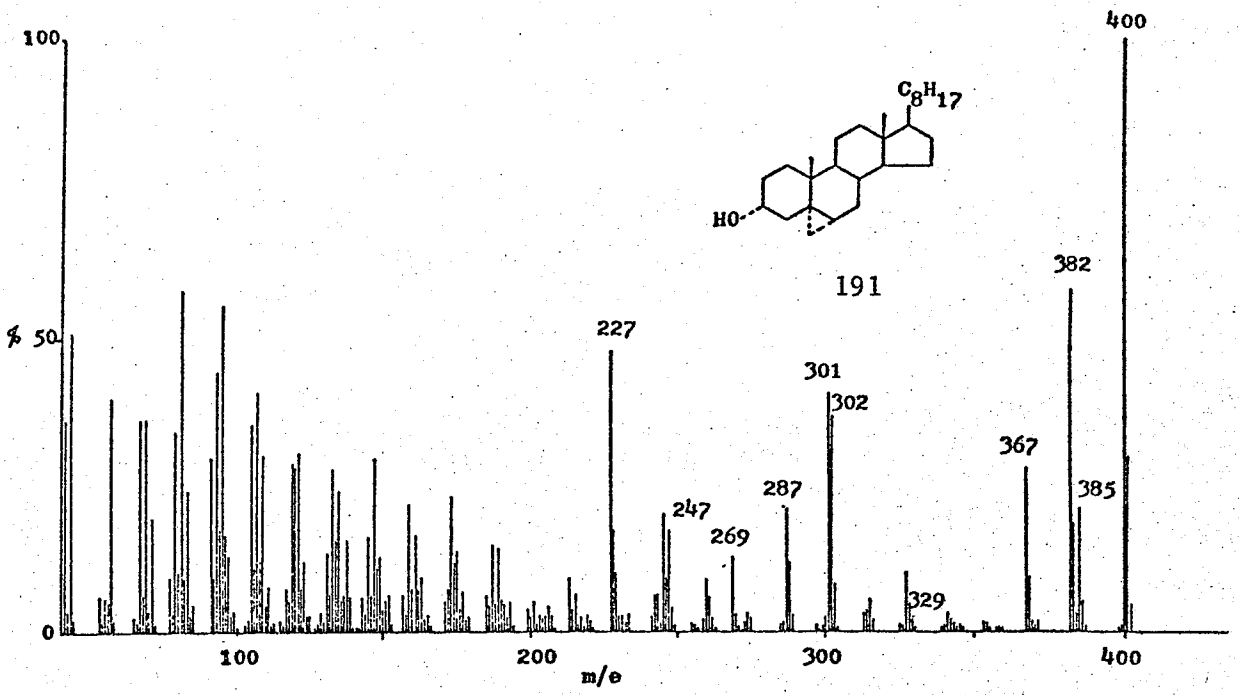

Figure IX. Mass spectrum of 3α-hydroxy-5,6α-cyclopropano-5α-cholestane.

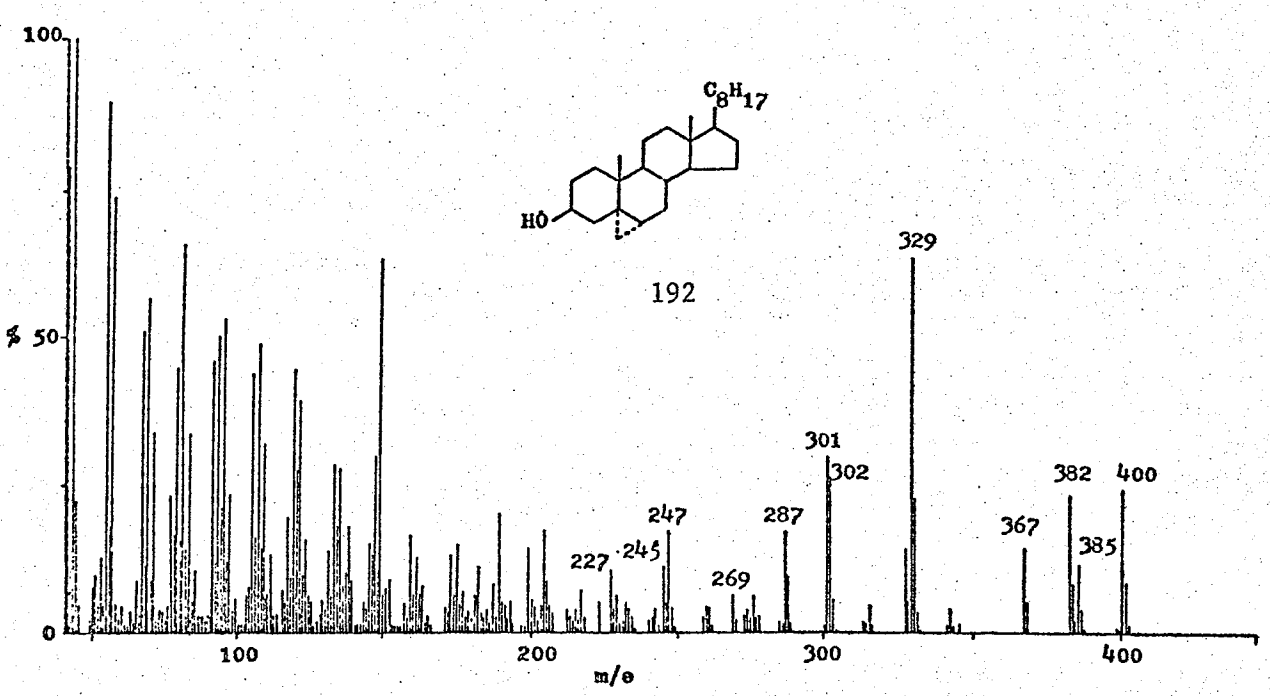

Figure X. Mass spectrum of 3β-hydroxy-5,6α-cyclopropano-5α-cholestane.

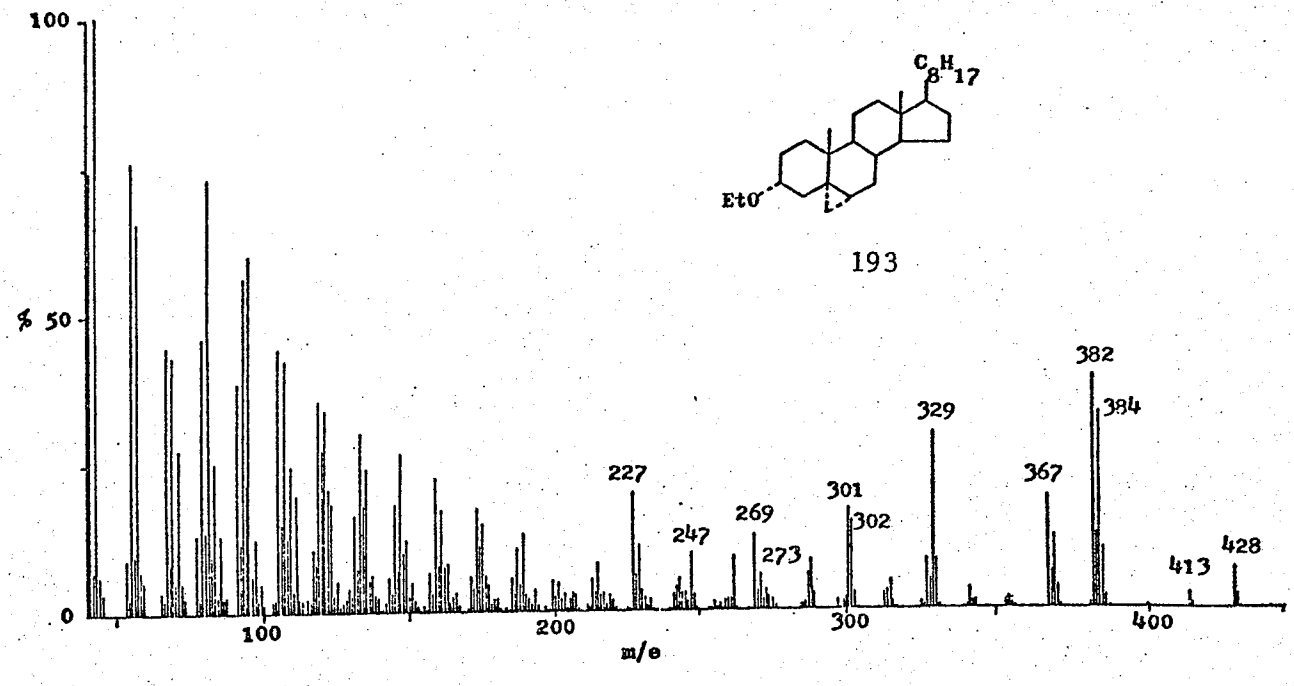

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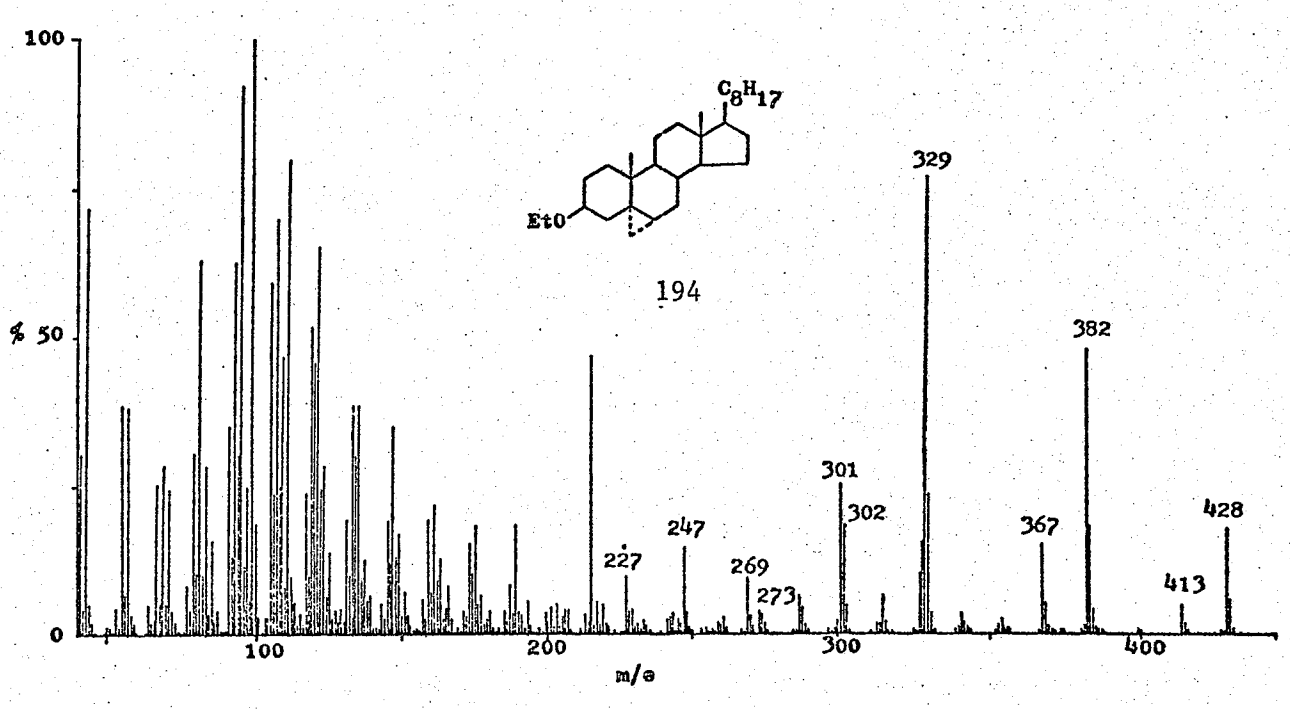
Figure XI. Mass spectrum of 3α-ethoxy-5,6α-cyclopropano-5α-cholestane.



194

Figure XII. Mass spectrum of 3β-ethoxy-5,6α-cyclopropano-5α-cholestane.

Table X. Mass spectral fragments of 5,6α-cyclopropano-5α-cholestane derivatives (relative intensity m/e)

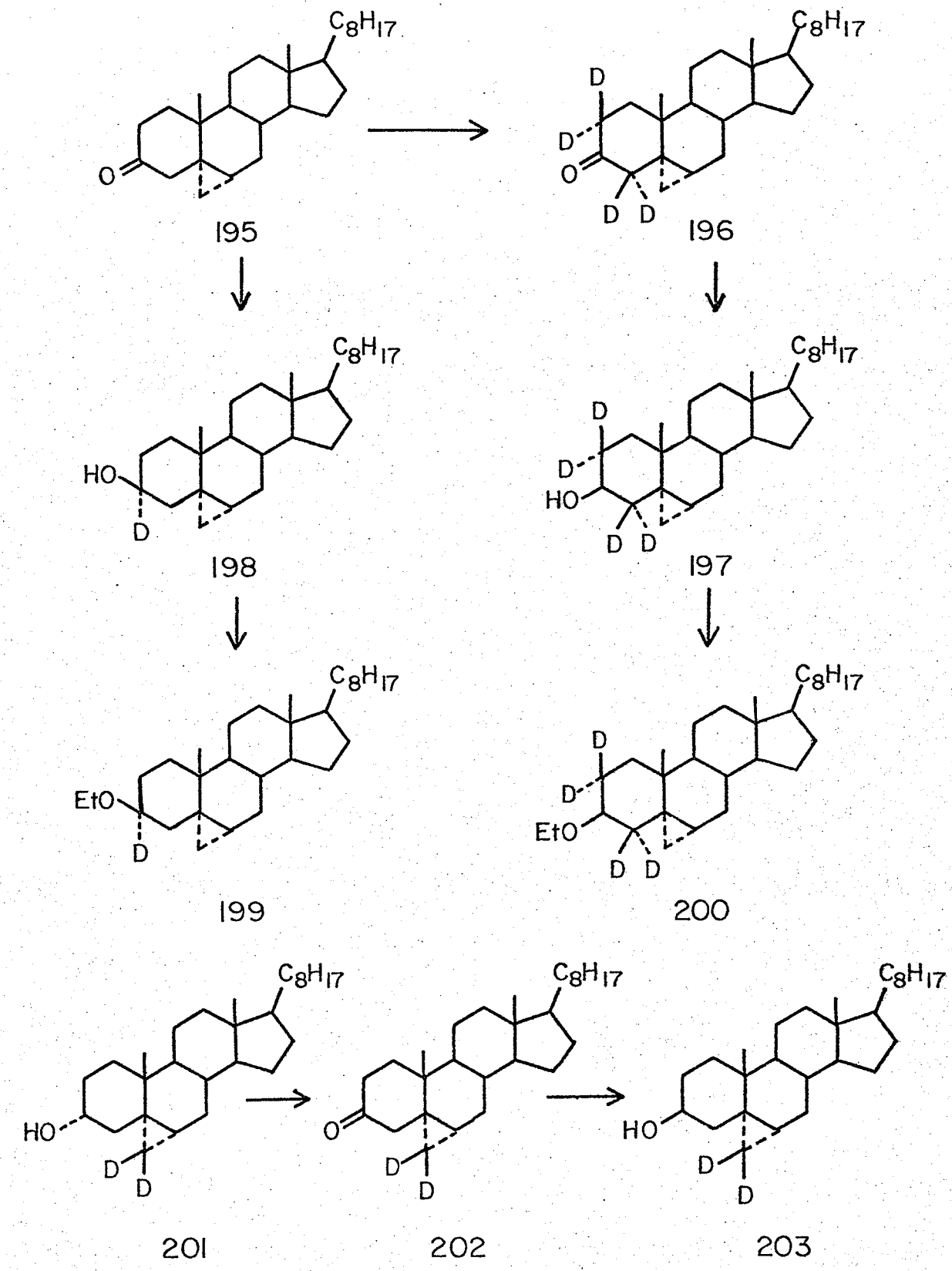
Substituents	M^+	$M^+ - CH_3$	$M^+ - R'OH$	$M^+ - (CH_3 + R'OH)$	$M^+ - R$	$M^+ - (R + R'OH)$	$M^+ - (R + 42)$	$M^+ - (R + 42 + R'OH)$	$M^+ - (ring A + ring B)$	$(C_{22}H_{37})$	$(C_{22}H_{38})$	$(C_{24}H_{41})$	$M^+ - C_2H_4O$
3α-OH	100 400	67.9 385	57.3 382	27.0 367	27.5 287	19.6 269	42.9 245	60.7 227	17.0 247	40.0 301	36.0 302	1.9 329	-
3β-OH	43 400	11.3 385	36.5 382	25.0 367	16.8 287	6.3 269	11.1 245	10.5 227	22.5 247	43.5 301	34.0 302	100.0 329	-
3α-OEt	7.0 428	2.5 413	39.0 382	19.0 367	5.0 315	12.5 269	7.0 273	18.5 227	10.0 247	17.0 301	15.0 302	30.0 329	33.0 384
3β-OEt	11.5 428	3.1 413	31.5 382	10.1 367	4.2 315	6.1 269	2.4 273	6.5 227	14.5 247	25.0 301	18.3 302	78.3 329	-
3α-OH (cyclopropyl-d_2)	55.0 402	16.0 387	35.5 384	11.0 369	11.3 289	10.5 271	31.2 247	36.1 229	31.2 247	30.0 301	29.5 302	8.5 331	-
3β-OH (cyclopropyl-d_2)	40.0 402	16.6 387	30.5 384	19.0 369	18.2 289	11.1 271	34.0 247	15.8 229	34.0 247	46.2 301	40.0 302	83.1 331	-
3β-OH (3α-d_1)	34.9 401	15.7 386	37.8 383	20.5 368	24.3 288	10.8 270	23.8 246	19.2 228	31.4 247	43.2 301	36.5 302	78.4 330	-
3β-OEt (3α-d_1)	6.2 429	7.5 414	26.7 383	30.5 368	7.3 316	13.0 270	8.0 274	19.0 228	20.4 247	40.0 301	27.5 302	88.9 330	-
3β-OH (2,2,4,4-d_4)	48.9 404	22.9 389	41.4 386	26.0 371	17.9 291	16.4 273	27.5 249	24.0 231	42.1 247	67.9 301	53.2 302	87.1 330	-
3β-OEt (2,2,4,4-d_4)	19.6 432	5.0 417	52.4 386	15.0 371	17.9 291	16.4 273	1.9 277	9.9 231	18.2 247	31.0 301	26.4 302	100.0 330	-

R = C_8H_{17}; R' = H, C_2H_5

302 (15-55%). A strong signal (>75%) at m/e 329 appears in the spectrum of the 3β-alcohol (Fig. X) and the 3β-ether (Fig. XII), whereas in the spectrum of the 3α-alcohol (Fig. IX) and the corresponding ether (Fig. XI), this ion is much less abundant (<30%). The 3α-ether (Fig. XI) also has a prominent signal at m/e 384 (33%) not present in the other spectra.

The existence of these particular fragment ions in the mass spectrum of 5,6α-cyclopropano-5α-cholestane derivatives and the general lack of information regarding the effect of the presence of a cyclopropane ring on the fragmentation of molecules prompted us to study further the mechanism of their fragmentations in order to determine if there is any major involvement of the cyclopropane ring in the formation of their fragment ions. Several deuterium labelled 5,6α-cyclopropano-5α-cholestane derivatives were prepared for this study as outlined (Scheme 47).

Scheme 47



B. Synthesis of deuterium labelled compounds

In addition to the 3-hydroxy and the 3α-ethoxy derivatives (191, 192, and 193) prepared previously [127], the 3β-ethoxy-5,6α-cyclopropano-5α-cholestane (194) was derived from the corresponding alcohol (192) by etherification using the method of Narayanan and Iyer [81]. Compound 194 showed cyclopropyl C-H and ether C-O stretching vibration at 3077 and 1105 cm^{-1}, respectively, in the i.r. spectrum. Treatment of the ketone (195) with a solution of sodium in dioxane and D_2O at reflux temperature gave the 5,6α-cyclopropano-5α-cholestan-3-one-2,2,4,4-d_4 (196). The isotope composition of the deuterated derivative was determined by mass spectral analysis to be 93% d_4 and 7% d_3. Reduction of 196 with lithium aluminum hydride in ether led to a mixture of the 3α- and 3β-alcohols. Chromatography on alumina gave the 3β-hydroxy-5,6α-cyclopropano-5α-cholestane-2,2,4,4-d_4 (197) as the major product, on elution with 75% benzene/petroleum ether. Compound 197 was composed of 82% d_4, 12% d_3, and 5% d_2. The 3β-ethoxy-5,6α-cyclopropano-5α-cholestane-2,2,4,4-d_4 (200) was then prepared from 197 by the same method used to prepare 194. The isotope composition of 200 was 81% d_4, 12% d_3, and 3% d_2.

Reduction of the ketone (195) with lithium aluminum deuteride afforded the 3β-hydroxy-5,6α-cyclopropano-5α-cholestane-3α-d_1 (198) after chromatographic separation from the 3α-epimer. The analytically pure sample of 198 contained 97% d_1 and 3% d_0. Treatment of 198 in the same way as 192 led to the 3β-ethoxy-5,6α-cyclopropano-5α-cholestane-3α-d_1 (199) which was composed of 96% d_1 and 4% d_0.

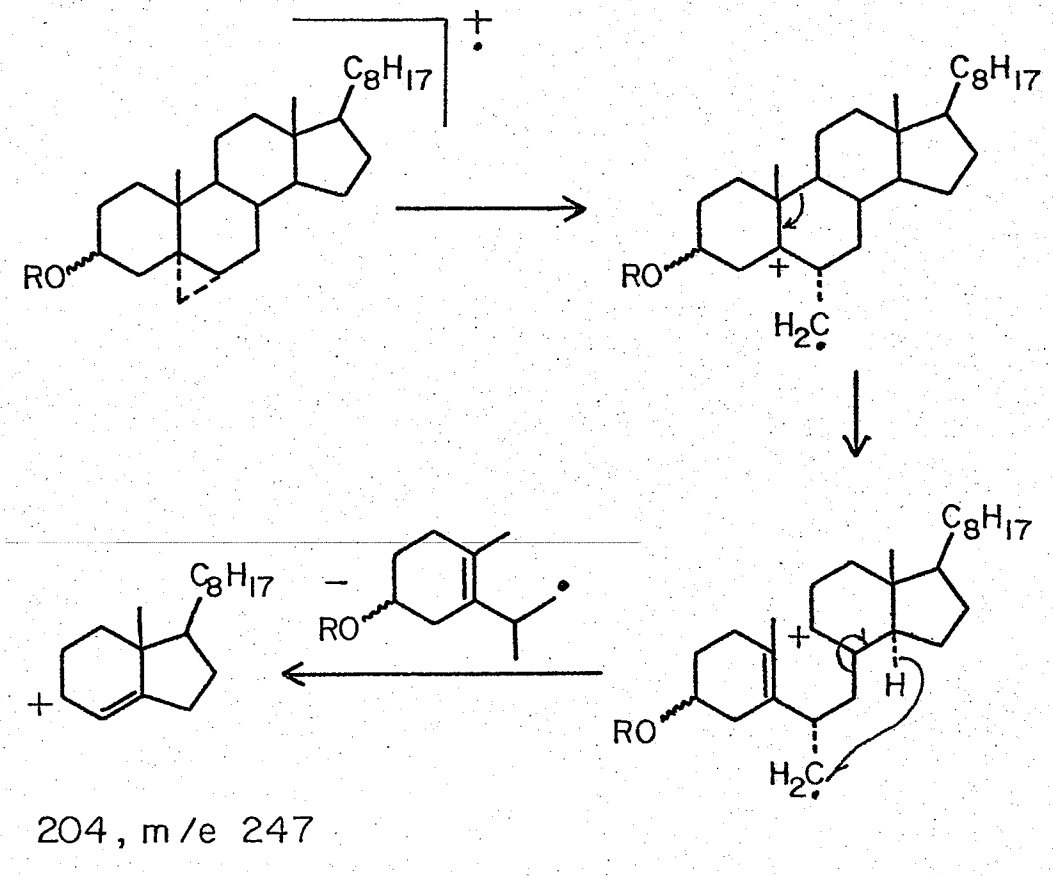
The 3α-hydroxy-5,6α-deuteriocyclopropano-5α-cholestane-d_2 (201)

was prepared by the modified Simmons-Smith reaction described by Rawson and Harrison [130] using diiodomethane-d_2. Chromatography of the crude product on alumina gave, on elution with 25-50% benzene/petroleum ether, the 3α-epimer (201) which was composed of 87% d_2, 7% d_1, and 6% d_0. Oxidation of 201 with Jones' reagent [43] afforded the 5,6α-deuteriocyclopropano-5α-cholestan-3-one-d_2 (202) which was further reduced with lithium aluminum hydride to give a mixture of the epimeric hydroxyl derivatives. Chromatography of the mixture on alumina gave, on elution with 50-100% benzene/petroleum ether, the 3β-hydroxy-5,6α-deuteriocyclopropano-5α-cholestane-d_2 (203) which was composed of 91% d_2, 5% d_1, and 4% d_0. Mass spectra of compounds 197 - 201 and 203 were determined for comparison with the hydrogen analogs (Table X).

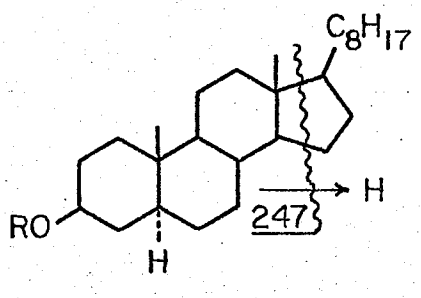
C. Results and discussion

By application of the Biemann's shift rule [131], the fragment ion at m/e 247 (204) present in all spectra of the 5,6α-cyclopropano-5α-cholestane compounds (Figs. IX-XII) was unmoved in the spectra of the deuterium labelled derivatives (Table X) showing that loss of the hydrogen atoms at C-2, C-3, C-4, and the cyclopropane ring had occurred. Cleavage of the C-7—C-8 and C-9—C-10 bonds with migration of a hydrogen atom from C-14 as outlined (Scheme 48) accounts for the formation of this fragment ion.

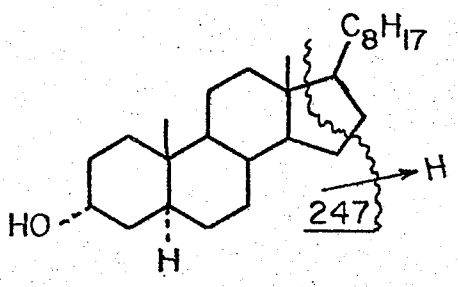
Scheme 48



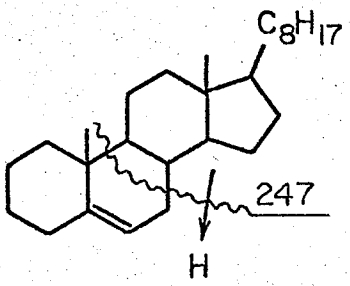
The m/e 247 fragment in the spectrum of 3β-methoxy-5α-cholestane (205a) [132,133] and the corresponding ion of 3α-hydroxy-5α-cholestane (206) [134] has been assigned to cleavage of the D-ring. This ion was shifted to m/e 250 in the spectrum of the 3β-deuteriomethoxy-5α-cholestane-d_3 (205b) [132]. The presence of a m/e 247 fragment in the mass spectra of 5-cholestene (207) [116] and 3β-methoxy-cholest-5-ene (208) [132] may be formed by an analogous process as described in Scheme 48 involving a cyclic five-membered transition state. This ion remains at m/e 247 in the spectrum of 3β-deuteriomethoxy-cholest-5-ene-d_3 (208b) [132].



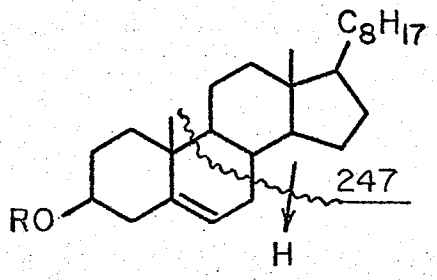
205 a, R = CH_3
b, R = CD_3



206



207



208 a, R = CH_3
b, R = CD_3

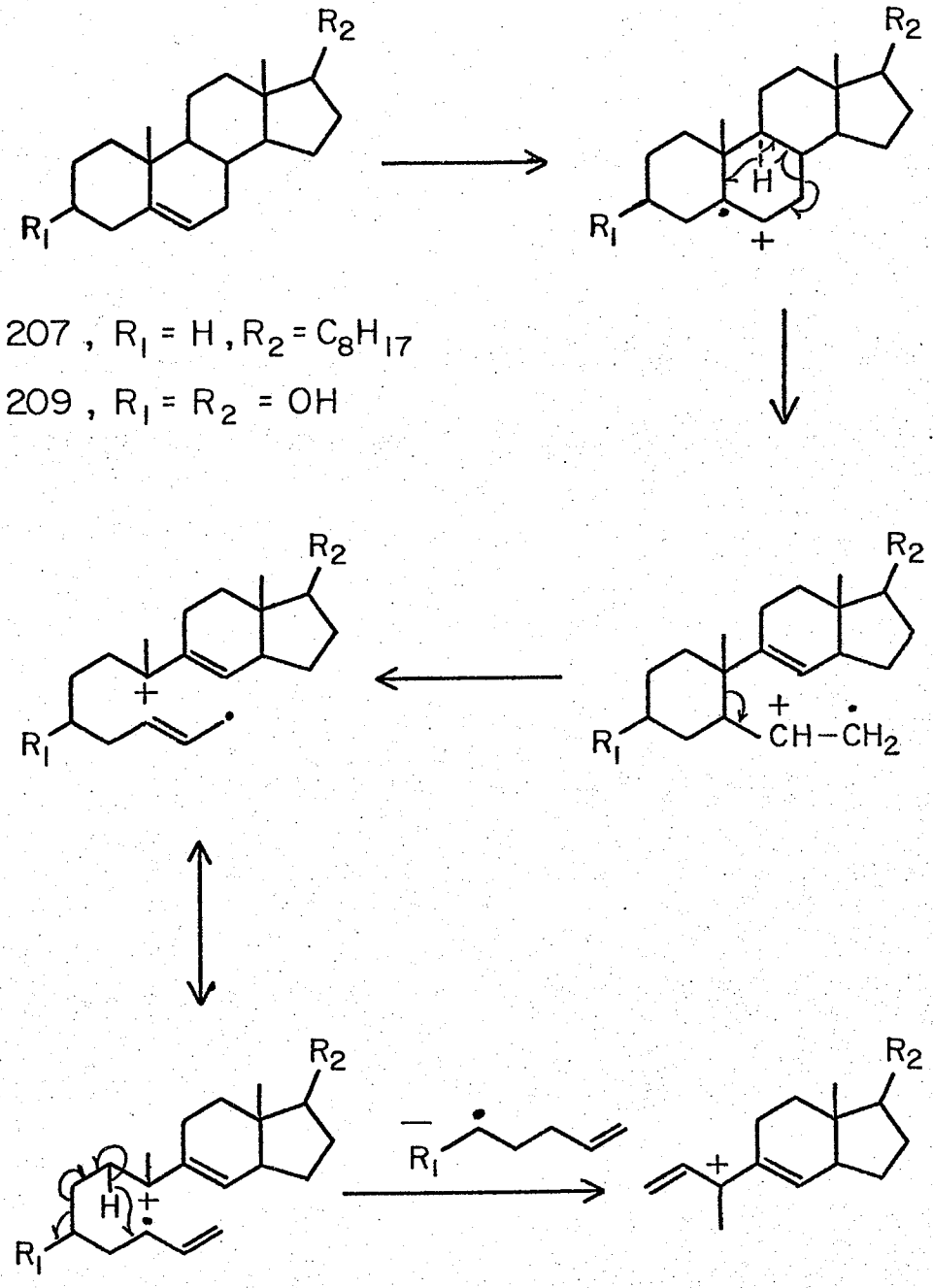
An ion of m/e 301 (210a) in the mass spectrum of 5-cholestene (207) [116] and the corresponding ion, m/e 205 (210b), in androst-5-ene-3β,17β-diol (209) [116] has been reported and a mechanism of formation (Scheme 49) proposed by Spiteller-Frideman and Spiteller [35].

The 5,6α-cyclopropano-5α-cholestane derivatives labelled at C-2 and C-4 (197 and 200) show that an analogous fragment is not formed from these molecules. If the ion m/e 301 in the 5,6α-cyclopropano-5α-cholestane derivatives was identical with that proposed [116] for the above, 197 and 200 should give rise to an equivalent ion at m/e 303. The ion at m/e 303 present in the mass spectra of 197 and 200 was calculated to be the ^{13}C isotopic contribution of the m/e 302 ion. Since the ions at m/e 301 (211) and 302 (212) were unmoved in the mass spectra of the deuterium labelled derivatives (197 - 201 and 203), fragmentation leading to their formation involving cleavage of the 5,6α-cyclopropane ring and ring A is proposed to occur with migration of the C-9 and C-2 hydrogen atoms by route a or with migration of the C-1 hydrogen atom by route b (Scheme 50). Accurate mass determinations of the m/e 301 and 302 fragments in the 3β-alcohol (Fig. X) show that these ions have a composition of $C_{22}H_{37}$ and $C_{22}H_{38}$, respectively, as expected from Scheme 50.

The mass spectra of 3β-methoxy-cholest-5-ene (208a) and the deuterium labelled derivative (208b) also contain the m/e 301 ion [132]. However, no structure was suggested by the authors.

A fragmentation pathway leading to the strong m/e 329 ion (>90%) (214) in the spectrum of cholesterol TMS ether (213) has been proposed (Scheme 51) [135]. A similar fragment (215) was also suggested [132] for

Scheme 49



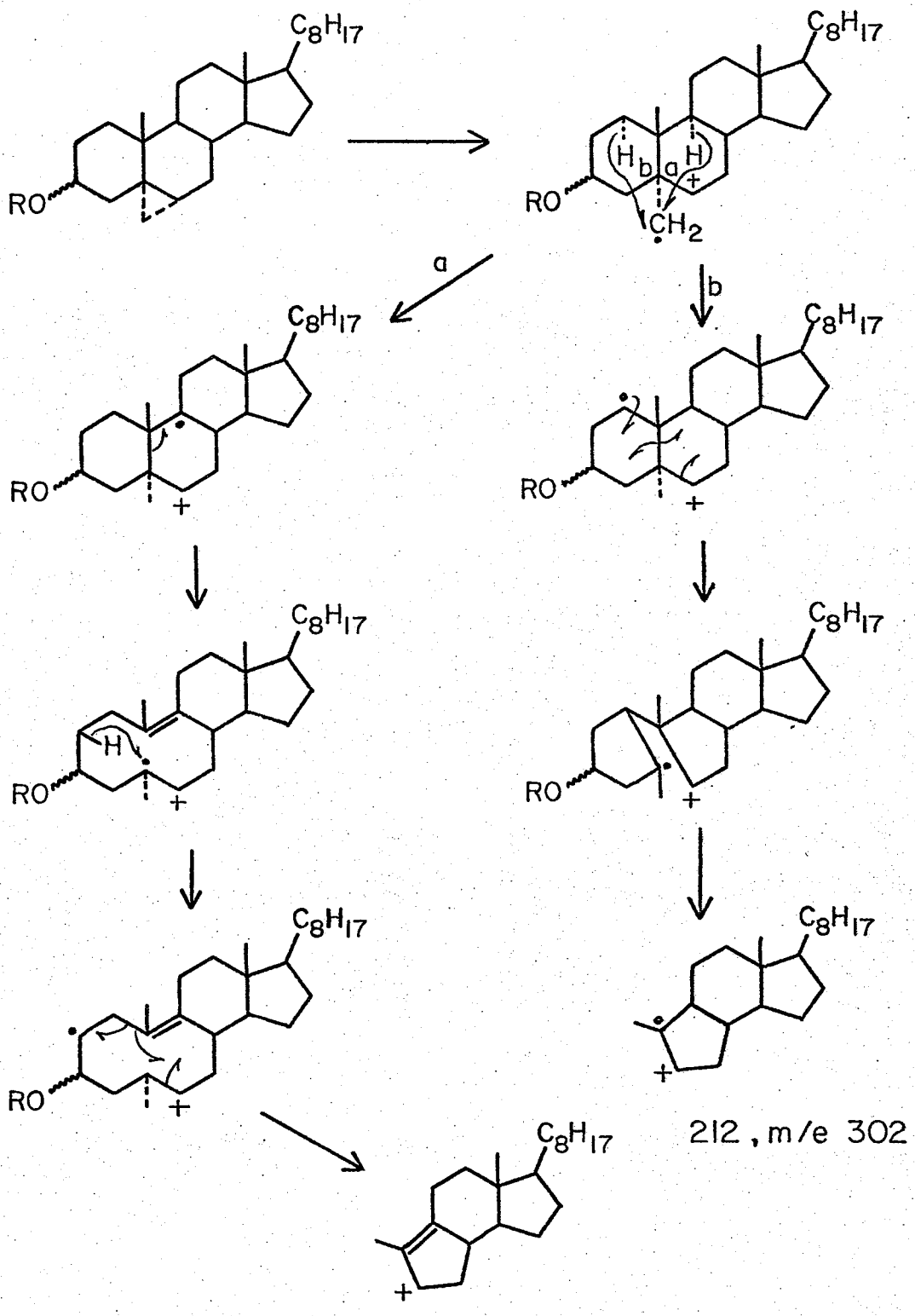
207 , $R_1 = H, R_2 = C_8H_{17}$

209 , $R_1 = R_2 = OH$

210a , $R_2 = C_8H_{17}$, m/e 301

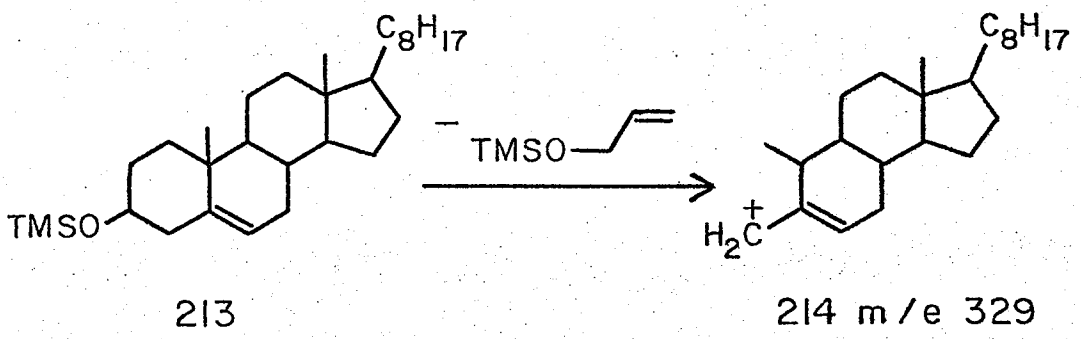
b , $R_2 = OH$, m/e 205

Scheme 50

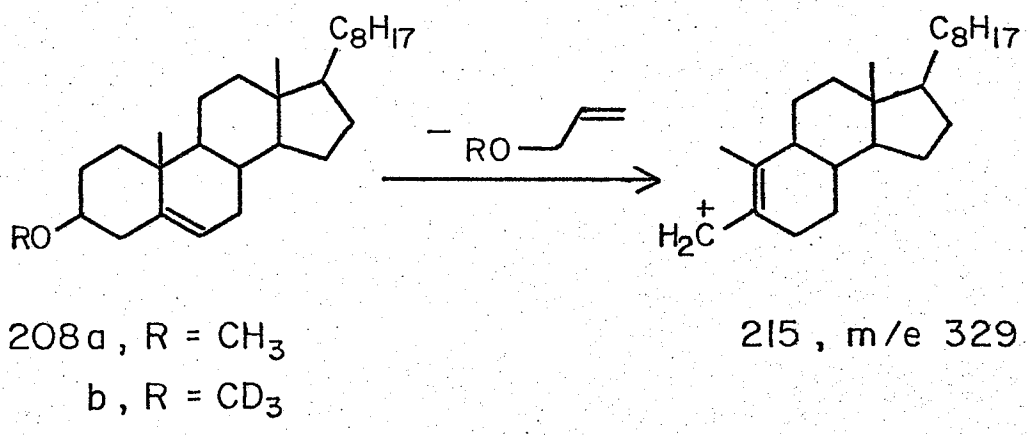


the m/e 329 ion (20-30%) in the spectrum of the unlabelled and labelled methyl ether (208a and 208b) (Scheme 52)

Scheme 51



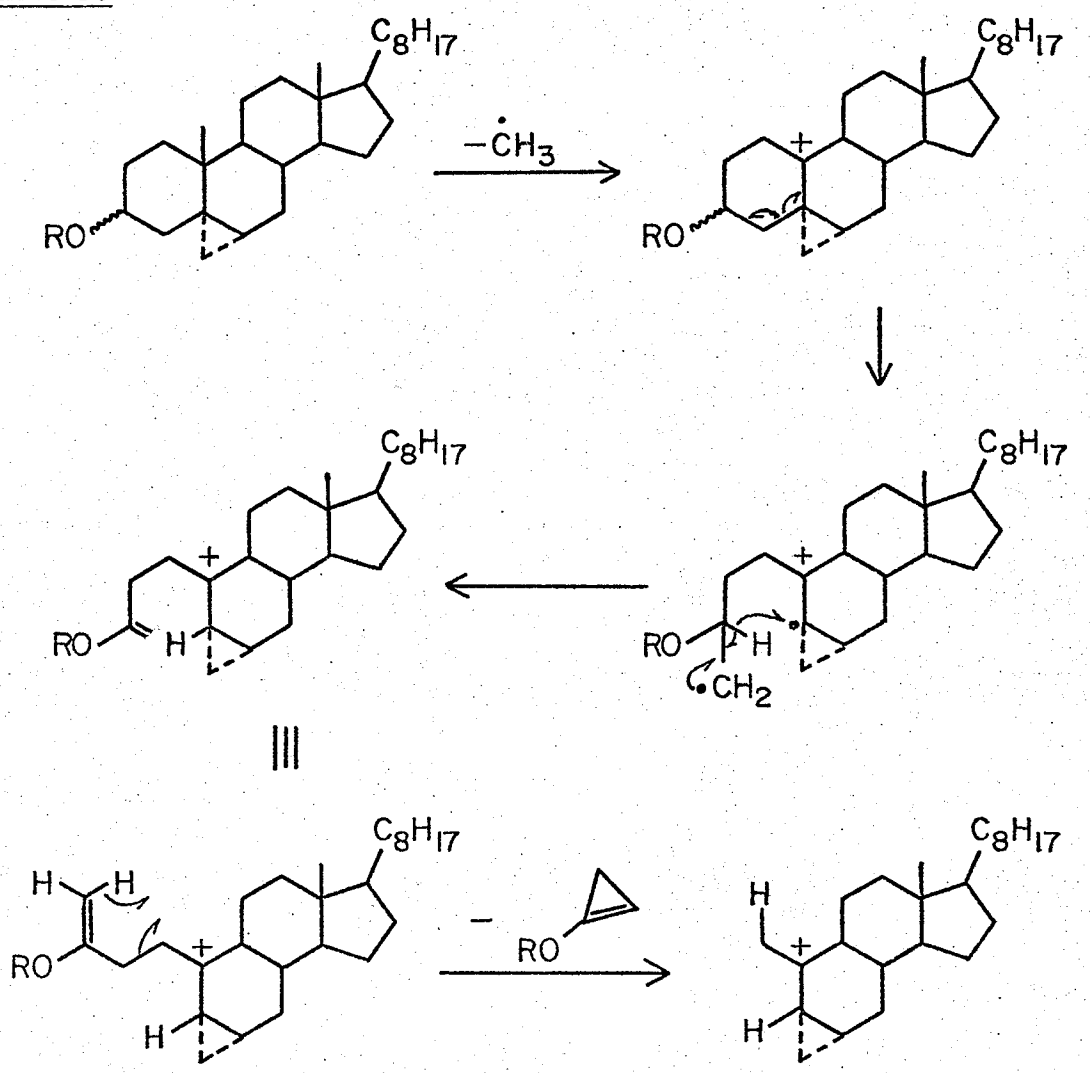
Scheme 52



The presence of a m/e 329 ion (216) in the mass spectra of the epimeric alsohols (Figs. IX and X) and the corresponding ethyl ethers (Figs. XI and XII) also implies loss of the C-3 oxygen function, possibly accompanied by cleavage of ring A. Compounds having a deuterium label at different positions show that the m/e 329 ion was increased by 1 a.m.u. in 197 and 200; 1 a.m.u. in 198 and 199; and 2 a.m.u. in 201 and 203 (Table X). These results show that the methylene protons

of the cyclopropane ring remain with the positively charged portion during the fragmentation process. High resolution mass measurement of the m/e 329 ion in the 3β-alcohol (Fig. X) and the 3α-ethyl ether (Fig. XI) gives a composition of $C_{24}H_{41}$ showing that loss of the oxygen function takes place. Cleavage of ring A followed by migration of the C-3 hydrogen atom and one of the two hydrogen atoms at C-4 toward the final positively charged fragment accounts for the fragment masses (Scheme 53).

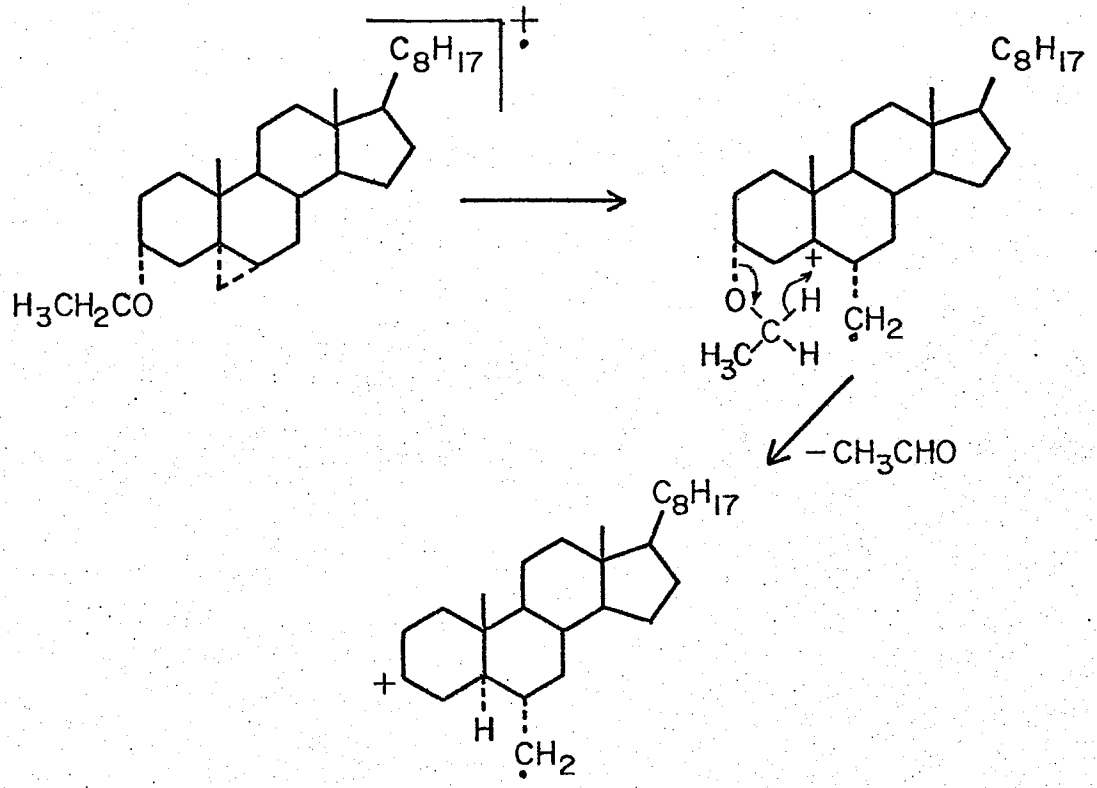
Scheme 53



The relative abundance of the m/e 329 ion is greater than 75% in the 3β-isomers (Figs. X and XII) and compounds 197, 198, 199, 200, and 203, and is less than 30% in the 3α-isomers (Figs. IX and XI) and compound 201. This difference in relative abundance of the ion at m/e 329 shows that the configuration of the hydrogen atom at C-3 affects the ease of formation of this ion. The mechanism shown in Scheme 53 accounts for all of the factors mentioned above for both epimeric alcohols (191 and 192) and the corresponding ethyl ethers (193 and 194). It is apparent that an ion fragment analogous to 214 proposed by Diekman and Djerassi [135] is not formed from the 5,6α-cyclopropano-5α-cholestane derivatives.

It is interesting that the 3α-ethyl ether (Fig. XI) is the only compound which shows an ion at m/e 384 (33%) in the 5,6α-cyclopropano-5α-cholestane derivatives studied. Accurate mass measurement shows that C_2H_4O has been lost from the molecular ion. The formation of this ion (217) may involve migration of one of the ethoxy methylene hydrogen atoms followed by elimination of acetaldehyde (Scheme 54), this would account for the lack of this ion in the mass spectra of the 3β-ethyl ether (Fig. XII) and the epimeric alcohols (Figs. IX and X). The methylene hydrogen atoms of the 3α-ethyl ether (193) exist in a closer stereochemical situation relative to the positively charged C-5 than those of the 3β-ethyl ether (194). The epimeric alcohols (191 and 192) do not possess an analogous hydrogen atom.

Scheme 54



217, m/e 384

D. Conclusion

Besides the well-known fragment ions of steroid derivatives [128, 129], mass spectrometric fragmentations of 5,6α-cyclopropano-5α-cholestane derivatives demonstrate the formation of additional characteristic fragment ions (m/e 247, 301, 302, 329, and 384) which have been related to the presence of the cyclopropane ring. In the case of the ion at m/e 247, 301, 302, and 384, fragmentations appear to be directed by initial cleavage of the cyclopropane ring, whereas for the ion at m/e 329, the cyclopropane ring may stabilize the intermediate fragment ions and/or radicals leading to the m/e 329 ion or provide greater stability to the m/e 329 ion itself.

These results do not support the generalization of Dias and Djerassi [121], however, it is not surprising that in the rigid steroid molecule stereochemical factors may favor one fragmentation pathway which does not occur predominantly in the analogous simple molecules.

III. EXPERIMENTAL

Unless otherwise stated the following instruments and procedures were used.

Melting point (m.p.) were carried out on a Thomas-Hoover capillary apparatus and are uncorrected. Optical rotations $|\alpha|_D$ were measured in chloroform solution on a Bellingham and Stanley (Model A) Polarimeter (concentration range 0.1 - 1.0%) in a 1 dm tube at 23^{o}. Infrared (i.r.) spectra were determined on a Perkin-Elmer (Model 267) Grating Infrared Spectrophotometer. Proton magnetic resonance (p.m.r.) spectra were determined on a Varian A56/60A instrument; 220 MHz spectra were recorded on a Varian HR 220 instrument by the Canadian 220 MHz NMR Centre, Ontario Research Foundation, Sheridan Park, Ontario; tetramethylsilane (TMS) was used as an internal standard. Ultraviolet (u.v.) spectra were determined on a Beckmann DU (Model 2400) Spectrophotometer using 1 cm silica cells. Mass spectra (m.s.) were recorded on a Finnigan Quadrapole (Model 1015) instrument at 70 eV using a direct probe method by the Chemistry Department, University of Manitoba. Mass spectra of the 5,6α-cyclopropano-5α-cholestane derivatives were determined by the Mass Spectroscopy Laboratory, Chemistry Department, University of Alberta, Edmonton, using a Metropolitan-Vickers MS-2 at a source temperature of 155^{o}. The mass spectrometers were operated at an accelerating voltage of 70 eV. Accurate mass measurements were carried out by the peak matching method. Elemental analysis were performed by either Parcher and Parcher, Microanalytical Laboratory, Bonn, West Germany or Mr. G. Crouch, School of Pharmacy, University of London, England.

Petroleum ether refers to the fraction boiling within the range

of 60-80°. Thin-layer chromatography (t.l.c.) was carried out on silica gel coated glass plates developed with the following solvent systems: (A) 5% ethyl acetate/chloroform; (B) 10% ethyl acetate/petroleum ether; (C) 25% ethyl acetate/petroleum ether, visulized by spraying with 4% v/v concentrated sulfuric acid in ethanol followed by heating at approximately 100° for 15-30 minutes. Gas-liquid chromatography (g.l.c.) was carried out on a Varian Aerograph Series 1800 chromatograph equipped with a hydrogen flame ionization detector and a Pyrex glass column packed with 2% OV-17 on Chromosorb GHP, 60-80 mesh, internal diameter 2 mm, length 180 cm was used. The detector temperature was 300°, column temperature 240°, and the carrier gas (N_2) flow rate was 40 ml/min. All compounds (where appropiate) were injected as their trimethylsilyl derivatives at a concentration of 10 µg/µl.

An authentic sample of 17β-hydroxy-2α-methyl-5α-androstan-3-one was obtained by basic hydrolysis[100] of the 17β-propionate ester (dromostanolone propionate) received from Eli Lilly Co. Inc., Indianapolis, Indiana, USA.

Acetylation: To a 10% solution of the steroid in dry pyridine was added one-half the volume of acetic anhydride. After standing overnight at room-temperature, the reaction was poured into an ice-water mixture and allowed to stand for 30 minutes followed by acidification with dilute hydrochloric acid and ether extraction.

Zinc-copper couple: A homogenous, consistently active, zinc-copper couple was prepared as follows. Finely powdered cupric acetate monohydrate (8 g, 0.04 mole) and zinc dust (130 g, 2 mole) were thoroughly

mixed with a pestle and mortar. To avoid excessive frothing the mixture was added portionwise to a mechanically stirred solution of boiling glacial acetic acid (275 ml). After addition of a further 200 ml of cold acetic acid, the slurry was filtered with suction and washed several times with ether without allowing all of the ether to evaporate. The ether-moist couple was kept under ether and used without delay.

Simmons-Smith Reaction with the Unsaturated Steroid: To the freshly prepared couple in dry ether (20 ml) was added dry diiodomethane (10 mmole) m.p. 5-6°, in dry ether (10 ml). Refluxing usually occurred spontaneously and the reaction was refluxed for 30-60 minutes.

The steroid (1 mmole) was dissolved in dry ether (10-25 ml), added to the reagent (10 mmole), and the mixture refluxed. The reaction was poured into excess saturated aqueous sodium bicarbonate and the resulting slurry extracted with ether. The ether solution was washed with water, brine, dried over anhydrous sodium sulfate, filtered, and evaporated at reduced pressure to give the crude reaction product.

2α,4α-Dibromo-17β-acetoxy-5α-androstan-3-one (71)

Prepared by the method of Jones and Ship [32]. Material of m.p. 184-190° was used for sodium borohydride reduction.

2α,4α-Dibromo-5α-androstane-3α,17β- and 3β,17β-diol 17-acetate (72a) and (73a)

Sodium borohydride (946 mg) in ethanol (50 ml) was added to a slurry of 2α,4α-dibromo-17β-acetoxy-5α-androstan-3-one (71) (15 g) in ethanol (200 ml). The mixture was stirred for five minutes at room temperature, during which time dissolution occurred, and poured into

ice-cold 1 N sulfuric acid (1 l). Extraction with dichloromethane yielded a crude product which on crystallization from chloroform gave 2α,4α-dibromo-5α-androstane-3β,17β-diol 17-acetate (73a) (527 mg); m.p. 248-252°, second crop (982 mg), m.p. 235-240°. Further recrystallization from dichloromethane gave 73a (746 mg), m.p. 253-255°; $|\alpha|_D$ -46°; i.r. ν_{max} (KBr): 3413 (O-H), 1706 (ester C=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.59 (1, t, J = 8.0 Hz, 17α-H), 4.10 (1, m, 2β-H), 3.94 (1, t, J = 10.0 Hz, 4β-H), 3.80 (1, t, J = 10.0 Hz, 3α-H), 2.39 (1, q, J = 13.0 and 5.0 Hz, 1β-H), 2.03 (3, s, acetyl-H), 0.88 (3, s, 19-H), 0.78 (3, s, 18-H) p.p.m.

Anal. Calcd. for $C_{21}H_{32}O_3Br_2$: C, 51.23; H, 6.55; Br, 32.25. Found: C, 51.08; H, 6.42; Br, 33.81.

The mother liquor was evaporated and crystallized from acetone/dichloromethane to give 2α,4α-dibromo-5α-androstane-3α,17β-diol 17-acetate (72a) (3.2 g), m.p. 202-205°. Further recrystallization gave 72a, m.p. 206-207°; $|\alpha|_D$ -24°; i.r. ν_{max} (KBr): 3472 (O-H), 1706 (ester C=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.59 (1, t, J = 8.0 Hz, 17α-H), 4.35 (1, m, 2β-H), 4.27 (1, t, J = 2.5 Hz, 3β-H), 4.20 (1, q, J = 11.0 and 2.5 Hz, 4β-H), 2.03 (3, s, acetyl-H), 0.89 (3, s, 19-H), 0.77 (3, s, 18-H) p.p.m.

Anal. Calcd. for $C_{21}H_{32}O_3Br_2$: C, 51.23; H, 6.55; Br, 32.25. Found: C, 51.09; H, 6.42; Br, 33.07.

Column chromatographic separation of the acetylated crude 2α,4α-dibromoalcohols gave a 3:1 ratio of 3α- to 3β-acetates.

2α,4α-Dibromo-5α-androstane-3α,17β-diol diacetate (72b)

2α,4α-Dibromo-5α-androstane-3α,17β-diol 17-acetate (72a) (95 mg)

was acetylated. The crude reaction product was recrystallized from methanol to give 2α,4α-dibromo-5α-androstane-3α,17β-diol diacetate (72b) (77 mg), m.p. 228-229°; $|\alpha|_D$ -19°; i.r. ν_{max} (CCl_4): 1761, 1742 (ester C=O) cm^{-1}; p.m.r. δ (CCl_4): 5.70 (1, t, J = 2.5 Hz, 3β-H),4.58 (1, t, J = 8.0 Hz, 17α-H), 4.33 (1, m, 2β-H), 4.16 (1, q, J = 11.0 and 2.5 Hz, 4β-H), 2.18 (3, s) and 2.02 (3, s, acetyl-H), 0.90 (3, s, 19-H), 0.76 (3, s, 18-H) p.p.m.; m.s. m/e: 532, 534, 536 (M^+, weak).

Anal. Calcd. for $C_{23}H_{34}O_4Br_2$: C, 51.70; H, 6.41; Br, 29.91. Found: C, 51.70; H, 6.36; Br, 29.90.

2α,4α-Dibromo-5α-androstane-3β,17β-diol diacetate (73b)

2α,4α-Dibromo-5α-androstane-3β,17β-diol 17-acetate (73a) (100 mg) was acetylated. Successive recrystallization from methanol gave 2α, 4α-dibromo-5α-androstane-3β,17β-diol diacetate (73b) (73 mg), m.p. 217-218°; $|\alpha|_D$ -34°; i.r. ν_{max} (CCl_4): 1761, 1742 (ester C=O) cm^{-1}; p.m.r. δ (CCl_4): 5.27 (1, t, J = 10.0 Hz, 3α-H), 4.60 (1, t, J =8.0 Hz, 17α-H), 4.28 (1, m, 2β-H), 3.92 (1, t, J = 10.0 Hz, 4β-H), 2.42 (1, q, J = 13.0 and 5.0 Hz, 1β-H), 2.12 (3, s) and 2.01 (3, s, acetyl-H), 0.88 (3, s, 19-H), 0.76 (3, s, 18-H) p.p.m.; m.s. m/e: 532, 534, 536 (M^+, weak).

Anal. Calcd. for $C_{23}H_{34}O_4Br_2$: C, 51.70; H, 6.41; Br, 29.91. Found: C, 51.91; H, 6.37; Br, 29.93.

2β,4β-Cyclo-5α-androstane-3β,17β-diol 17-acetate (76a)

To a slurry of 2α,4α-dibromo-5α-androstane-3β,17β-diol 17-acetate (73a) (600 mg) in ethanol (18 ml) was added freshly prepared 2% zinc-copper couple (9 g). The mixture was mechanically stirred for 1/2 hour at room temperature. The reaction was filtered, the ethanol eva-

porated at reduced pressure with minimal heating. Cold aqueous sodium bicarbonate was added and the products extracted with ether. The crude product showed one major spot on t.l.c. (solvent A) and contained >98% 2β,4β-cyclo-5α-androstane-3β,17β-diol 17-acetate (76a) on g.l.c. Successive recrystallization from methanol gave the alcohol (76a) (222 mg), m.p. 130-132°; $|\alpha|_D$ +24° (dioxane); i.r. ν_{max} (KBr): 3546 (O-H), 3040 (cyclopropyl C-H), 1727 (ester C=O) cm^{-1}; m.s. m/e: 332 (M^+).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.85; H, 9.70. Found: C, 75.97, H, 9.75.

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate (75a)

2α,4α-Dibromo-5α-androstane-3α,17β-diol 17-acetate (72a) (1 g) was treated with zinc-copper couple by the same method described above. The crude product showed three spots on t.l.c. and three peaks on g.l.c. On stirring with petroleum ether (7 ml), the insoluble material was collected (456 mg, m.p. 134-136°). Successive recrystallization from acetone/ether gave 2β,4β-cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) (350 mg), m.p. 143-146°; $|\alpha|_D$ +17° (dioxane); i.r. ν_{max} (KBr): 3509 (O-H), 3049 (cyclopropyl C-H), 1727 (ester C=O) cm^{-1}; m.s. m/e: 332 (M^+).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.85; H, 9.70. Found: C, 76.12, H, 9.63.

From the petroleum ether solution was recovered 85 mg, m.p. 105-127° of crystalline material which was stirred with petroleum ether (10 ml). The petroleum ether soluble portion was filtered through alumina, the p.m.r. spectrum of the combined fraction (41 mg) corresponded to a mixture of the 17β-acetoxy-5α-androst-2- and -3-ene (74) [34];

p.m.r. δ (CCl_4): 5.14-5.62 (2, m, olefinic-H), 4.56 (1, t, J = 8.0 Hz, 17α-H), 1.96 (3, s, acetyl-H), 0.76 (6, s, 18- and 19-H) p.p.m. [34]; m.s. m/e: 316 (M^+), 262 (M^+ - 54) [35]. The petroleum ether insoluble material (12 mg) was recrystallized from methanol to give a sample which was identical to 76a by mixed melting point and i.r. and m.s. spectra.

Higher overall yields of the cyclopropanols were obtained by treating the mixture of 3α- and 3β-dibromoalcohols (72a and 73a) directly with the zinc-copper couple. Separation of the products was carried out by extraction of the crude solid residue with petroleum ether (10% w/v). The 3α-cyclopropanol was recrystallized from the petroleum ether insoluble portion. The 3β-cyclopropanol and olefinic mixture was fractionally recrystallized from the petroleum ether soluble portion.

Debromination with zinc powder in ethanol

A mixture of the dibromoalcohols (72a and 73a) (20 mg) and zinc powder (300 mg) in ethanol (5 ml) was stirred for several hours at room temperature. The reaction was followed by t.l.c. (solvent A) which showed a single spot corresponding to the starting material and no cyclopropanols (75a and 76a) were detected after 24 hours of reaction.

Debromination of 2α,4α-dibromo-5α-androstane-3α,17β- and 3β,17β-diol diacetate (72b and 73b) with zinc-copper couple

A mixture of the dibromoacetate (72b and 73b) (10 mg) in ethanol (3 ml) was treated with zinc-copper couple by the same method described before. After 5 hours of reaction, t.l.c. (solvent B) showed an additional spot equivalent to the isomeric 17β-acetoxy-5α-androst-2- and

-3-ene (74). The reaction was allowed to stand overnight at room temperature. I.r. spectrum of the crude product was identical to that of 74.

2β,4β-Cyclo-5α-androstane-3β,17β-diol diacetate (76b)

2β,4β-Cyclo-5α-androstane-3β,17β-diol 17-acetate (76a) (120 mg) was acetylated. The crude reaction product showed a single spot on t.l.c. (solvent A). Recrystallization of the crude product from methanol gave the 2β,4β-cyclo-5α-androstane-3β,17β-diol diacetate (76b) (100 mg), m.p. 142-143°; $|\alpha|_D$ +23°; i.r. ν_{max} (CCl_4): 3050 (cyclopropyl C-H), 1736 (ester C=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.51 (1, t, J = 8.0 Hz, 17α-H), 3.63 (1, unresolved t, 3α-H), 1.94 (3, s) and 1.90 (3, s, acetyl-H), 0.85 (3, s, 19-H), 0.74 (3, s, 18-H) p.p.m.; m.s. m/e: 332 ($M^+ - CH_2CO$).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.93; H, 9.07.

2β,4β-Cyclo-5α-androstane-3β,17β-diol 17-acetate 3-tosylate (76c)

To a solution of 2β,4β-cyclo-5α-androstane-3β,17β-diol 17-acetate (76a) (500 mg) in dry pyridine (5 ml) was added p-toluenesulfonyl chloride (600 mg). The mixture was allowed to stand overnight at room temperature and the brown solution was poured into ice-water. The product was extracted with ether and the ether solution was washed with cold 1 N sulfuric acid. The crude reaction product was dissolved in acetone and the solution was stirred with activated charcoal and filtered. Successive recrystallization from acetone at room temperature gave 2β,4β-cyclo-5α-androstane-3β,17β-diol 17-acetate 3-tosylate (76c) (450 mg), m.p. 155-156°; $|\alpha|_D$ +19°; i.r. ν_{max} (CCl_4): 3030 (broad,

cyclopropyl and aromatic C-H), 1730 (ester C=O), 1595 (aromatic C=C), 1188 and 1176 (tosyl S=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 7.62 (4, q, $J_{AX} = J_{A'X'}$ = 8.0 Hz, p-substituted aromatic-H), 4.61 (1, t, J = 8.0 Hz, 17α-H), 3.56 (1, unresolved t, 3α-H), 2,47 (3, s, aromatic methyl-H), 2.01 (3, s, acetyl-H), 0.75 (6, s, 18- and 19-H) p.p.m.; m.s. m/e: 426 (M^+ - HOAc).

Anal. Calcd. for $C_{28}H_{38}O_5S$: C, 69.10; H, 7.87; S, 6.59. Found: C, 69.00; H, 7.86; S, 6.50.

2β,4β-Cyclo-5α-androstane-3α,17β-diol diacetate (75b)

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) (115 mg) was acetylated. Successive recrystallization of the crude product from methanol gave the 2β,4β-cyclo-5α-androstane-3α,17β-diol diacetate (75b) (58 mg), m.p. 147-148°; $|\alpha|_D$ +14°; i.r. ν_{max} (CCl_4): 3049 (cyclopropyl C-H), 1736 (ester C=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.60 (1, t, J = 8.0 Hz, 17α-H), 4.25 (1, t, J = 6.0 Hz, 3β-H), 2.12 (3, s) and 2.01 (3, s, acetyl-H), 0.94 (3, s, 19-H), 0.78 (3, s, 18-H) p.p.m.; m.s. m/e: 332 (M^+ - CH_2CO).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.54; H, 9.17.

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate 3-tosylate (75c)

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) (1.9 g) was treated with p-toluenesulfonyl chloride (2.4 g) in dry pyridine (20 ml) by the procedure used for 76c. The crude product was recrystallized from acetone to give the tosylate (75c) (1.7 g), m.p. 111-112°; $|\alpha|_D$ +36°; i.r. ν_{max} (CCl_4): 3020 (broad, cyclopropyl and aromatic C-H), 1730 (ester C=O), 1595 (aromatic C=C), 1190 and 1175

(tosyl S=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 7.62 (4, q, $J_{AX} = J_{A'X'}$ = 8.0 Hz, p-substituted aromatic-H), 4.63 (1, t, J = 8.0 Hz, 17α-H), 4.53 (1, t, J = 6.0 Hz, 3β-H), 2.45 (3, s, aromatic methyl-H), 2.02 (3, s, acetyl-H), 0.83 (3, t, 19-H), 0.75 (3, s, 18-H) p.p.m.; m.s. m/e: 314 (M^+ - TsOH).

Anal. Calcd. for $C_{28}H_{38}O_5S$: C, 69.10; H, 7.87; S, 6.59. Found: C, 69.05; H, 7.66; S, 6.54.

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) with zinc acetate

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) (100 mg) was dissolved in a saturated solution of zinc acetate in ethanol (30 ml). The reaction was allowed to stand at room temperature for five days, poured into water, and the products extracted with ether. The crude product which gave two major spots on t.l.c. (solvent A) corresponding to 75a and 76a was acetylated. The acetylation product was chromatographed on alumina, from which a combined fraction of 64 mg was obtained. Recrystallization from methanol gave 30 mg of material which was identical to 76b by mixed melting point and i.r. spectrum.

2β,4β-Cyclo-5α-androstane-3β,17β-diol 17-acetate (76a) with methanolic potassium hydroxide

2β,4β-Cyclo-5α-androstane-3β,17β-diol 17-acetate (76a) (100 mg) in methanolic potassium hydroxide (0.36 M, 5 ml) was refluxed for one hour. No starting material was detected on t.l.c. (solvent A) after 30 minutes of reaction. The solution was poured into water and neutralized with dilute sulfuric acid. The products (85) were extracted with ether and showed i.r. ν_{max} (CCl_4): 3620 (O-H), 2800 and 2700 (aldehyde

C-H), 1720 (aldehyde C=O) cm^{-1}; p.m.r. δ (CCl_4): 9.62 (aldehyde-H), 3.58 (17α-H), 0.78 (19-H), 0.70 (18-H) p.p.m.

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) with methanolic potassium hydroxide

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) (100 mg) was treated with methanolic potassium hydroxide by the same method described above. Spectra (i.r. and p.m.r.) of the crude product were identical to the above product from 76a.

Acetolysis of 2β,4β-cyclo-5α-androstane-3β,17β-diol 17-acetate 3-tosylate (76c)

A solution of anhydrous sodium acetate (400 mg) and acetic anhydride (1 ml) in acetic acid (10 ml) was refluxed for one hour. The solution was cooled to 100-105°, the tosylate (76c) (100 mg) was added and the resulting mixture was stirred and maintained at 100-105° on an oil-bath. The reaction was followed by t.l.c. (solvent A) at appropriate time intervals. After 20 hours the solution was poured into ice-water (100 ml) and the product immediately crystallized. The crystals were filtered and washed with water, dried in vacuo over phosphorous pentoxide at room temperature for 4 days. The crude product (98 mg, m.p. 153.5-155.5°) was further recrystallized from acetone to give a sample (82 mg) of m.p. 155-156°; the mixed m.p. of this sample with the starting material showed no depression, and give an identical i.r. spectrum with that of the starting material.

Acetolysis of 2β,4β-cyclo-5α-androstane-3α,17β-diol 17-acetate 3-tosylate (75c)

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate 3-tosylate (75c) (500 mg) was treated with a solution of anhydrous sodium acetate (2.2

g) and acetic anhydride (5.5 ml) in acetic acid (55 ml) by the same method described above. No starting material was detected on t.l.c. (solvent A) after 15 minutes of reaction time. The reaction was poured into ice-water and the products were extracted with ether. The crude product (394 mg) was chromatographed on alumina. Elution with 2-5% benzene/petroleum ether gave 93 mg crystalline material, p.m.r. δ (CCl_4): 5.75-5.32 (m, olefinic-H), 4.52 (t, 17α-H), 1.95 (s, acetyl-H), 0.91 (s, 19-H), 0.77 (s, 18-H) p.p.m.; which on crystallization from methanol gave 17β-acetoxyandrosta-2,4-diene (86), m.p. 98-99°; u.v. λ_{max} (EtOH): 266 (log ε, 3.69), 272-3 (log ε, 3.65) nm, lit.[42] m.p. 102-104°; λ_{max} (EtOH): 266 (log ε, 3.78), 272-3 (log ε, 3.74) nm.

Elution with 25-50% benzene/petroleum ether gave 113 mg which was recrystallized from methanol to give 5α-androst-2-ene-4α,17β-diol diacetate (87), m.p. 188-189°; $|\alpha|_D$ +153°; i.r. ν_{max} (CCl_4): 3030 (vinylic C-H), 1735 (ester C=O), 1728 (α,β-unsaturated ester C=O), 1650 (alkene C=C) cm^{-1}; p.m.r. δ ($CDCl_3$): 5.86 (1, m, olefinic-H), 5.73 (1, m, olefinic-H), 5.20 (1, m, 4β-H), 4.57 (1, t, J = 8.0 Hz, 17α-H), 2.02, 2.01 (6, 2s, acetyl-H), 0.95 (3, s, 19-H), 0.77 (3, s, 18-H) p.p.m.; m.s. m/e: 374 (M^+).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.86; H, 8.92.

Elution with 50-75% benzene/petroleum ether gave 67 mg which was recrystallized from methanol to give 5α-androst-3-ene-2α,17β-diol diacetate (88), m.p. 147-148°; $|\alpha|_D$ -47°; i.r. ν_{max} (CCl_4): 3030 (vinylic-C-H), 1735 (ester C=O), 1728 (α,β-unsaturated ester C=O), 1650 (alkene C=C) cm^{-1}; p.m.r. δ ($CDCl_3$): 5.59 (2, m, olefinic-H), 5.27 (1, m, 2β-

H), 4.57 (1, t, J = 8.0 Hz, 17α-H), 2.02, 2.01 (6, s, acetyl-H), 0.86 (3, s, 19-H), 0.77 (3, s, 18-H) p.p.m.; m.s. m/e: 374 (M^+).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.71; H, 9.00.

5α-Androst-2-ene-4,17-dione (89)

5α-Androst-2-ene-4α,17β-diol diacetate (87) (60 mg) was refluxed with methanolic potassium hydroxide (0,36 M, 1.5 ml) for one hour. The reaction was poured into water and the product was extracted with ether. The crude product was oxidized with Jones' reagent [43], and the resulting dione (89) recrystallized from ether (24 mg), m.p. 180-180.5° (lit.[136] m.p. 172-175°); i.r. ν_{max} (KBr): 3040 (vinylic C-H), 1730 (5-membered ring C=O), 1675 (α,β-unsaturated 6-membered ring C=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 6.81 (1, o, J = 10.0, 6.0 and 2.5 Hz, C-2 olefinic-H), 6.01 (1, o, J = 10.0, 3.0 and 1.0 Hz, C-3 olefinic-H), 0.90 (3, s, 19-H), 0.88 (3, s, 18-H) p.p.m.; m.s. m/e: 286 (M^+).

5α-Androst-3-ene-2,17-dione (90)

5α-Androst-3-ene-2α,17β-diol diacetate (88) (50 mg) was treated with methanolic potassium hydroxide and Jones' reagent [43] by the procedure described above. The crude product was recrystallized from ether to give the dione (90) (21 mg), m.p. 146-147°; i.r. ν_{max} (KBr): 3030 (vinylic C-H), 1745 (5-membered ring C=O), 1667 (α,β-unsaturated 6-membered ring C=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 6.59 (1, d, J = 10.0 and 2.0 Hz, C-4 olefinic-H), 5.99 (1, o, J = 10.0, 3.0 and 1.0 Hz, C-3 olefinic-H), 0.92 (3, s, 19-H), 0.88 (3, s, 18-H) p.p.m.; m.s. m/e: 286 (M^+).

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) with zinc-copper couple

To 2β,4β-cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) (100 mg) in ethanol (5 ml) was added freshly prepared 2% zinc-copper couple (1.5 g). Conversion of 75a to 76a was monitored by t.l.c. (solvent A) and g.l.c. After 48 hours the reaction was filtered, poured into aqueous sodium bicarbonate, and ether extracted. The crude product was acetylated followed by chromatography on alumina. A combined fraction of 58 mg corresponding to 76b was obtained. Recrystallization from methanol gave 26 mg of material identical to 76b by mixed melting point and i.r. spectrum.

2β,4β-Cyclo-5α-androstane-3α,17β-diol 17-acetate 3-succinate (75d)

A solution of succinic anhydride (1.5 g) in dry pyridine (8 ml), made by warming, was cooled to room temperature. To this solution was added 2β,4β-cyclo-5α-androstane-3α,17β-diol 17-acetate (75a) (500 mg), and the mixture was allowed to stand overnight at room temperature. The reaction was poured into 1 N sulfuric acid and the product was extracted with ether. The ether solution was washed with water, saturated sodium chloride solution, and dried over anhydrous sodium sulfate. Excess ether was evaporated under reduced pressure. The crude product was recrystallized from petroleum ether/ether to give 2β,4β-cyclo-5α-androstane-3α,17β-diol 17-acetate 3-succinate (75d) (370 mg), m.p. 134-135°; $|\alpha|_D$ +19°; i.r. ν_{max} (CCl_4): 3515 (free O-H), 3300-2500 (bonded O-H), 3030 (cyclopropyl C-H), 1736 (ester C=O), 1712 (carboxylic C=O), 1240, 1160 (C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 10.96 (1, s, carboxylic-H), 4.63 (1, t, J = 8.0 Hz, 17α-H), 4.32 (1, t, J = 6.0 Hz, 3β-H), 2.70 (4, s, succinoyl methylene-H), 2.02 (3, s, acetyl-H), 0.94 (3, s, 19-H), 0.78 (3, s, 18-H) p.p.m.; m.s. m/e: 332 (M^+ - $HOOCCH_2CH$=C=O).

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.41; H, 8.38. Found: C, 69.17; H, 8.32.

2β,4β-Cyclo-5α-androstane-3β,17β-diol 17-acetate 3-succinate (76d)

2β,4β-Cyclo-5α-androstane-3β,17β-diol 17-acetate (76a) (500 mg) was treated with succinic anhydride (1.5 g) in dry pyridine (8 ml) by the procedure described above. The crude product was recrystallized from petroleum ether/ether to give 2β,4β-cyclo-5α-androstane-3β,17β-diol 17-acetate 3-succinate (76d) (421 mg), m.p. 157-158°; $|\alpha|_D$ +13°;

i.r. ν_{max} (CCl_4): 3510 (free O-H), 3300-2500 (bonded O-H), 3040 (cyclopropyl C-H), 1736 (ester C=O), 1712 (carboxylic C=O), 1240, 1165 (C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 10.98 (1, s, carboxylic-H), 4.63 (1, t, J = 8.0 Hz, 17α-H), 3.77 (1, unresolved t, 3α-H), 2.60 (4, s, succinoyl methylene-H), 2.02 (3, s, acetyl-H), 0.86 (3, s, 19-H), 0.76 (3, s, 18-H) p.p.m.; m.s. m/e: 332 (M^+ - $HOOCCH_2CH=C=O$).

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.41; H, 8.38. Found: C, 69.59; H, 8.57.

Chromatographic separation of 2α,4α-dibromo-5α-androstane-3α,17β- and 3β,17β-diol diacetates (72b and 73b)

2α,4α-Dibromo-17β-acetoxy-5α-androstan-3-one (71) (2 g) was reduced with sodium borohydride (130 mg) in ethanol (100 ml) by the procedure used before. The crude dibromoalcohols was acetylated with acetic anhydride (20 ml) in dry pyridine (40 ml). Chromatography of the crude acetylated product (2.8 g) on neutral alumina (70 g) gave, on elution with 15-25% benzene/petroleum ether, 2α,4α-dibromo-5α-androstane-3α,17β-diol diacetate (72b) (1.7 g). Elution with benzene gave 2α,4α-dibromo-5α-androstane-3β,17β-diol diacetate (73b) (517 mg). These isomeric acetates were recrystallized from acetone and identified by mixed melting point and i.r. spectra comparison with the samples obtained previously.

Tribromination of 17β-hydroxy-5α-androstan-3-one (70)

17β-Hydroxy-5α-androstan-3-one (70) (1.0 g) in glacial acetic acid (100 ml) was treated with phenyltrimethylammonium perbromide (4.27 g) [32]. The crude product (1.95 g) was reduced with sodium borohydride (130 mg) in ethanol (80 ml) by the procedure used before.

The crude product (1.84 g) was acetylated with acetic anhydride (10 ml) in dry pyridine (20 ml). The diacetate(s) (1.95 g) was adsorbed from 5% benzene/petroleum ether onto neutral alumina (66 g). The second and third fractions from 5% benzene/petroleum ether (650 mg) was recrystallized from acetone to give 2,2,4α-tribromo-5α-androstane-3β, 17β-diol diacetate (132) (48 mg), m.p. 256-260°; $|\alpha|_D$ -15°; i.r. ν_{max} (CCl_4): 1761, 1736 (ester C=O), 1238, 1211 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 5.39 (1, d, J = 10.0 Hz, 3α-H), 4.59 (1, t, J = 8.0 Hz, 17α-H), 4.14 (1, q, J = 12.0 and 10.0 Hz, 4β-H), 3.15 (1, d, J = 15.0 Hz, 1β-H), 2.60 (1, d, J = 15.0 Hz, 1α-H), 2.26 (3, s) and 2.05 (3, s, acetyl-H), 1.20 (3, s, 19-H), 0.80 (3, s, 18-H) p.p.m.; m.s. m/e: 550, 552, 554, 556 (M^+ - HOAc).

Anal. Calcd. for $C_{23}H_{33}O_4Br_3$: C, 45.04; H, 5.42. Found: C, 45.20; H, 5.44.

P.m.r. spectrum showed that the mother liquor was a mixture of the 2,2,4α-tribromo-5α-androstane-3β,17β-diol diacetate (132) and the 2α,4α-dibromo-5α-androstane-3α,17β-diol diacetate (72b) (see below).

Elution with 5-10% benzene/petroleum ether gave an additional 543 mg crystalline material which was recrystallized from ether to give a sample which melted at 227-228°; mixed melting point with 2α, 4α-dibromo-5α-androstane-3α,17β-diol diacetate (72b) did not depress, i.r. spectra were identical.

Elution with 25-50% benzene/petroleum ether gave a total fraction (337 mg) which was recrystallized from acetone to afford the 2α,4,6β-tribromo-androst-4-ene-3β,17β-diol diacetate (133) (128 mg), m.p.

202-203°; $|\alpha|_D$ -250°; i.r. ν_{max} (CCl_4): 1754, 1736 (ester C=O), 1235, 1211 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 5.87 (1, d, J = 9.0 Hz, 3α-H), 5.59 (1, q, J = 4.4 and 2.1 Hz, 6α-H), 4.61 (1, t, J = 8.3 Hz, 17α-H), 4.37 (1, o, J = 13.8, 9.0 and 3.5 Hz, 2β-H), 2.38 (1, q, J = 13.0 and 4.0 Hz, 1β-H), 2.19 (3, s) and 2.05 (3, s, acetyl-H), 1.52 (3, s, 19-H), 0.88 (3, s, 18-H) p.p.m.; m.s. m/e: 529, 531, 533 (M^+ - Br).

Anal. Calcd. for $C_{23}H_{31}O_4Br_3$: C, 45.19; H, 5.11; Br, 39.22. Found: C, 45.37; H, 5.11; Br, 39.21.

17β-Hydroxy-5α-estran-3-one (99)

A solution of 17β-hydroxy-estr-4-en-3-one (98) (15 g) in dioxane/ether (1:1) (150 ml) was added in a steady stream to a solution of lithium (1.5 g) in anhydrous liquid ammonia (300 ml) with vigorous stirring. At the end of the addition, the solution was stirred for a further ten minutes and solid ammonium chloride was added in portions (15 g) until the blue color was discharged. The ammonia was allowed to evaporate at room temperature overnight yielding a crude solid which was extracted with ether, washed with water and dried. The ether solution was evaporated to afford a gum which was adsorbed from benzene onto alumina (560 g). Elution with 10-25% benzene/ether (7.7 l) afforded 17β-hydroxy-5α-estran-3-one (99) (9.9 g), m.p. 131-132° (lit.[46] 130-131°); i.r. ν_{max} ($CHCl_3$): 3610 (O-H), 1760 (C=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 3.64 (1, t, 17α-H), 0.77 (3, s, 18-H) p.p.m.

2ξ,4ξ-Dibromo-17β-acetoxy-5α-estran-3-one(s) (105, 106, 107)

To a solution of 17β-hydroxy-5α-estran-3-one (99) (3.7 g) in glacial acetic acid (370 ml) was added solid phenyltrimethylammonium

perbromide (11.1 g) The solution was allowed to stir at room temperature overnight and poured into ice-water (4 l). The precipitate was collected and dissolved in ether. The ether solution was washed with saturated sodium bicarbonate solution, water and dried. Evaporation of the ether under reduced pressure afford 2ξ,4ξ-dibromo-17β-acetoxy-5α-estran-3-one(s) (105, 106, 107) (6.4 g). P.m.r. δ ($CDCl_3$): 4.64 (1, t, 17α-H), 4.50-4.30 (2, m, 2ξ- and 4ξ-H), 2.03 (3, s, acetyl-H), 0.83 (3, s, 18-H) p.p.m. The crude product was reduced with sodium borohydride.

A small portion of the crude product (90 mg) was recrystallized from ether to give a sample (50 mg) of m.p. 175-178° dec., which showed the following i.r. ν_{max} ($CHCl_3$): 1724 (broad, C=O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{28}O_3Br_2$: C, 50.44; H, 5.93; Br, 33.56. Found: C, 48.75; H, 6.03; Br, 37.23.

2ξ,4ξ-Dibromo-5α-estrane-3ξ,17β-diol 17-acetate(s) (111, 112, 113)

To a solution of 2ξ,4ξ-dibromo-17β-acetoxy-5α-estran-3-one(s) (105, 106, 107) (6.3 g) in ethanol (100 ml) was added a solution of sodium borohydride (507 mg) in ethanol (10 ml). The resulting mixture was stirred for 10 minutes at room temperature and poured into ice-cooled dilute sulfuric acid. The products were extracted with dichloromethane, the extract was washed with saturated sodium bicarbonate, water, and dried over anhydrous sodium sulfate and evaporated. The crude product (6.7 g) which showed three spots on t.l.c. (solvent C) was divided into three homogeneous portions (1.3, 2.0 and 3.4 g).

Attempts at crystallization of the mixture (2.0 g) of 2ξ,4ξ-dibromo-5α-estrane-3ξ,17β-diol 17-acetate(s) from acetone, ether, and methanol was unsuccessful.

Debromination of 2ξ,4ξ-dibromo-5α-estrane-3ξ,17β-diol 17-acetate(s) (111,112,113) with zinc-copper couple

The mixture of dibromoalcohols (111, 112, 113) (3.4 g) was treated with zinc-copper couple (23.31 g) in ethanol (100 ml) by the procedure used for 73a. The reaction was worked up after 15 minutes to give a clear gum (2.33 g) which showed three spots on t.l.c. (solvent A). Extraction of the crude product with petroleum ether failed to separate the mixture. The total crude product was acetylated with acetic anhydride (12 ml) in dry pyridine (24 ml). The resulting material (2.47 g) was chromatographed on neutral alumina (87 g). Elution with petroleum ether gave 800 mg crystalline material which was not further purified. However, spectroscopic data of this fraction showed that it was a mixture of 17β-acetoxy-5α-estr-2- and -3-ene (114). I.r. ν_{max} (CCl_4): 3021 (vinylic C-H), 1733 (ester C=O), 1238 (ester C-O) cm^{-1}; p.m.r. δ (CCl_4): 5.67-5.24 (2, m, olefinic-H), 4.62 (1, t, J = 8.0 Hz, 17α-H), 1.98 (3, s, acetyl-H), 0.80 (3, s, 18-H) p.p.m.; m.s. m/e: 302 (M^+).

Elution with 5% benzene/petroleum ether gave a total fraction (300 mg) which was recrystallized from methanol to afford 2β,4β-cyclo-5α-estrane-3β,17β-diol diacetate (115) (55 mg), m.p. 117-118°; $|\alpha|_D$ +16°; i.r. ν_{max} (CCl_4): 3040 (cyclopropyl C-H), 1739 (ester C=O), 1227 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.58 (1, t, J = 8.5 Hz, 17α-H), 3.61 (1, unresolved t, $W_{½}$ = 3.0 Hz, 3α-H), 2.02 (3, s) and 1.98 (3, s, acetyl-H), 0.77 (3, s, 18-H) p.p.m.; m.s. m/e: 318 (M^+ - CH_2CO), 300 (M^+ - HOAc).

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.39; H, 9.07.

The later fractions (662 mg) eluted from the column were a mixture by t.l.c. (solvent A), however, some material crystallized from ethanol (246 mg), m.p. 125-128°; further recrystallization from ethanol gave 2β-bromo-5α-estrane-3β,17β-diol diacetate (116) (203 mg), m.p. 129-130°; $|\alpha|_D$ -1°; i.r. ν_{max} (CCl_4): 1733 (ester C=O), 1235 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.64 (1, m, $W_{\frac{1}{2}}$ = 35.0 Hz, 3α-H), 4.59 (1, t, J = 8.0 Hz, 17α-H), 4.52 (1, unresolved m, $W_{\frac{1}{2}}$ = 6.0 Hz, 2α-H), 2.09 (3, s) and 2.03 (3, s, acetyl-H), 0.80 (3, s, 18-H) p.p.m.; m.s. m/e: 380, 382 (M^+ - HOAc), 320, 322 (M^+ - 2HOAc), 301 (M^+ - (Br + HOAc)).

Anal. Calcd. for $C_{22}H_{33}O_4Br$: C, 59.86; H, 7.54; Br, 18.11. Found: C, 60.19; H, 7.57; Br, 18.40.

2ξ,4ξ-Dibromo-5α-estrane-3ξ,17β-diol diacetate(s) (147, 148, 149)

The minor portion (1.3 g) from the reduction of 105/106/107 was acetylated with acetic anhydride (7 ml) in dry pyridine (14 ml). The crude product (1.4 g) was adsorbed from petroleum ether onto neutral alumina (45 g).

The first two fractions from 10% benzene/petroleum ether were combined (237 mg) and crystallized from ether to give 72 mg crystalline material, m.p. >240° dec. Further recrystallization from ether afforded the 2α,4α-dibromo-5α-estrane-3α,17β-diol diacetate (147) (40 mg), m.p. 270-273° dec.; $|\alpha|_D$ -13°; i.r. ν_{max} (CCl_4): 1759, 1732 (ester C=O), 1235, 1215 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 5.68 (1, t, J = 2.5 Hz, 3β-H), 4.59 (1, t, J = 8.0 Hz, 17α-H), 4.09 (1, o, J = 12.5, 4.5 and 2.5 Hz, 2β-H), 3.85 (1, q, J = 11.5 and 2.5 Hz, 4β-H), 2.20 (3, s) and 2.03 (3, s, acetyl-H), 0.79 (3, s, 18-H)

p.p.m.; m.s. m/e: 458, 460, 462 (M^+ - HOAc).

Anal. Calcd. for $C_{22}H_{32}O_4Br_2$: C, 50.78; H, 6.20, Br, 30.72. Found: C, 50.64; H, 6.20; Br, 31.16.

From the mother liquor was recovered 110 mg of residue which was further recrystallized from ether to give the 2α,4β-dibromo-5α-estrane-3β,17β-diol diacetate (148) (41 mg), m.p. 179-180°; $|\alpha|_D$ -29°; i.r. ν_{max} (CCl_4): 1735 (ester C=O), 1224 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$) : 4.78 (1, q, J = 10.8 and 3.3 Hz, 3α-H), 4.60 (1, t, J = 8.0 Hz, 17α-H), 4.47 (1, q, J = 3.5 and 1.9 Hz, 4α-H), 4.39 (1, m, J = 11.6, 10.4 and 5.0 Hz, 2β-H), 2.62 (1, q, J = 10.0 and 5.0 Hz, 1β-H), 2.16 (3, s) and 2.04 (3, s, acetyl-H), 0.80 (3, s, 18-H) p.p.m.; m.s. m/e: 458, 460, 462 (M^+ - HOAc).

Anal. Calcd. for $C_{22}H_{32}O_4Br_2$: C, 50.78; H, 6.20; Br, 30.72. Found: C, 51.00; H, 6.41; Br, 30.42.

Elution with 50% benzene/petroleum ether gave 376 mg which was recrystallized from ether to afford the 2β,4α-dibromo-5α-estrane-3β, 17β-diol diacetate (149) (94 mg), m.p. 214-215°; $|\alpha|_D$ +39°; i.r. ν_{max} (CCl_4): 1736 (ester C=O), 1224 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.81 (1, q, J = 10.0 and 3.8 Hz, 3α-H), 4.66 (1, t, J = 3.6 Hz, 2α-H), 4.61 (1, t, J = 8.5 Hz, 17α-H), 4.09 (1, t, J = 10.5 Hz, 4β-H), 2.45-2.30 (2, m, 1α- and 1β-H), 2.16 (3, s) and 2.05 (3, s, acetyl-H), 0.81 (3, s, 18-H) p.p.m.; m.s. m/e: 458, 460, 462 (M^+ - HOAc).

Anal. Calcd. for $C_{22}H_{32}O_4Br_2$: C, 50.78; H, 6.20; Br, 30.72. Found: C, 50.77 ; H, 6.24; Br, 30.82.

Tribromination of 17β-hydroxy-5α-estran-3-one (99)

17β-Hydroxy-5α-estran-3-one (99) (1.1 g) in glacial acetic acid (110 ml) was treated with phenyltrimethylammonium perbromide (4.95 g) by the same method described before. The crude product (2.0 g) which showed a single spot on t.l.c. (solvent A) was reduced with sodium borohydride (150 mg) in ethanol (80 ml). The crude product from reduction was acetylated with acetic anhydride (10 ml) in dry pyridine (20 ml). The resulting diacetate (2.2 g) was chromatographed on neutral alumina (77 g). Elution with 10-25% benzene/petroleum ether gave a total fraction of 1.28 g, which was recrystallized from acetone to give the 2,2,4α-tribromo-5α-estrane-3β,17β-diol diacetate (150) (650 mg), m.p. 247-249° dec.; $|\alpha|_D$ +61°; i.r. ν_{max} (CCl_4): 1764, 1736 (ester C=O), 1230, 1209 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 5.30 (1, d, J = 10.0 Hz, 3α-H), 4.61 (1, t, J = 8.5 Hz, 17α-H), 3.91 (1, t, J = 10.5 Hz, 4β-H), 3.04 (1, q, J = 14.8 and 2.5 Hz, 1β-H), 2.24 (3, s) and 2.05 (3, s, acetyl-H), 0.81 (3, s, 18-H) p.p.m.; m.s. m/e: 536, 538, 540, 542 (M^+ - HOAc).

Anal. Calcd. for $C_{22}H_{31}O_4Br_3$: C, 44.09; H, 5.22; Br, 40.01. Found: C, 44.09; H, 5.34; Br, 39.78.

P.m.r. spectrum of the mother liquor showed no evidence for the presence of other bromo-derivatives.

5α-Androst-16-ene-3β,17-diol diacetate (23b)

5α-Androst-16-ene-3β,17-diol diacetate (23b) was prepared essentially by the procedure of Leeds et al.[68], m.p. 170-171° (lit.[68] 170-172°); i.r. ν_{max} (CCl_4):1718 (ester C=O), 1608 (alkene C=C), 1198, 1020, 1005 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 5.41 (1, m, olefinic-

H), 4.61 (1, broad m, 3α-H), 2.12 (3, s) and 1.99 (3, s, acetyl-H), 0.88 (3, s, 18-H), 0.84 (3, s, 19-H) p.p.m.

16α,17-Cyclopropano-5α-androstane-3β,17β-diol diacetate (24b)

5α-Androst-16-ene-3β,17-diol diacetate (23b) (1.1 g) was treated with the Simmons-Smith reagent as described before except that after addition of the steroid the reaction was heated in a "bomb" at 77° for 19 hours. The reaction was worked up as before and the crude product was crystallized from methanol to give 16α,17-cyclopropano-5α-androstane-3β,17β-diol diacetate (24b) (224 mg), m.p. 170-175°. Chromatography of the mother liquor on neutral alumina gave, on elution with 25-50% benzene/petroleum ether, a further 105 mg, m.p. 178-181°. Recrystallization from ether/methanol gave a sample, m.p. 179.5-182° (lit.[9] 184-185°); i.r. ν_{max} (CCl_4): 3077 (cyclopropyl C-H), 1715 (ester C=O), 1220,1020,1005 (ester C-O) cm^{-1}; p.m.r. δ (CCl_4): 4.65 (1, broad m, 3α-H), 1.92 (6, s, acetyl-H), 0.86 (3, s, 18-H), 0.82 (3, s, 19-H), 0.68-0.74 (m, cyclopropyl-H) p.p.m.

5α-Androst-2-ene-3,17β-diol diacetate (153a)

5α-Androst-2-ene-3,17β-diol diacetate (153a) was prepared by the method of Hartshorn and Wallis[58], m.p. 168-169° (lit.[67] 168-175°); i.r. ν_{max} (CCl_4): 1739 (ester C=O), 1706 (alkene C=C), 1222 (ester C-O) cm^{-1}; p.m.r. δ (CCl_4): 5.17 (1, m, olefinic-H), 4.56 (1, t, J = 8.0 Hz, 17α-H), 2.02 (3, s) and 1.96 (3, s, acetyl-H), 0.85 (3, s, 19-H), 0.78 (3, s, 18-H) p.p.m.

Simmons-Smith treatment of 5α-androst-2-ene-3,17β-diol diacetate (153a)

This reaction was carried out under the general procedure outlined before with the following variations:

	Enol acetate	Temperature	Reaction time
1.	1.0 g	35°	18 hours
2.	1.12 g	35°	45 hours
3.	1.12 g	35°	8 days
4.	0.565 g	100°	20 hours
5.	1.12 g	75-88°	18 hours
6.	1.12 g	70-75°	15 hours
7.	1.12 g	71°	36 hours

From reactions 1-3 largely starting material was recovered. Chromatography on neutral alumina gave no major fractions which indicated the presence of cyclopropane containing products in their p.m.r. spectra.

Reactions 4-7 gave similar complex mixtures indicating the presence of a little starting material by t.l.c. (solvent C). Chromatography over neutral alumina showed a similar weight distribution of fractions, however, with no major bands.

3,3-Dimethoxy-5α-androstane-17β-yl acetate (154a)

17β-Acetoxy-5α-androstan-3-one (151b) (7 g) was dissolved in dry methanol (90 ml) and a solution of p-toluenesulfonic acid monohydrate (450 mg) in dry methanol (10 ml) was added. After refluxing for 5 minutes the reaction was cooled and the resulting crystalline material filtered and washed with cold methanol containing a trace of pyridine to give 3,3-dimethoxy-5α-androstane-17β-yl acetate (154a) (4.82 g), m.p. 134-138°; recrystallization from methanol raised the m.p. to 142.5-144° (lit.[72] 143-147°); i.r. ν_{max} (CCl_4): 1730 (ester C=O), 1242,1105, 1058, 1031 (C-O) cm^{-1}; p.m.r. δ (CCl_4): 4.50 (1, unresolved t, 17α-H), 3.08 (3, s) and 3.03 (3, s, methoxy methyl-H),

1.95 (3, s, acetyl-H), 0.78 (3, s, 19-H), 0.73 (3, s, 18-H) p.p.m.

Anal. Calcd. for $C_{23}H_{38}O_4$: C, 72.98; H, 10.12. Found: C, 73.09; H, 10.22.

3-Methoxy-5α-androst-2-ene-17β-yl acetate (153b)

A solution of 3,3-dimethoxy-5α-androstane-17β-yl acetate (154a) (2.34 g) was refluxed in dry p-cymene (1.6 ml). At intervals an aliquot was spotted on a t.l.c. plate (solvent C) and the disappearance of the starting material and development of a faster spot was noted. When no starting material remained (6 hours) the solvent was evaporated at reduced pressure (1 mm) and the crystalline residue was recrystallized from ether/methanol in the presence of a trace of pyridine to afford 3-methoxy-5α-androst-2-ene-17β-yl acetate (153b) (983 mg), m.p. 126.5-129.5° (lit.[73] 127-129°); i.r. ν_{max} (CCl_4): 3096 (vinylic C-H), 1730 (ester C=O), 1672 (alkene C=C), 1242, 1227, 1075 (C-O) cm^{-1}; p.m.r. δ (CCl_4): 4.70-4.28 (2, m, olefinic and 17α-H), 3.44 (3, s, methoxy methyl-H), 1.98 (3, s, acetyl-H), 0.78 (6, s, 18- and 19-H) p.p.m.

3β-Methoxy-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155a)

3-Methoxy-5α-androst-2-ene-17β-yl acetate (153b) (2.15 g) was treated with the Simmons-Smith reagent as described previously and the reaction refluxed for 65 hours. Work up as described followed by chromatography on neutral alumina gave, on elution with petroleum ether, a crystalline product showing one spot on t.l.c. (solvent B) slower than the starting material. Recrystallization from ether/methanol gave 3β-methoxy-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155a) (792 mg), m.p. 130-135°; further recrystallization as before

yielded 720 mg, m.p. 138-140°; $|\alpha|_D$ +36°; i.r. ν_{max} (CCl_4): 3096 (cyclopropyl C-H), 1730 (ester C=O), 1242, 1036 (C-O) cm^{-1}; p.m.r. δ (CCl_4 with and without TMS): 4.53 (1, t, J = 8.0 Hz, 17α-H), 3.15 (3, s, methoxy methyl-H), 1.95 (3, s, acetyl-H), 0.80 (3, s, 19-H), 0.70 (3, s, 18-H), 0.08 (1, t, J = 4.0 Hz, cyclopropyl-H) p.p.m.; m.s. m/e: 360 (M^+).

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.61; H, 10.07. Found: C, 76.39; H, 10.28.

3β-Methoxy-2α,3-cyclopropano-5α-androstan-17β-ol (155b)

3β-Methoxy-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155a) (100 mg) was mixed with methanol (5 ml) containing concentrated hydrochloric acid (1.2 ml) followed by ether (5 ml) to effect desolution of the steroid. The reaction was stirred at room temperature and t.l.c. (solvent B) run at intervals. After 22 hours all starting material had gone and a slower spot had developed. The reaction was then poured into water and extracted with ether. The ether was washed with excess bicarbonate solution, water and dried over anhydrous sodium sulfate. Removal of the ether at reduced pressure gave a crude product (87 mg). Several recrystallization from petroleum ether gave 3β-methoxy-2α,3-cyclopropano-5α-androstan-17β-ol (155b), m.p. 109-110°; $|\alpha|_D$ +27°; i.r. ν_{max} (CCl_4): 3623 (free O-H), 3480 (bonded O-H), 3067 (cyclopropyl C-H) cm^{-1}; p.m.r. δ (CCl_4): 3.47 (1, t, J = 8.0 Hz, 17α-H), 3.16 (3, s, methoxy methyl-H), 0.84 (3, s, 19-H), 0.67 (3, s, 18-H), -0.02 (1, t, J = 4.0 Hz, cyclopropyl-H) p.p.m.; m.s. m/e: 318 (M^+).

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.05; H, 10.87.

Acid treatment of 3β-methoxy-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155a)

The steroid (155a) (121 mg) was dissolved in dioxane (4 ml) and methanol (12 ml) containing concentrated hydrochloric acid (1.44 ml). T.l.c. (solvent B) was run at intervals; as the starting material decreased two slower spots developed, one corresponding to 3β-methoxy-2α,3-cyclopropano-5α-androstan-17β-ol (155b) and the other one slower than both. After 4½ hours only the slowest spot remained. The reaction was worked up as in the previous experiment to yield after several recrystallization from ether/petroleum ether, 17β-hydroxy-2α-methyl-5α-androstan-3-one (156) (62 mg), m.p. 151.5-153° (lit.[77] 153-154°); i.r. ν_{max} (KBr): 3509 (bonded O-H), 1706 (6-membered ring C=O) cm^{-1}; p.m.r. δ ($CDCl_3$): 3.62 (1, t, J = 8.0 Hz, 17α-H), 1.05 (3, s, 19-H), 0.99 (3, d, J = 6.0 Hz, 2α-methyl-H), 0.76 (3, s, 18-H) p.p.m.

Attempted oxidation of the methoxy group in 3β-methoxy-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155a)

1. Finely divided chromic anhydride (113 mg) was added to a stirred solution of steroid (20.6 mg) in glacial acetic acid (1.1 ml). After stirring for 1 hour the reaction was added carefully to excess cold saturated aqueous sodium bicarbonate (25 ml). The mixture was extracted with ether. The ether washed with water and dried over anhydrous sodium sulfate gave no residue.

2. The above reaction was repeated on the steroid (400 mg) except that the temperature was maintained at 20° by cooling in a water bath. Extraction gave no residue. Continuous ether extraction for 20 hours gave 20 mg of uncrystallizable product. I.r. ν_{max} (CCl_4):

3571-2381 (carboxylic O-H), 1754-1695 (C=O) cm^{-1}.

3. Reaction No. 1 was repeated on the steroid (150 mg) using 1 mole equivalent of chromic anhydride (42 ml) in glacial acetic acid (5 ml) and the temperature maintained at approximately 20°. After 2 hours the reaction was worked up as before to give a residue which showed one spot equivalent to starting material on t.l.c. (solvent B). On crystallization from methanol yielded the starting material (87 mg), m.p. 132.5-136.5°.

4. Reaction No. 3 was repeated except that after 5 minutes the reaction was allowed to proceed at room temperature for 6 hours. T.l.c. (solvent B) showed only one spot with an equivalent R_f to the starting material. Worked up as before to yield starting material (107 mg), m.p. 134-136°. Mixed melting point was not depressed.

Conditions were varied so that good yields of starting material were recovered or the substance was totally converted to water soluble acidic substances. Intermediate conditions gave no evidence of formate formation.

Boron trifluoride etherate/acetic anhydride treatment of 3β-methoxy-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155a)

3β-Methoxy-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155a) (120 mg) was dissolved in acetic anhydride (4 ml) and cooled in an ice-salt bath. Boron trifluoride etherate (0.7 ml) was added and solution allowed to stand at approximately 0° for 20 hours. A t.l.c. (solvent B) at this time showed no starting material and one major slower spot. The reaction was poured into ice-water and extracted with ether, washed with excess saturated aqueous sodium bicarbonate,

water and dried over anhydrous sodium sulfate. Removal of the ether at reduced pressure yielded a brown crystalline residue (127 mg). Filtration through neutral alumina in petroleum ether gave 80 mg which on recrystallization from ether/methanol gave 41 mg, m.p. 101-105°. Further recrystallization from the same solvent gave 3-acetoxymethyl-5α-androst-2-ene-17β-yl acetate (153c) (18 mg), m.p. 106-108°; i.r. ν_{max} (CCl_4): 1745, 1738 (ester C=O) cm^{-1}; p.m.r. δ (CCl_4): 5.65 (1, m, olefinic-H), 4.59 (1, t, J = 8.5 Hz, 17α-H), 4.45 (2, s, acetoxymethyl methylene-H), 2.07 (3, s) and 2.05 (3, s, acetyl-H), 0.78 (3, s, 19-H), 0.73 (3, s, 18-H) p.p.m.; m.s. m/e: 388 (M^+).

Anal. Calcd. for $C_{24}H_{36}O$: C, 74.19; H, 9.34. Found: C, 74.27; H, 9.42.

Attempted preparation of the 3-pyranyl enol ether of 17β-hydroxy-5α-androstan-3-one (151a)

1. Steroid (0.1 mmole), p-toluenesulfonic acid monohydrate (0.01 mole) in dry, freshly distilled 3,4-dihydropyran (0.37 ml) were allowed to stand at room temperature. T.l.c. (solvent B) were ran after 1, 2, and 24 hours. The mixture contained largely starting material with the gradual development of many other products. No crystalline material was isolated.

2. Steroid (1 mmole), p-toluenesulfonic acid monohydrate (0.1 mole) were dissolved in carbon tetrachloride (50 ml) and 3,4-dihydropyran (1.2 mmole) was added. The solution was refluxed for 5 hours during which time it became brown and the t.l.c. (solvent B) showed no new products formed. At this stage 3,4-dihydropyran (5 ml) was added and refluxing continued for a further 17 hours. T.l.c. still indi-

cated a large amount of starting material and was very dark brown. No crystalline material was isolated.

3. Steroid (1 mmole) was dissolved in dry carbon tetrachloride (100 ml) and 3,4-dihydropyran (10 ml) added followed by 70% w/w perchloric acid (0.5 ml). This mixture was stirred at room temperature for 20 hours. No crystalline material was isolated from this black reaction mixture.

4. 3,4-Dihydropyran (0.2 ml) was added to carbon tetrachloride (10 ml) containing phosphorous oxychloride (10 drops) followed by steroid (1 mmole). The mixture was refluxed for 5 hours. No crystalline product was obtained.

Attempted addition of the Simmons-Smith reagent to an enolate anion

To dry 17β-acetoxy-5α-androstan-3-one (151b) (1.66 g) dissolved in dry ether (25 ml) in a dry oxygen free nitrogen atmosphere was added a 53% oil suspension of sodium hydride (264 mg) and the mixture refluxed for 3 hours *. At this time an ether solution of the Simmons-Smith reagent prepared for zinc (3 g) as described before was decanted into the mixture and refluxing continued for a further 17 hours. The reaction was then poured into water and extracted into ether solution. Evaporation yielded a residue which after chromatography over neutral alumina yielded on elution with benzene (793 mg) and crystallized from methanol, 5α-androstane-3β,17β-diol 17-acetate (152b), m.p. 146-147.5°; i.r. ν_{max} (CCl_4): 3650 (free O-H), 3500 (bonded O-H), 1736 (ester C=O), 1242, 1041 (C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.62 (1, t, 17α-H), 3.59 (1,

* A similar reaction worked up at this stage contained only starting material identified by t.l.c., i.r. and mixed m.p.

m, 3α-H), 2.02 (3, s, acetyl-H), 0.82 (3, s, 19-H), 0.80 (3, s, 18-H) p.p.m.

Oxidation of 5α-androstane-3β,17β-diol 17-acetate (152b)

Steroid (152b) (100 mg) was dissolved in acetone (6 ml) and Jones' reagent [43] added dropwise until an excess was present. Excess methanol and water were added and the organic solvents evaporated by reduced pressure. Sufficient chloroform was added and subsequently evaporated. Filtration of the solid and recrystallization from ether/methanol yielded 17β-acetoxy-5α-androstan-3-one (151b), m.p. 155-157°, which was not depressed admixture with an authentic sample. I.r.(CCl_4) were identitical.

3,3-Bis(2-chloroethoxy)-5α-androstane-17β-yl acetate (154b)

17β-Acetoxy-5α-androstan-3-one (151b) (1.1 g) was added to a solution of benzene (90 ml) and 2-chloroethanol (10 ml) containing p-toluenesulfonic acid monohydrate (100 mg). The solution was filtered with a Dean-Stark apparatus and refluxed for 3 hours, cooled, and poured into excess cold aqueous sodium bicarbonate. Washed with water, dried and evaporated at reduced pressure with minimal heating. The crude product was recrystallized from ether/methanol containing a trace of pyridine to give 3,3-bis(2-chloroethoxy)-5α-androstane-17β-yl acetate (154b) (615 mg); which on recrystallization gave an analytical sample, m.p. 94-95°; $|\alpha|_D$ +11°; i.r. ν_{max} (CCl_4): 1735 (ester C=O), 1238, 1105, 1087 (C-O), 671 (C-Cl) cm^{-1}; p.m.r. δ (CCl_4): 4.46 (1, t, J = 8.0 Hz, 17α-H), 3.54 (8, m, chloroethoxy ethylene-H), 1.93 (3, s, acetyl-H), 0.78 (3, s, 19-H), 0.72 (3, s, 18-H) p.p.m.; m.s. m/e: 474, 476, 478 (M^+).

Anal. Calcd. for $C_{25}H_{42}O_4Cl_2$: C, 63.15; H, 8.48; Cl, 14.91. Found: C, 62.90; H, 8.67; Cl, 14.74.

3(2-Chloroethoxy)-5α-androst-2-ene-17β-yl acetate (153d)

A flask containing 3,3-bis(2-chloroethoxy)-5α-androstane-17β-yl acetate (154b) (600 mg) was immersed in an oil-bath (200-210°) under reduced pressure (water pump). After 10 minutes reaction ceased and the crude product recrystallized from methanol containing a trace of pyridine to give 3(2-chloroethoxy)-5α-androst-2-ene-17β-yl acetate (153d) (200 mg), m.p. 123-126°; $|\alpha|_D$ +44°; i.r. ν_{max} (CCl_4): 1735 (ester C=O), 1675 (alkene C=C), 1238, 1196, 1087 (C-O), 671 (C-Cl) cm^{-1}; p.m.r. δ (CCl_4): 4.44 (2, m, olefinic and 17α-H), 3.68 (4, m, chloroethoxy ethylene-H), 1.94 (3, s, acetyl-H), 0.75 (6, s, 18- and 19-H) p.p.m.; m.s. m/e: 394 (M^+).

Anal. Calcd. for $C_{23}H_{35}O_3Cl$: C, 69.94; H, 8.93; Cl, 8.96. Found: C, 70.36; H, 9.08; Cl, 8.56.

3β(2-Chloroethoxy)-2α,3-cyclopropano-5α-androstane-17β-yl acetate (155d)

3(2-Chloroethoxy)-5α-androst-2-ene-17β-yl acetate (153d) (1.6 g) in dry ether (30 ml) was added to a 10 molar excess of the Simmons-Smith reagent prepared from zinc-copper couple (4 g) and methylene iodide (3.32 ml). After 24 hours reflux the i.r. spectrum still showed the enolic double bond absorption and a second 10 molar excess of reagent prepared as before was added. A further 10 molar excess was added after 24 hours and the reaction refluxed for a total of 3 days. The reaction was poured into aqueous sodium bicarbonate and ether extracted. The residue from the ether extraction was taken up in petroleum ether and chromatographed over neutral alumina.

Elution with petroleum ether gave a major fraction (733 mg) which on crystallization from methanol yielded an analytical sample (433 mg) of 3β(2-chloroethoxy)-2α,3-cyclorpopano-5α-androstane-17β-yl acetate (155d), m.p. 129-130°; $|\alpha|_D$ +23°; i.r. ν_{max} (CCl_4): 3075 (cyclopropyl C-H), 1735 (ester C=O), 1235, 1031 (C-O), 669 (C-Cl) cm^{-1}; p.m.r. δ (CCl_4 with and without TMS): 4.51 (1, t, J = 8.0 Hz, 17α-H), 3.56 (4, m, chloroethoxy ethylene-H), 1.95 (3, s, acetyl-H), 0.85 (3, s, 19-H), 0.74 (3, s, 18-H), 0.06 (1, t, J = 4.0 Hz, cyclopropyl-H) p.p.m.; m.s. m/e: 408 (M^+).

Anal. Calcd. for $C_{24}H_{38}O_3Cl$: C, 70.30; H, 9.34; Cl, 8.64. Found: C, 70.38; H, 9.28; Cl, 8.31.

2α,3-Cyclopropano-5α-androstane-3β,17β-diol (155e)

The 2-chloroethoxycyclopropane ether (155d) (1 g) in dry ether (20 ml) was cooled in an ice-bath under a nitrogen atmosphere. n-Butyl lithium in petroleum ether (6 ml) was added and the mixture stirred. After 15 minutes the reaction was poured into one equivalent of cold dilute acetic acid (200 ml). The reaction was extracted with ether, the ether was washed with aqueous sodium bicarbonate, water, dried and evaporated under reduced pressure at room temperature. The crude product was dissolved in acetone at room temperature and concentrated in a stream of nitrogen to yield the cyclopropanol (155e) (390 mg), m.p. 135-144°. Recrystallization of the heat labile substance gave an analytical sample, m.p. 145-155°. This material showed only one substance on t.l.c. in solvent C, 50% ether/benzene, 5% acetone/chloroform, and 5% methanol/carbon tetrachloride. G.l.c. analysis showed one peak (RRT 0.70); $|\alpha|_D$ +18° (dioxane); i.r. ν_{max} (KBr): 3400

(broad, O-H), 3090 (cyclopropyl C-H), 1058 (C-O) cm^{-1}; heating this disc at 180° gave a diminished 3400 peak and a new absorption at 1725 (ketone C=O) cm^{-1}; p.m.r. δ (pyridine with and without TMS): 3.83 (1, t, J = 8.5 Hz, 17α-H), 1.97 (3, s, acetyl-H), 0.93 (3, s, 19-H), 0.90 (3, s, 18-H), 0.32 (1, m, cyclopropyl-H) p.p.m.; m.s. m/e: 304 (M^+).

Anal. Calcd. for $C_{20}H_{32}O_2 \cdot \frac{1}{2}H_2O$: C, 76.69; H, 10.31. Found: C, 76.62; H, 10.61.

Anal. Calcd. for $C_{20}H_{32}O_2 \cdot \frac{1}{2}CHCl_3$: C, 67.61; H, 9.00; Cl, 14.60. Found: C, 68.60; H, 9.11; Cl, 12.01.

2α,3-Cyclopropano-5α-androstane-3β,17β-diol diacetate (155c)

2α,3-Cyclopropano-5α-androstane-3β,17β-diol (155e) was acetylated. After standing at room temperature overnight the reaction was poured into ice-water and extracted with ether to give a crude product which was recrystallized from cold acetone to give an analytical sample of 155c, m.p. 162-163°; $|\alpha|_D$ +15°; i.r. ν_{max} (CCl_4): 3090 (cyclopropyl C-H), 1735 (ester C=O), 1235, 1176, 1087 (ester C-O) cm^{-1}; p.m.r. δ ($CDCl_3$): 4.58 (1, t, J = 8.0 Hz, 17α-H), 2.02 (3, s) and 1.95 (3, s, acetyl-H), 0.92 (3, s, 19-H), 0.78 (3, s, 18-H), 0.50 (2, m, cyclopropyl-H) p.p.m.; m.s. m/e: 388 (M^+).

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 74.35; H, 9.24.

Acid treatment of 2α,3-cyclopropano-5α-androstane-3β,17β-diol (155e)

2α,3-Cyclopropano-5α-androstane-3β,17β-diol (155e) (170 mg) was dissolved in a solution (17 ml) of concerntrated hydrochloric acid in methanol (15% v/v). The resulting solution was refluxed for one hour. The reaction was poured into excess aqueous saturated sodium bicarbo-

nate and extracted with ether, the ether was washed with water, dried and evaporated under reduced pressure. The crude product (108 mg) showed one spot equivalent to 17β-hydroxy-2α-methyl-5α-androstan-3-one (156) on t.l.c. (solvent C). Recrystallization from cold acetone gave a sample, m.p. 152.5-153.5°; mixed m.p. with an authentic sample of 17β-hydroxy-2α-methyl-5α-androstan-3-one was not depressed. I.r. and p.m.r. spectra were identical to those of 156.

Base treatment of 2α,3-cyclopropano-5α-androstane-3β,17β-diol (155e)

2α,3-Cyclopropano-5α-androstane-3β,17β-diol (155e) (60 mg) was refluxed for two hours with a solution (100 ml) of methanolic potassium hydroxide (2% w/v). The reaction which showed one spot faster than the starting material on t.l.c. (solvent C) was concentrated and dilute hydrochloric acid was added. Extraction with ether gave a residue which was recrystallized from cold acetone, m.p. 146-148°; i.r. and p.m.r. spectra were identical to those of 156.

3β-Ethoxy-5,6α-cyclopropano-5α-cholestane (194)

To a solution of 3β-hydroxy-5,6α-cyclopropano-5α-cholestane (93 mg) in dry benzene (20 ml), potassium metal (280 mg) was added and the mixture refluxed for 1 hour with vigorous stirring. Ethyl iodide (4 ml) was added and reflux continued for a further 4 hours. Excess t-butanol was added to the cooled reaction mixture, and the slurry evaporated to dryness at reduced pressure. Water and dilute mineral acid were added to the residue and the resulting aqueous mixture extracted with ether. The crude reaction product was dissolved in petroleum ether and filtered through alumina. Recrystallization from acetone gave 3β-ethoxy-5,6α-cyclopropano-5α-cholestane (194), m.p. 53.5-54.5°;

$|\alpha|_D$ -50°; i.r. ν_{max} (KBr): 3077 (cyclopropyl C-H), 1105 (C-O) cm^{-1}; p.m.r. δ (CCl_4): 3.36 (2, q, J = 7.0 Hz, ethoxy methylene-H), 1.06 (3, s, 19-H), 0.60 (3, s, 18-H), 0.41-0.07 (m, cyclopropyl-H) p.p.m.

Anal. Calcd. for $C_{30}H_{52}O$: C, 84.04; H, 12.23. Found: C, 83.93; H, 12.11.

5,6α-Cyclopropano-5α-cholestan-3-one-2,2,4,4-d_4 (196)

Sodium metal (57 mg) was dissolved in dioxane (2 ml) and D_2O (0.5 ml). 5,6α-Cyclopropano-5α-cholestan-3-one (195) (100 mg) was added and the mixture was refluxed for 2 hours. The reaction mixture was poured into an aqueous buffer solution (pH 6.5) and extracted with ether. The ether solution was washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated at reduced pressure to give the deuterated derivative having 93% d_4, 7% d_3 isotope composition by mass spectrometric analysis.

3β-Hydroxy-5,6α-cyclopropano-5α-cholestane-2,2,4,4-d_4 (197)

5,6α-Cyclopropano-5α-cholestan-3-one-2,2,4,4-d_4 (196) (100 mg) was refluxed with lithium aluminum hydride (43 mg) in dry ether (10 ml) for 3 hours. Excess hydride was destroyed with ethyl acetate. The reaction mixture was poured into water acidified with dilute hydrochloric acid, and extracted with ether. The crude product gave two spots on t.l.c. (solvent C) corresponding to the 3α- and 3β-alcohols. Chromatography on alumina gave 197 on elution with 75% benzene/petroleum ether, m.p. 156-156.5°; (82% d_4, 12% d_3, 5% d_2).

3β-Hydroxy-5,6α-cyclopropano-5α-cholestane-3α-d_1 (198)

A solution of 5,6α-cyclopropano-5α-cholestan-3-one (195) (150 mg) in dry ether (10 ml) was treated with lithium aluminum deuteride (57

mg) by the same method as 196. Chromatography on alumina gave, on elution with 75% benzene/petroleum ether, 3β-hydroxy-5,6α-cyclopropano-5α-cholestane-3α-d_1 (198) (80 mg), recrystallized from petroleum ether, m.p. 155-156.5°; (97% d_1, 3% d_0).

3β-Ethoxy-5,6α-cyclopropano-5α-cholestane-3α-d_1 (199)

3β-Hydroxy-5,6α-cyclopropano-5α-cholestane-3α-d_1 (198) (37 mg) was treated in the same way as 3β-hydroxy-5,6α-cyclopropano-5α-cholestane. The crude reaction product was filtered through a column of alumina, and recrystallized from acetone to give 199, m.p. 53-54°; (96% d_1, 4% d_0).

3β-Ethoxy-5,6α-cyclopropano-5α-cholestane-2,2,4,4-d_4 (200)

3β-Hydroxy-5,6α-cyclopropano-5α-cholestane-2,2,4,4-d_4 (197) (55 mg) was treated by the same method as 3β-hydroxy-5,6α-cyclopropano-5α-cholestane. The crude product in petroleum ether was filtered through a column of alumina, and recrystallized from acetone to give 200, m.p. 53-54°; (81% d_4, 12% d_3, 3% d_2).

3α-Hydroxy-5,6α-deuteriocyclopropano-5α-cholestane-d_2 (201)

A mixture of zinc dust (3.93 g) and cuprous chloride (595 mg) in dry ether (10 ml) was refluxed for 30 minutes. To this mixture CD_2I_2 (2.4 ml) was added and reflux continued for another 30 minutes. Epicholesterol (1.15 g) was added and the reaction mixture refluxed for 3 hours. The reaction was poured into excess sodium bicarbonate and the products extracted with ether. The ether solution was washed with water, saturated sodium chloride solution, dried over sodium sulfate, and evaporated at reduced pressure. Chromatography of the crude product on alumina gave, on elution with benzene/petroleum ether, 3α-

hydroxy-5,6α-deuteriocyclopropano-5α-cholestane-d_2 (201) (944 mg) which was recrystallized from acetone, m.p. 120-121°; (87% d_2, 7% d_1, 6% d_0).

5,6α-Deuteriocyclopropano-5α-cholestan-3-one-d_2 (202)

3α-Hydroxy-5,6α-deuteriocyclopropano-5α-cholestane-d_2 (201) (617 mg) was dissolved in acetone (40 ml) and oxidized with Jones' reagent. Recrystallization of the crude product from methanol afforded 202 (500 mg), m.p. 144°.

3β-Hydroxy-5,6α-deuteriocyclopropano-5α-cholestane-d_2 (203)

5,6α-Deuteriocyclopropano-5α-cholestan-3-one-d_2 (202) (490 mg) in dry ether (50 ml) was reduced with lithium aluminum hydride (250 mg) by the same method as 196. Chromatography of the crude product gave, on elution with benzene/petroleum ether, 203 (362 mg), recrystallization from petroleum ether afforded 236 mg, m.p. 155-156°; (91% d_2, 5% d_1, 4% d_0).

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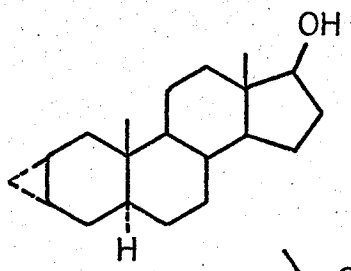
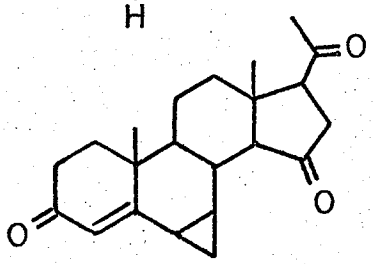
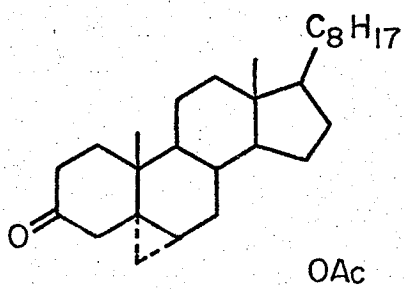
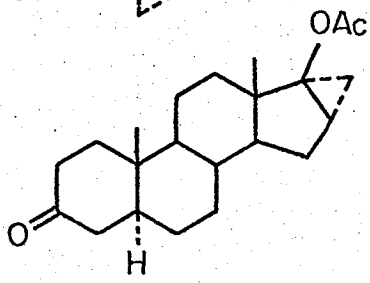
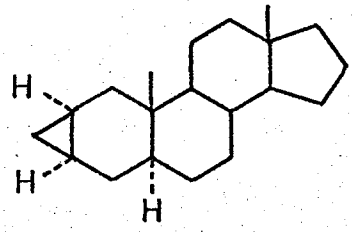
APPENDIX

In the nomenclature of cyclopropane containing steroids, ambiguity arose in that different terms have been used in literatures by several authors to designate the additional "CH_2" fragment, which is attached to two adjacent carbon atoms of the parent steroid molecule. These are listed in the following:

Structure	Nomenclature	Reference
	2α,3α-Methano-5α-androstan-17β-ol	75
	15-Keto-6β,7β-methyleneprogesterone	94
	5,7β-Cyclo-B-homo-5α-cholestan-3-one	115
	17β-Acetoxy-3'H-cycloprop|16α,17|-5α-androstan-3-one	9, 93
	2α,3α-Dihydro-2'H-cyclopropa|2,3|-5α-androstane	137

The term "cyclopropano" has been employed in a review article [118] and is used most frequently in practice. This term was chosen in naming the compounds reported because of its frequency in literatures, its simplicity and its clarity in indicating the presence of a three-membered ring.