STUDIES IN THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF A-RING STEROID DERIVATIVES

by

Volker G. Paslat

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ABSTRACT

The isosteric and isoelectric relationships between the carbon-carbon double bond and the cyclopropane carbon-carbon bond of cyclic fusion of a variety of steroid derivatives are examined. These relationships may alter the biological activity and/or the biological potency of pharmacologically active steroid derivatives. The effect of the cyclopropane ring and the carbon-carbon double bond on the biological activity of pharmacologically active steroids is contrasted. Evidence suggests that the contribution of the cyclopropane ring and the carbon-carbon double bond to the biological activity of pharmacologically active steroids may be correlated, in part, to the contribution which these substituents make to the partition coefficient of the steroid molecule.

The preparation of 2α,3-dibromocyclopropano-5α-androstane-3β,17β-diol diacetate (94) from 5α-androst-2-ene-3,17β-diol diacetate (93) and dibromocarbene, prepared by the phase transfer technique, is described. Reduction of the dibromide (94) with the Zn/Cu couple and/or Raney nickel gave, exclusively, the monobromide 2α,3-(endo)-bromocyclopropano-5α-androstane-3β,17β-diol diacetate (97). Attempts to effect

complete dehalogenation utilizing a variety of other reductive procedures was unsuccessful in all cases.

The addition of the Simmons-Smith reagent to the enol acetate 93 and 3-trimethysilyloxy-17β-acetoxy-5α-androst-2-ene (115) is described.

The proton magnetic spectra of the brominated cyclopropanosteroids 94 and 97 and other C-2 cyclopropanosteroid derivatives indicated that the cyclopropane ring demonstrates a shielding effect which appears to operate through large distances. The mass spectra of the brominated cyclopropanosteroids 94 and 97, and other C-2 cyclopropanosteroid derivatives showed, with few exceptions, similar fragmentation patterns.

The preparation of 3,3-deuterio-17β-hydroxy-5α-androstane-d_2 (125) and 17β-hydroxy-5α-androstane (126) is described. Similarly, the preparation of 2α,3α-cyclopropano-17β-hydroxy-5α-androstane (118) and 2α,3α-cyclopropano-2β-4,4-deuterio-17β-hydroxy-5α-androstane-d_3 (121) is described.

Androgenic/myotropic assays of 118 and 121 suggested that metabolic activation at C-2 and/or C-4 accounted for the enhanced biological activity of 118 relative to 121. Androgenic/myotropic assays of 125 and 126 indicated that factors other than metabolic activation (of the A-ring) may account for the enhanced biological activity of 125 relative to 126.

TABLE OF CONTENTS

Page

B. PROGESTINS Continued

Page

B. PROGESTINS Continued

Page

C. ANTI-ANDROGENS Continued

Page

Page

Page

Page

Page

III. EXPERIMENTAL

Page

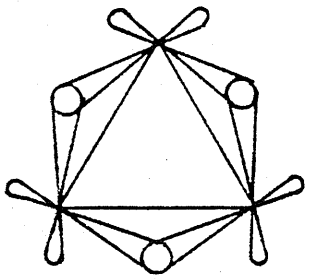
I. INTRODUCTION

A. THE CYCLOPROPANE RING

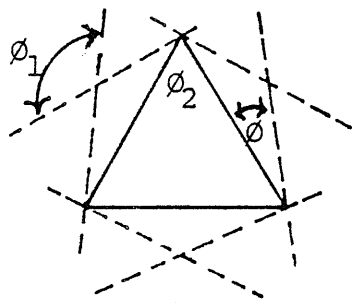
(i) The pseudo-unsaturated carbon-carbon bonds of the cyclopropane ring.

(a) Correlation with the carbon-carbon double bond.

The Coulson and Moffit model of the cyclopropane ring (Figure Ia) shows that the molecular orbitals which constitute the cyclopropane carbon-carbon bonds do not pass through the axis of the carbon atoms but are bent away from the axis of the carbon atoms (Figure Ib) by an angle of $22^{\circ}(\emptyset)$. Therefore, the angle between the carbon-carbon bonds of the cyclopropane fing ($\emptyset_1$) is 104° rather than $60^{\circ}(\emptyset_2)$. A 60° bond angle is the expected value if the molecular orbitals which constitute the bonds of the cyclopropane ring passed along the axis of the carbon atoms [1,2].



a



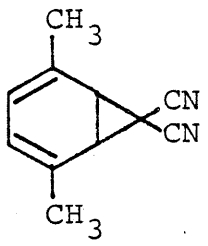
b

$\emptyset = 22^{\circ}$; $\emptyset_1 = 104^{\circ}$

$\emptyset_2 = 60^{\circ}$

Figure I. The Coulson and Moffit model of the molecular orbitals of the cyclopropane ring.

Electron density X-ray diffraction studies confirm the "bent bond" concept for the cyclopropane ring[1]. For example, the molecular orbitals that constitute the carbon-carbon bonds of the substituted cyclohexadiene derivative I have been shown to be bent away from the axis of the carbon atoms by 20°, i.e. $\phi = 20^{\circ}$. Therefore, as these molecular orbitals are bent, they are strained. It has been shown that each carbon-carbon bond of the cyclopropane ring possesses 8.68 kcal/mole strain energy. This excess bond energy accounts for the high reactivity of the cyclopropane ring.



I

The bent carbon-carbon bonds of the cyclopropane ring do not permit perfect overlap of the s molecular orbitals. Therefore, a considerably large proportion of the molecular orbital picture for the cyclopropane carbon-carbon bond constitutes p molecular orbitals. Calculations obtained by valence bond perfect-pairing approximation and minimization of the bond energies indicate that the carbon-carbon bonds of the cyclopropane ring are in fact $sp^{4.12}$ hybridized[1,2] and the carbon-

hydrogen bonds are $sp^{2.28}$ hybridized. Therefore, the molecular orbitals that constitute the carbon-carbon bonds of the cyclopropane ring, bear resemblance to the molecular orbitals that constitute the carbon-carbon double bond (sp^2 hybridized).

(b) The symmetrically bent cyclopropane carbon-carbon bond.

The symmetrically bent carbon-carbon bond[2] of the cyclopropane ring is the bond that is most frequently encountered in cyclopropane substituted cyclic and polycyclic systems. The bent bond can be referred to as the Type I cyclopropane bond, Figure II.

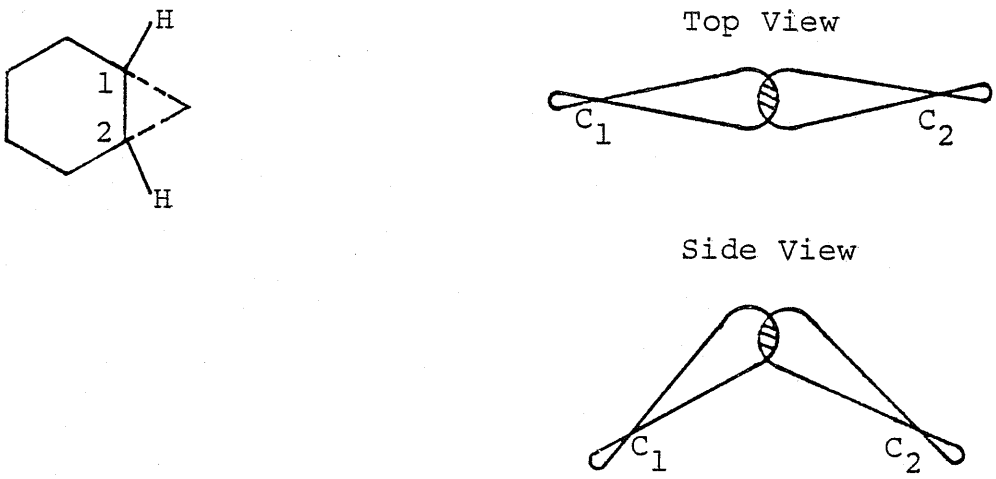


Figure II. Side and top view of the C-1(2) bond of a cis-substituted cyclopropane ring derivative.

(c) The non-symmetrically bent or twist bent cyclopropane carbon-carbon bond.

The twist bent cyclopropane carbon-carbon bond[2,3] represents a highly strained bond. The strain in these bicyclic structures arise from the inherent torquing which results when the cyclopropane ring is trans-fused to a cyclic molecule (Figure III). Twist bent, Type II, cyclopropane carbon-carbon bonds

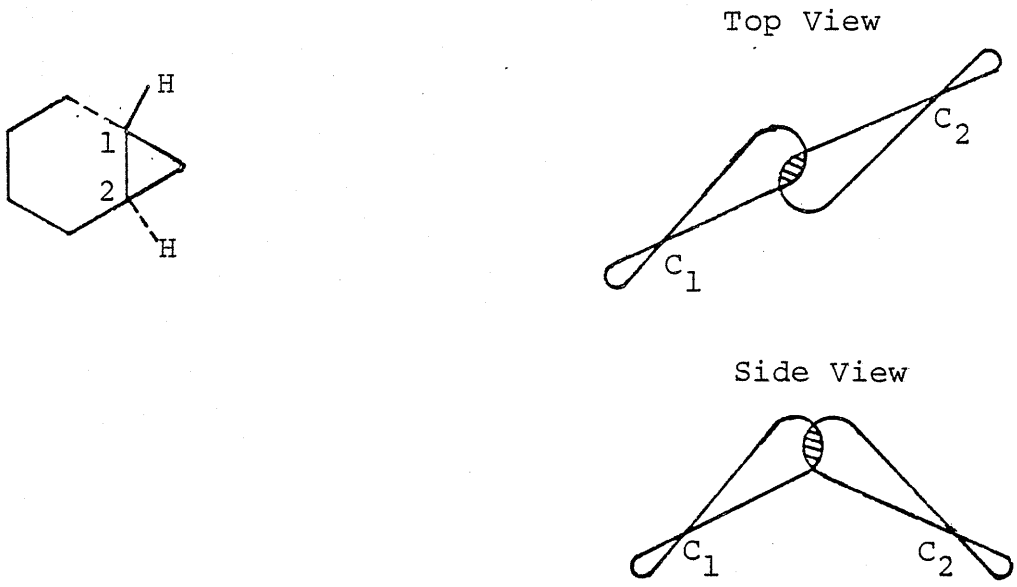
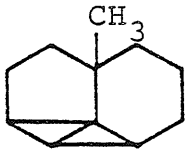


Figure III. Side and top view of the C-1(2) bond of a trans-substituted cyclopropane ring derivative.

demonstrate exceptionally high reactivity towards nucleophilic substances.[2] The polycyclic compound 2 and the bicyclic compound 3 are examples of substances demonstrating a Type II cyclopropane carbon-carbon bond [2,3].



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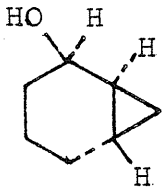


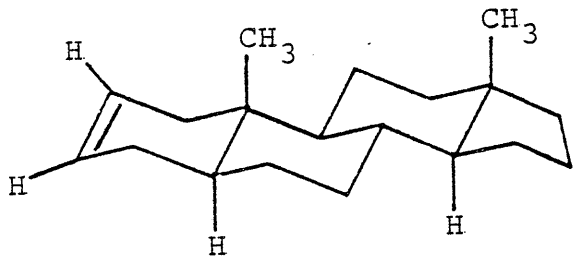
3

(ii) The isosteric and isoelectric relationship of the carbon-carbon bonds of the cyclopropane ring and the carbon-carbon unsaturated bond.

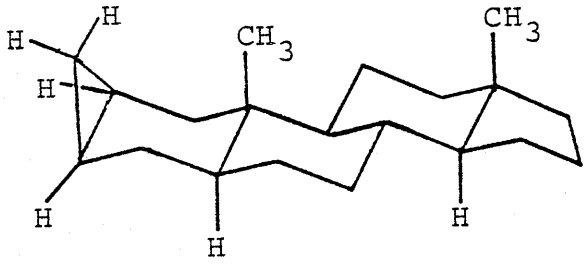
The carbon-carbon double bond possesses spatial geometry. The terms cis and trans express this geometric variance. Nevertheless, no stereochemical terms are required to define the carbon-carbon double bond. In contradistinction, the cyclopropane ring fused to a cyclic compound requires stereochemical identification. That is, the cyclopropane ring may lie above or below the plane of the cyclic structure to which it is fused and the configurational terms of β or α, respectively, must be employed.

Cyclic structures substituted with a cyclopropane ring can therefore demonstrate stereoisomerism, that is, isomers that differ only in the spatial arrangements of their atoms. The stereoisomerism associated with the cyclopropane ring fused to a cyclic substance allows therefore only one carbon-carbon bond and, furthermore, only one side of that carbon-carbon bond to be isoelectric

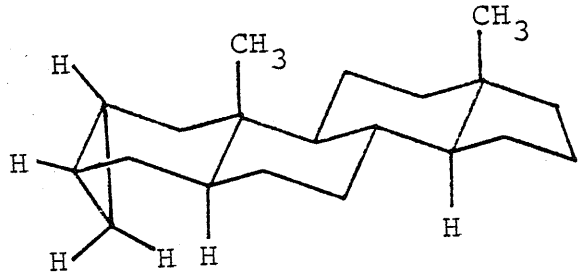
and isosteric with a carbon-carbon unsaturated bond. This bond is the one which is common to both the cyclopropane ring and the cyclic structure, i.e., the carbon-carbon bond of cyclic fusion (Figure IV). The stereochemical and isomeric relationship between the cyclopropane carbon-carbon bond and the carbon-carbon unsaturated bond may have a profound effect on the biological activity and potency of steroidal olefins and their cyclopropyl substituted analogues.



5α-androst-2-ene



2β, 3β-cyclopropano-5α-androstane. The C-2 bond is isoelectric and isosteric on the α-surface with the C-2 site of unsaturation of 5α-androst-2-ene.



2α, 3α-cyclopropano-5α-androstane. The C-2 bond is isoelectric and isosteric on the β-surface with the C-2 site of unsaturation of 5α-androst-2-ene.

Figure IV. The isosteric and isoelectric relationship between the carbon-carbon double bond and the bond of cyclic fusion.

(iii) Physical properties of the cyclopropane ring in relation to the carbon-carbon double bond.

(a) Substituent partition coefficient

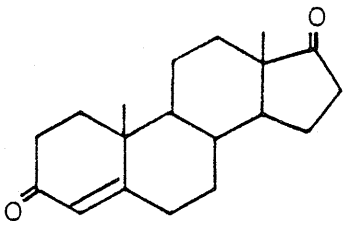
The substituent partion coefficient [4,5] of the cyclopropane ring designated $\pi_{cyclopropane}$, is 1.21 log units[6] whereas the substitutent partition coefficient of the carbon-carbon unsaturated bond is 0.73 log units[5,7]. Therefore, when a cyclopropane ring is substituted for a carbon-carbon double bond, the partition coefficient of the substance is enhanced by approximately 0.5 log units. As the partition coefficient of a (drug) substance is known to alter the distribution, metabolism and excretion of that substance, the effect on the partition coefficient of a substance by the cyclopropane ring may have a profound effect on the biological activity and/or potency of a biologically active substance.

(b) Isomerization

(i) The carbon-carbon double bond

The isolated carbon-carbon double bond has been shown to isomerize *in vivo*[8,9]. The isomerization process may be enzyme induced. For example, androst-4-ene-3,17-dione(4) is a major metabolite of 3β-hydroxy-androst-5-en-17-one(5)[8]. The C-5 labile site of unsaturation may be protected from *in vivo* biotransformation through substitution with an isosteric and

isoelectric C-5 cyclopropane ring, for example as in 6.



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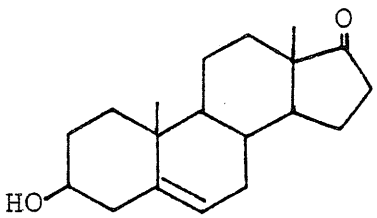

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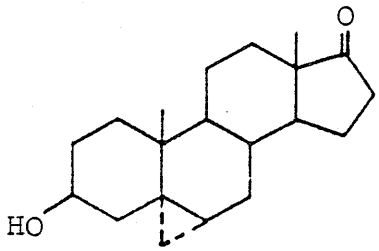

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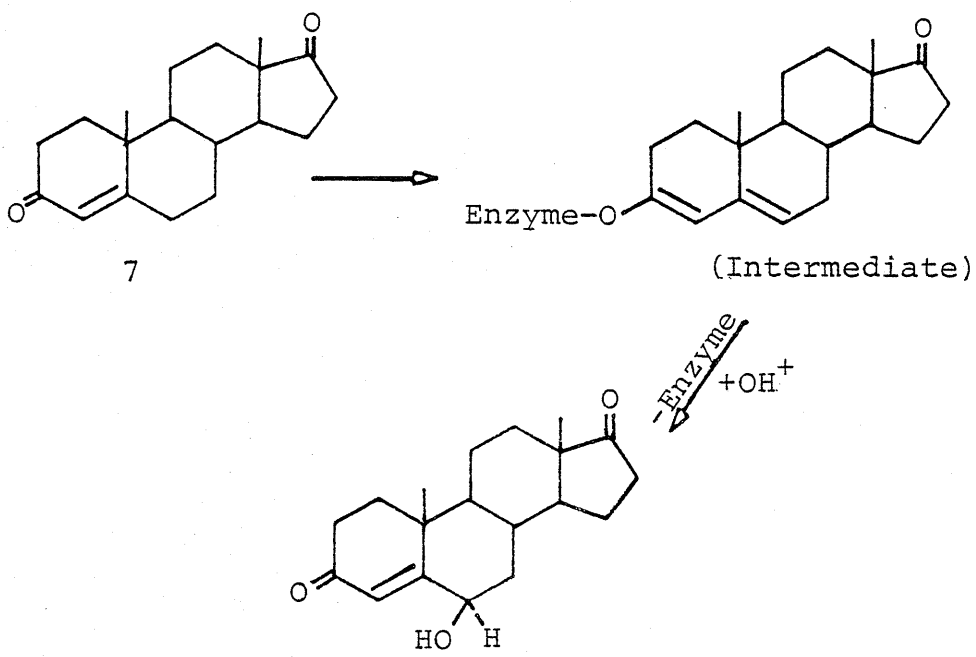
(ii) α,β-Unsaturated ketones

The 6β-hydroxylation of androst-4-ene-3,17 dione (7) is believed to proceed through the enzyme induced enolization mechanism depicted in Scheme I[9]. Substitution of the C-4 double bond or the C-5 double bond with an isosteric and isoelectric cyclopropane ring would impair or prevent the enolization step (Scheme I) and thereby impair or prevent the C-6 oxidation of this substance by this mechanism. The cyclopropane ring is known to demonstrate orbital overlap with the molecular orbitals

of the carbonyl substitutent [10,11,12] and therefore biological activity attributable to this parameter is maintained.

Scheme I



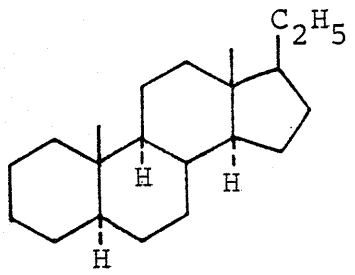
7 (Intermediate)

B. PROGESTINS

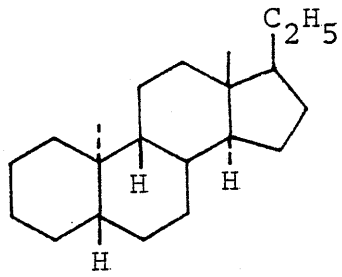
(i) 17α-acetoxy-(9β,10α)-pregnane derivatives

(a) Biological significance of the (9β,10α)-stereochemistry

Pregnane derivatives with the stereochemistry inverted at C-9 and C-10 (9β,10α) are potent progestational agents in the rat (Figure V)[13,14,15,16,17]. The natural stereoisomers (9α,10β) of these retro-pregnanes are the cyproterone acetate series of potent anti-androgens[19]. Natural[19] and retro-pregnane derivatives [15,16,17] substituted with a cyclopropane ring in the A or B-rings, also demonstrate anti-androgenic and progestational activity, respectively. The cyclopropane ring in the natural pregnane series is in the α-configuration whereas the cyclopropane ring in the retro-pregnane series has the β-configuration[17,18].



(9α,10β)-Pregnane
Natural Steroid
Nucleus

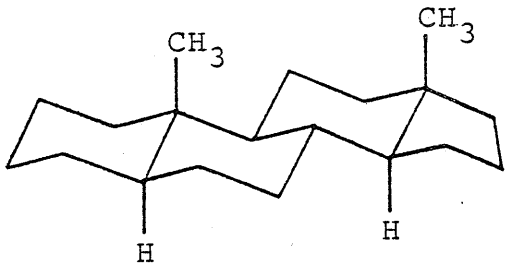


(9β,10α)-Pregnane
Retro-Steroid
Nucleus

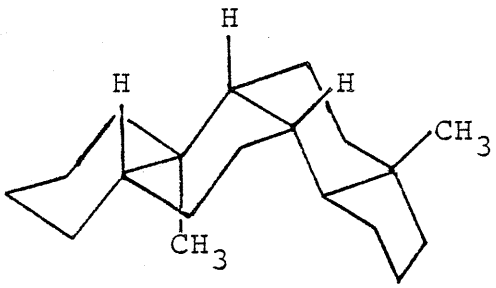
Figure V. Stereochemistry of the natural and retro-pregnane steroids.

Figure VI is a representation of the configuration of the natural and retro-pregnane steroids. The stereochemistry at C-9 and C-10 significantly alters the configuration of the two stereoisomeric pregnane derivatives. The 9β,10α-configuration of the retro-pregnane nucleus may be best described as concave whereas the 9α,10β-configuration of the natural pregnane nucleus may be best described as planar[13].

A concave configuration in the steroid nucleus appears to be associated with progestational activity whereas a planar configuration in the steroid nucleus appears to be associated with anti-androgenic or androgenic activity. For example, the 5β-androstane derivative 16α,17α-difluorocyclopropano-5β-androstane-3β,17β-diol diacetate(8) is a progestational agent[20]. The 5β-androstane progestin 8 has the A/B-ring junction in the cis configuration and demonstrates a configuration of the A-ring which is similar to the configuration of the retro-pregnane nucleus shown in Figure VI. The 5α-androstane derivative 16α,17α-difluorocyclopropano-5α-androstane-3β,17β-diol-diacetate(9)[21], which demonstrates androgenic activity, has the A/B-ring junction in the trans configuration and demonstrates a configuration of the A-ring which is identical to that of the natural pregnane nucleus shown in Figure VI. Recent evidence suggests that the (9β,10α)-steroid nucleus (Figure VI) may take on a more planar conformation[22]. Nevertheless, a planar conformation as that observed for the natural series of steroids would not be anticipated.

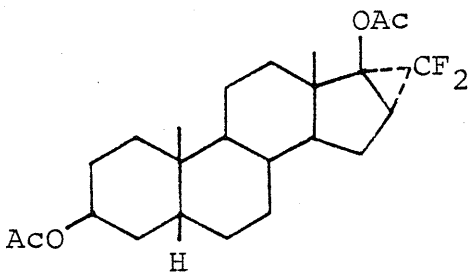


Natural (9α,10β)-
Steroid Nucleus
Configuration



Retro-(9β,10α)-
Steroid Nucleus
Configuration

Figure VI. The planar and concave configurations of the natural (9α,10β) and retro-(9β,10α)-pregnane steroid nuclei.



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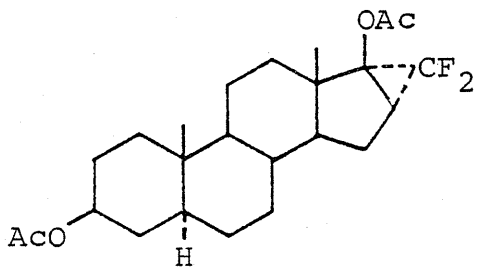

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Figure VII shows the concave nature of the A-ring of the 5β-androstane progestin 8. Consistent with the above discussion is the observation that the planar structure pregna-4,16-diene-3,20-dione(10) is completely void of progestational activity[13]. Compound 10 is the 16,17-dehydro derivative of the naturally occurring progestin, progesterone.

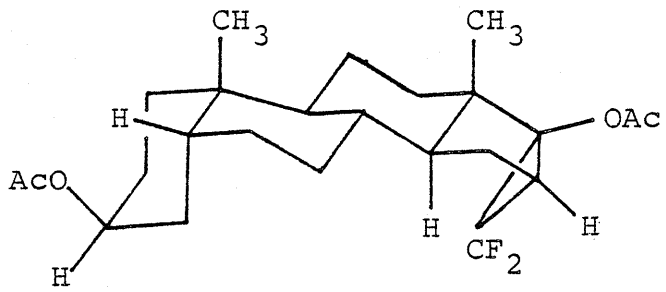

Figure VII. The concave configuration of the 5β-androstane derivative 8.

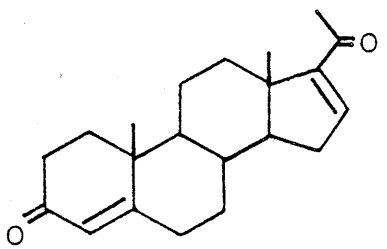

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Table I shows the progestational potencies of a series of related retro-pregnane derivatives.

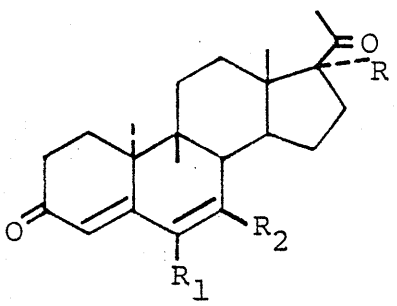
TABLE I

Progestational potencies of a series of related retro-pregnane derivatives relative to the natural pregnane progestin, progesterone[14].

Compound	Progestational[a] Potency
1	6.42
2	34.3
3	28.0
4	78.7
progesterone	1.0

[a]The progestational potency of progesterone was arbitrarily set at 1.0.

Key to Table I



1; R = H, R_1 = R_2 = 2H
2; R = H, R_1 = R_2 = H
3; R = OAc, R_1 = R_2 = 2H
4; R = OAc, R_2 = R_2 = H
5; progesterone

Table I shows that the retro-pregnane (9β,10α)-pregn-4-ene-3,20-dione(1) demonstrates 6.4X the progestational activity of its natural stereoisomer progesterone pregn-4-ene-3,20-dione, in the Clauberg progestational assay. Therefore, the (9β,10α)-stereochemistry of the retro-pregnane progestins enhances the progestational activity relative to the natural progestin progesterone. The 17α-acetoxy substitutent enhances the progestational activity of both the natural and retro-pregnane derivatives[23].

(b) The effect of unsaturation on the progestational activity of the (9β,10α)-pregnanes.

The retro-pregnane 17α-acetoxy-(9β,10α)-pregna-4,6-diene-3,20-dione(11) demonstrates 2.8X the progestational activity of the C-6 saturated analogue 17α-acetoxy-(9β,10α)pregn-4-ene-3,20-dione(12). The introduction of a C-6 site of unsaturation has therefore an enhancing effect on the progestational activity of these C-4 unsaturated retro-pregnanes[14,16].

(i) Alteration of the partition coefficient.

The substituent partition coefficient for the conjugated carbon-carbon double bond ($\pi_{-CH:CH-CH:CH-}$) has a substituent partition coefficient value of 1.36 log units[5]. A methylene carbon atom has π=0.50 (see Appendix I). Therefore, the calculated partition coefficient of

11 is approximately 0.40 log units lower than the calculated partition coefficient of 12. The increased hydrophilic property of 11 may therefore diminish the overall vascular penetration[13] by two mechanisms, (a) decreased affinity for lipophilic tissues, and (b) increased affinity for plasma (carrier) proteins. Hydrophilic steroid derivatives have been shown to bind more firmly to epidermal stratum corneum than their more lipophilic analogues[25]. In a similar sense, the increased hydrophilic character of 11 may concomitantly enhance the binding affinity for progestin receptors.

Hepatic metabolic transformations require that the substrate leave plasma bound proteins. As 11 may demonstrate higher affinity for plasma proteins than hepatic (lipid rich) cell membranes the catabolism attributable to hepatic biotransformation may be diminished, thereby extending the biological half-life of this substance. Alternatively, the enhanced progestational activity of 11 may be mediated through the ability of the C-6 site of unsaturation to function as a nucleophilic site to which charged progestin receptor sites may bind[26].

The polyunsaturated retro-pregnane 17β-acetoxy-(9β,10α)-pregna-1,4,6-triene-3,20-dione(13) demonstrates 0.25X the progestational activity of the C-4/C-6 unsaturated diene 11[16]. The calculated partition

coefficient of 13 is 0.27 log units lower than the calculated partition coefficient of 11. Therefore, 13 will tend to remain with solubilized plasma proteins to a greater extent than 11. This behavior is a reflection of the hydrophilic character of the highly unsaturated A-ring of 13[5]. As the optimum partition coefficient for potent progestational potency in these unsaturated retro-pregnanes is that one associated with 11, alteration from this optimum partition coefficient may be expected to result in the alteration of the distribution and/or the metabolism of these substances[24]. The tendency, i.e. the free energy[27,28,29] of 13 to leave the aqueous environment of the solubilized plasma proteins and partition onto lipid rich (target) cell membranes may be very low[5a]. Hence, 13 should demonstrate diminished affinity for the progestin receptor. Table II summarizes the progestational potencies of the retro-pregnane progestins and shows the partition coefficients for these substances. Figure VIII shows that there is no simple relationship between the partition coefficient of these three molecules and their biological response[7]. Nevertheless, combined with the data from other related compounds, the biological response is consistent with a linear correlation with the partition coefficient (Figure XI).

TABLE II

Progestataional activities and partition coefficients of a series of related retro-pregnanes [16].

Compound	Progestational[a] Activity	Partition Coefficient
11	2.8	2.36
12	1.0	2.73
13	0.25	2.09

[a]The progestational potencies are normalized relative to the progestational activity of 12.

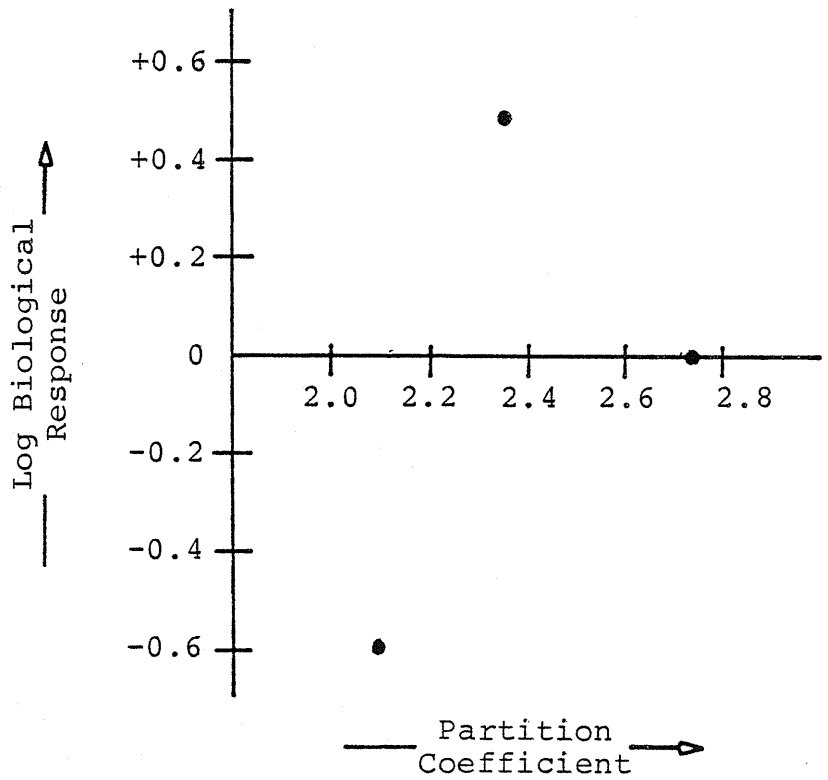
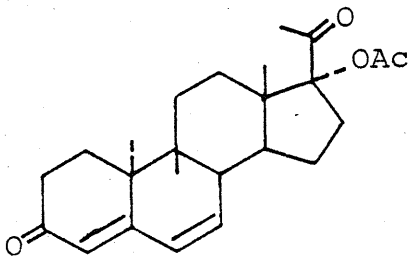


Figure VIII. Plot of the partition coefficient versus the log of the biological response of a series of related retro-pregnane derivatives.

(ii) Metabolic transformations.

It is known that 1,2-dehydrosteroids, e.g. 11β, 17α,21-trihydroxy-pregna-1,4-diene-3,20-dione(14), prednisolone, demonstrate biological half-lives ($t^{\frac{1}{2}}$) which are 2X to 2.5X greater than the biological $t^{\frac{1}{2}}$ of the corresponding 1,2-dihydro analogues, e.g. 11β,17α, 21-trihydroxy-pregn-4-en-3,20,dione(15), cortisol[13]. The diminished $t^{\frac{1}{2}}$ of 15 can be attributed to an enhanced rate of A-ring reduction relative to the diene 14. The C-1 site of unsaturation therefore impairs the A-ring reduction of these substances. By analogy, the $t^{\frac{1}{2}}$ of the C-1 unsaturated progestin 13 should be 2X to 2.5X greater than the $t^{\frac{1}{2}}$ of the C-1 saturated analogue 11. As 13 is significantly less effective a progestational agent than 11,A-ring metabolic transformation may be a pre-requisite to progestational activity. The biological activity of progesterone is believed to involve biologically active metabolites[30].



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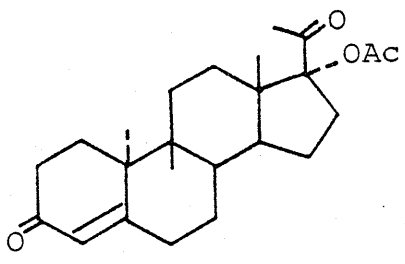

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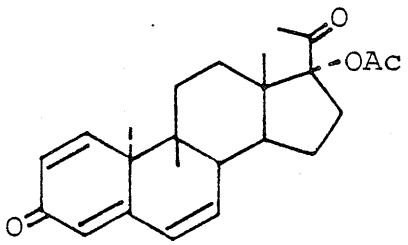

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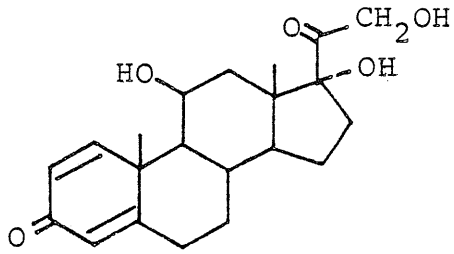

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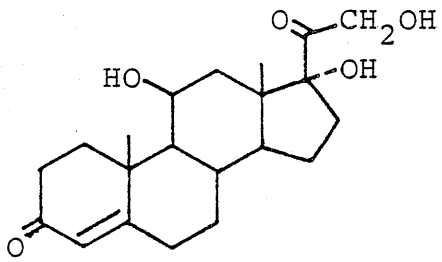

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(iii) Correlation of the A-ring conformation with the progestational potency of A/B-ring unsaturated retro-pregnanes.

The planarity of the A-ring and the angle ($\emptyset$) between the A-ring plane and the C-3 carbonyl function vary throughout the unsaturated retro-pregnanes (11-13) discussed above. Similarly, the angle ($\emptyset_1$) between the C-10 methyl substituent and the C-5(10) bond varies in these progestin derivatives. The variations in $\emptyset$ are greater than those for $\emptyset_1$. Nevertheless, there appears to be a relationship between the progestational activity of these unsaturated retro-pregnanes and the two parameters, $\emptyset$ and $\emptyset_1$, discussed above. Figure XI

shows the most stable Dreiding model conformations of these A/B ring unsaturated retro-pregnanes. The data of Figure IX are tabulated in Table III.

The data of Table III suggests that low progestational activity is associated with a planar A-ring conformation and a C-3 carbonyl substituent in close proximity to the A-ring plane, e.g. 13. Conversly, the data of Table III suggests that potent progestational activity is associated with a non-planar A-ring conformation and a C-3 carbony substituent in close proximity to the A-ring plane, e.g. 11. Furthermore, the intermediate progestational activity of 12 correlates with an A-ring conformation and an angle Ø that is intermediate to that of 11 and 13.

The conformational analysis discussed above suggests that the ability of these unsaturated retro-pregnane derivatives to bind with target cell recognition sites and/or progestin receptors decreases as the planarity of the A-ring increases and Ø becomes smaller[31]. Conversly, the binding effectiveness appears to increase as the A-ring conformation approaches the boat conformation in which Ø becomes large.

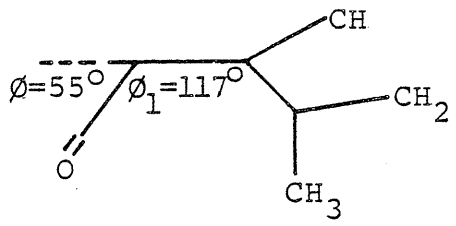
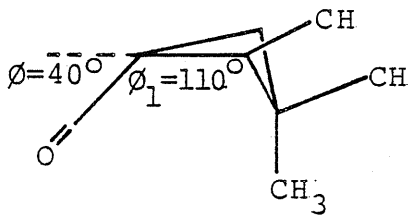
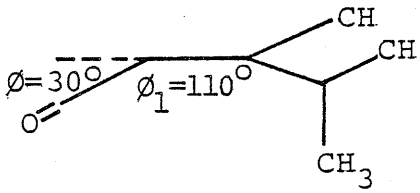
Compound	A-Ring Conformation
11	
12	
13	

Figure IX. Most stable Dreiding model conformations of the retro-pregnanes 11, 12 and 13 as seen through the imaginary axis passing through C-2 and C-4.

TABLE III

Correlation of ϕ and ϕ_1 with the progestational potencies[16] of a series of related unsaturated retro-pregnanes.

Compound	ϕ	ϕ_1	Progestational Potency	A-ring Conformation
11	55°	117°	2.8	skewed-boat
12	40°	110°	1.0	skewed-chair
13	30°	110°	0.25	planar

(c) The effect of a 1β,2β-cyclopropano substituent

The cyclopropano retro-pregnane 1β,2β-cyclopropano-17β-acetoxy-(9β,10α)-pregna-4,6-diene-3,20-dione(16) demonstrates potent progestational activity in the rat[16]. Table IV shows the progestational potency of 16 and other related unsaturated retro-pregnanes. The data of Table IV indicates that the 1β,2β-cyclopropano substituent significantly enhances the progestational potency of these unsaturated retro-pregnane derivatives.

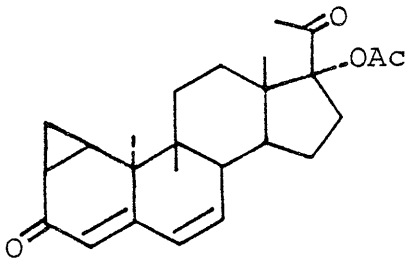


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TABLE IV

Progestational potencies of a series of related retro-pregnane progestins.

Compound	Progestational[a] Potency
11	4
13	1
16	30

[a]The progestational potencies are normalized to that of progesterone (1.0)[16].

(i) Alteration of the partition coefficient

Table V shows the partition coefficients of 16 and the partition coefficients of the unsaturated retro-pregnanes discussed above. The data of Table V shows that 16 has a partition coefficient value which is greater than the partition coefficient values of the other retro-pregnane progestins. For example, the partition coefficient of 16 is 0.48 log units greater than the partition coefficient of 13 and 0.21 log units greater than the partition coefficient of 11. The enhanced hydrophobic character of 16 may mediate enhanced interactions with lipophilic biological constituents and compartments, e.g. cell membrane receptors and target tissue. Progesterone, for example, demonstrates a partition coefficient that is 0.43 log units higher than 16. Progesterone is known to be extensively absorbed onto and/or into lipophilic biological compartments[13]. The enhanced lipophilic character of 16 may alter the distribution of this substance relative to the unsaturated analogues of 16 (Table V). The altered distribution may favour the progestin target tissue and/or receptor.

TABLE V

The partition coefficients and progestational potencies of a series of related retro-pregnane progestins.

Compound	Progestational Potency	Partition Coefficient	Δ^a
11	4	2.36	-0.21
13	1	2.09	-0.48
16	30	2.57	-

[a]The difference in partition coefficients relative to 16.

Target cell (membrane) recognition sites are proteinacious in nature. Furthermore, these recognition sites are contiguous with cell membranes rich in lipophilic constitutents such as cholesterol and phospholipids. The partitioning of a hydrophobic substance[28,29], e.g. 16 from plasma and/or solubilized plasma carrier proteins, onto the proteinacious target cell recognition sites should be favored process, i.e. the process should demonstrate a large negative free energy of formation[5]. Changes in the partition coefficient, which a specific substitutent may bring about, may also alter the catabolic pathways of a substance[24]. Nevertheless, alterations in the detoxification of a substance which may be attributable to a substituent, e.g. the cyclopropane ring, can be mediated through two parameters; (a) the substituent alters the partition coefficient which in turn results in the divergence of the substance from the

catabolic pathway, an indirect effect, or (b) the substitutent directly impairs the catabolic function of substrate specific enzyme systems,a direct effect[13]. The 1β,2β-cyclopropane ring in 16 may function to increase the progestational potency of 16 through both (a) and (b) or either one of (a) or (b).

(ii) Isosteric and isoelectric relationship of the 1β,2β-cyclopropano substituent and a C-1 site of unsaturation

The β-configuration of the 1,2-cyclopropano substituent of 16 causes the β-face of the C-1 bond of 16 to be isoelectric and isosteric with the C-1 site of unsaturation of 13. Therefore, the β-face (nucleophilic) surface at the C-1 bond of 13 is now (a) not nucleophilic and (b) more hydrophobic through substitution with the 1,2-cyclopropano substituent.

There is no convincing evidence to suggest that progestins bind to progestin receptors on the β-face of the steroid nucleus. Nevertheless, by analogy to the androgens which are thought to bind to androgen receptors on the β-face of the steroid nucleus[30], the possibility that β-face interaction between progestins and progestin receptors may not be completely discounted. The enhanced lipophilic character of the C-1 bond of 16 relative to 13, should enhance the effectiveness of

this substance to bind both to progestin cytosol receptor and progestin target cell recognition sites. The more polar substance 13 should display enhanced interactions with agueous (polar) phase biological compartments and may bind with greater affinity to solubilized plasma proteins[25].

(iii) The effect of the 1β,2β-cyclopropano substituent on the A-ring conformation

The most stable Dreiding model conformation of 16 indicates that the 1,2-cyclopropano substituent enhances the conformational rigidity of the A-ring. Nevertheless, the most stable Dreiding model of 13 (Table III, Figure IX) indicates that the A-ring conformation is more rigid and furthermore more planar than that of 16. Figure X shows that the A-ring conformation and the angle (Ø) between the A-ring plane and the C-3 carbonyl substituent of 16 more closely approximates that of 11. The progestin 11 demonstrates enhanced progestational activity relative to 13.

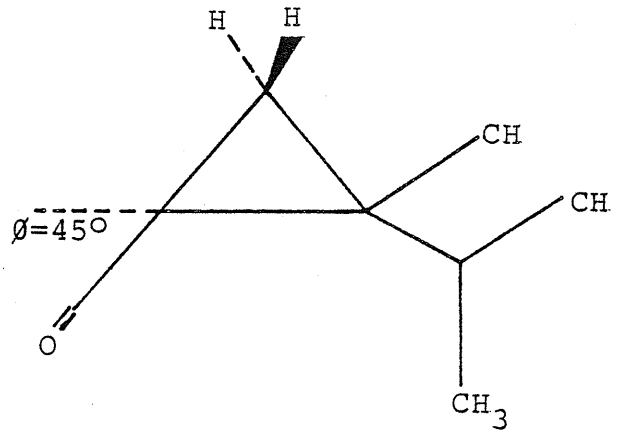
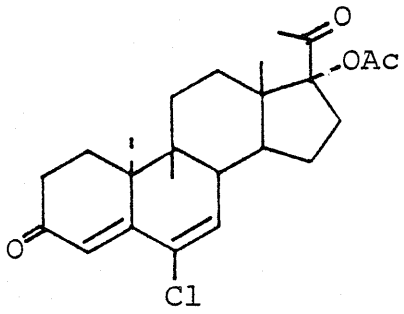


Figure X. The most stable Dreiding model, A-ring conformation of the retro-pregnane 16 as seen through the imaginary axis passing through C-2 and C-4.

(d) The effect of a 6β,7β-cyclopropano substituent

The 6-chloro analogue of 11, 6-chloro-17α-acetoxy-(9β,10α)-pregna-4,6-diene-3,20-dione(17) demonstrates progestational activity that is significantly greater than that of 11[16]. Nevertheless, the 6β,7β-cyclopropano derivative of 17, 6α-chloro-6β,7β-cyclopropano-17α-acetoxy-(9β,10α)-pregn-4-ene-3,20-dione(18), demonstrates significantly diminished progestational activity relative to the unsaturated 6-chloro analogue 17[16]. The 6β,7β-cyclopropano substituent therefore diminishes the progestational activity of the retro-pregnane progestin 17. Table VI summarizes the above data.



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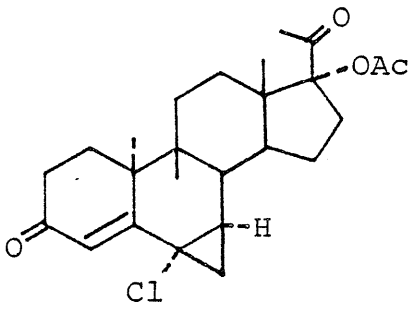

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TABLE VI

Progestational potencies[16] of a series of related retropregnane progestins.

Compound	Progestational[a] Potency
11	4
17	90
18	45

[a]The progestational potency is relative to the progestational activity of progesterone (1.0).

(i) Alteration of the partition coefficient.

The partition coefficient of 18 is significantly greater than the partition coefficient of 11 and 17 (Table VII). The vascular penetration of 18 may therefore be more extensive than the vascular penetration of 11 and 17. Non-target tissues, e.g. lipophilic adipose tissue, and emulsified plasma lipids (chylomicrons) may serve as depot sites for this highly lipophilic molecule. The altered distribution of 18 may therefore serve to decrease the availability of this substance for progestin target tissue(s). Furthermore, as alterations in the partition coefficient of a (drug) substance has been shown to influence the metabolic transformation of that substance, 18 may display a catabolic pathway which enhances the inactivation of this substance relative to 17[24].

TABLE VII

Progestational potency[16] and partition coefficients of a series of related retro-pregnane derivatives.

Compound	Progestational[a] Potency	Partition Coefficient	Δ^b
11	4	2.36	-0.97
17	90	2.75	-0.58
18	45	3.33	-

[a]Relative to the progestational potency of progesterone(1.0).
[b]The difference in the partition coefficient of 18 relative to the other substances.

(ii) Isosteric and isoelectric relationship of the 6,7-cyclopropano substituent and a C-6 site of unsaturation.

The β-configuration of the 6,7-cyclopropane ring in 18 gives rise to an α-face isoelectric and isosteric relationship with the C-6 bond of 17. The α-surface, C-6 nucleophilic centre is therefore lost and is replaced with a hydrophobic substituent. The carbon-carbon double bond may serve as a nucleophilic binding site for receptor and/or enzyme charged (electrophilic) binding sites. The hydrophobic character of the β-surface of C-6 bond in 18 is not compatable for inter-actions with charged (electrophilic) receptor binding sites. Steroid-receptor interactions at the C-6 bond may now demonstrate a positive, free energy of formation. This may diminish the affinity of 18 for the progestin receptor and compromise the effectiveness of 18 to bind with the progestin receptor.

(iii) The effect of the 6α-chloro and the 6β,7β-cyclopropano substituent on metabolic transformations.

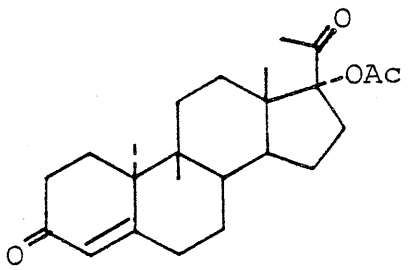
It is believed that large substituents at C-6, e.g. chloro, 6α- and 6β-methyl, of C-4 unsaturated 3-keto-steroids, diminish the rate of A-ring reduction[13]. The C-4 unsaturated, 3-keto function of the A-ring is believed essential for the maintainance of the biological

activity of most steroids. Recently, although evidence suggests the involvement of progesterone metabolites in the progestational response to progesterone. The 6α-chloro and the 6β,7β-cyclopropane substituents may be expected, by analogy, to diminish the rate of A-ring reduction in 18. The biotransformation of these substances to active metabolites may be a prerequisite for progestational activity, as it has been suggested for the natural pregnane series of progestins[30]. It may be therefore anticipated that 18 may demonstrate a diminished rate of conversion to A-ring reduced metabolites. This in turn may result in a diminuation of the progestational activity. Accordingly, the enhanced progestational activity of 17 may be attributed to the lack of either a 6α- or a 6β-substituent and to the presence of biologically active A-ring reduced steroids.

(iv) The effect on C-6 hydroxylation.

C-6 hydroxylation of A-ring, α,β unsaturated steroidal C-3 ketones has been suggested to proceed by an enzyme induced isomerization process outlined in Scheme I[9]. The progestin 19 may be expected to undergo C-6 hydroxylation through this mechanism. The cyclopropano steroid 18 may not readily undergo this isomerization mechanism due to the presence of the 6,7-cyclopropano and the 6α-chloro substituent. As the enzyme induced

isomerization process may be significantly impaired, the C-6 hydroxylation process is similarly impaired. This effect may diminish the rate of biotransformation and/or elimination.



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(v) Conformational changes in the retro-pregnane nucleus induced by 6α-substituents.

Retro-pregnane steroid substituents positioned at C-6 and in the α-configuration, e.g. the 6α-chloro substituent of 18, are believed to undergo 1,3-diaxial interactions with the 10α-methyl substituent[22]. This 1,3-diaxial interaction appears to be large enough to significantly alter the conformation of the B-ring. The altered B-ring conformation should therefore inturn alter the A-ring conformation to some extent. As the A-ring conformation appears to be related to the progestational potencies of these retro-pregnane derivatives (Table III), the 1,3-diaxial interactions

of the 10α and 6α-substituents may result in an A-ring conformation that is not compatable with potent progestational activity.

(e) The effect of 1β,2β;6β,7β-bis(methylene) substitution

The bis(methylene) substituted retro-pregnane derivatives 1β,2β;6β,7β-bis(methylene)-17α-acetoxy-(9β,10α)-pregn-4-ene-3,20-dione(20) and 1β,2β; 6β,7β-bis (methylene)-6α-chloro-17α-acetoxy-(9β,10β)-pregn-4-ene-3,20-dione(21) demonstrate enhanced progestational activity relative to their cyclopropano substituted analogues 16 and 18, respectively.[16] Substitution at C-1(2) and C-6(7) of these substances with bis(methylene) substiuents of the β-configuration enhances the biological potencies of these substances.

(i) Alteration of the partition coefficient.

The partition coefficients of 20 and 21 are significantly greater than those of 16 and 18, respectively. Table VIII shows the progestational potencies and partition coefficients 20 and 21 and other related retro-pregnane derivatives discussed above. The data of Table VIII indicates that an increase in the partition coefficient is associated with a concomitant increase in the biological potency. The notable and only exception to this observation is compound 17. Figure XI suggests

that the log of the progestational potencies of these retro-pregnane derivatives is linearly related to the partition coefficient.

TABLE VIII

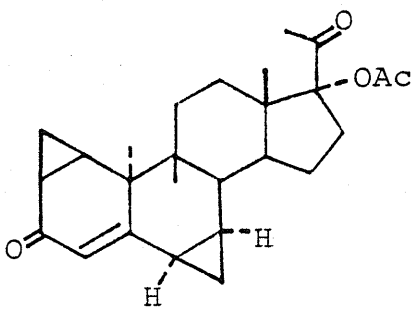
Progestational potencies and partition coefficients of the bis (methylene) retro-pregnanes 20 and 21 and other related retro-pregnane derivatives [16].

Compound[a]	Progestational[b] Potency	Partition Coefficient	Δ^c
progesterone	1.0	-	-
12	1	2.09	-1.47
11	4	2.36	-1.20
16	30	2.57	-0.99
18	45	2.95	-0.61
17	90	2.75	-0.81
20	200	3.17	-0.39
21	430	3.56	0

[a]The compounds have been arranged in order of increasing progestational activity.

[b]Relative to progesterone.

[c]Partition coefficient difference from that of 20.



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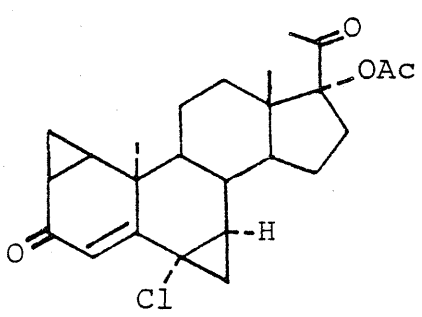

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The partition coefficients of both 20 and 21 (Table VIII) suggest that these substances favor lipophilic interactions[29]. As these partition coefficients are optimal for potent progestational activity, this suggests that the distribution of these substances must favor progestin target tissue(s) and therefore lipophilic target cell recognition sites.

The greater than two-fold increase in the progestational activity of 21 relative to 20 may be attributed to the enhanced lipophilic character of 21 relative to 20. The lipophilic character of 21 is 0.39 log units greater than that of 20. This enhancement of the hydrophobic property in 21 is attributable to the C-6 chloro substituent (π_{Cl}=0.39)

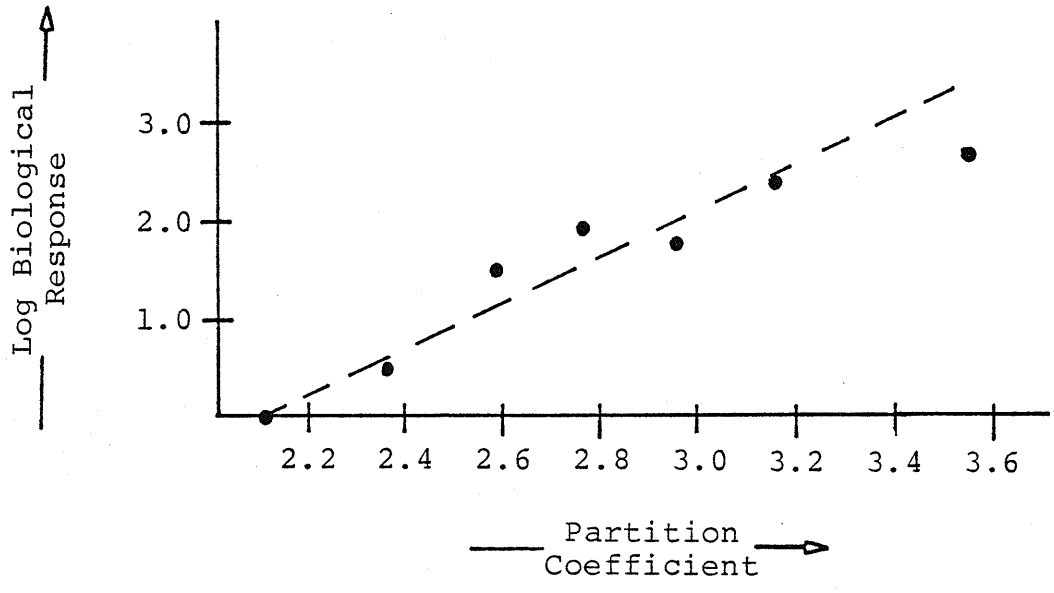


Figure XI. A plot of the log of the progestational potency versus the partition coefficient of a series of related retro-pregnane derivatives.

(ii) 19-Nor-5α-androstane derivatives

(a) The effect of the 15α,16α-cyclopropano substituent

Norgestrol, 17α-ethynyl-18-methy-19-nor-5α-androst-4-en-3-one-17-ol(22), exhibits progestational activity greater than the natural progestin, progesterone 32. The 19-nor-5α-androstane progestin 15α,16α-cyclopropano-17α-ethynyl-18-methyl-19-nor-5α-androst-4-en-3-one-17-ol(23) demonstrates significantly enhanced progestational activity in the Clauberg progestational assay relative to the C-15(16) non-substituted analague 22[32]. The 15α,16α-cyclopropano substitutent has therefore an enhancing effect on the progestational activity of the 19-nor-5α-androstane progestins.

(i) Alteration of the partition coefficient.

The partition coefficient of 23 is 0.21 log units greater than the partition coefficient of 22. This is attributable to the contribution that the cyclopropane ring makes to the hydrophobic character[29] of the molecule. The increase in the partition coefficient may alter the distribution and diminish the rate of biological elimination of 23 relative to 22. The contribution of the cyclopropane ring to the hydrophobic properties of 23 may also enhance the affinity of this substance for the progestational receptor.

(ii) The effect on metabolism.

The second most prominent metabolite of corticosterone, 11β,21-dihydroxy-pregna-3,20-dione(24), in the rat is 3β,11β,15α,21-tetrahydroxy-5α-pregn-20-one (25)[33]. This suggests that C-15 oxidation is a major metabolic pathway in the biological detoxification of corticosterone. The major metabolite of norgestrol, 17α-ethynyl-18-methyl-19-nor-5α-androst-4-en-3-one-17-ol (22) in man which accounted for approximately 30% of all urinary metabolites, was 17α-ethynyl-16β,17β-dihydroxy-18-methyl-19-nor-5α-androst-4-en-3-one(26)[34].

These observations suggest that the metabolic transformation of 22 may lead to C-15 and/or C-16 hydroxylated metabolites. These metabolites may be expected to exhibit diminished biological activity relative to the parent compound. The 15α,16α-cyclopropano substituent may impair hydroxylation both at C-15 and C-16. The introduction of α-substituents at C-16 is known to diminish the rate at which the C-17 alkyl side chain of C-21 steroids undergoes metabolic transformation[13]. The 15α,16α-cyclopropano ring may therefore also impair the catabolism of the C-17 alkynyl side chain. The presence of a 17α-substituent appears necessary for the maintenance of progestational activity whereas the configuration of the C-15(16) cyclopropane ring does not appear critical. For example, the C-15(16)

steroisomeric analogue of 23, 15β,16β-cyclopropano-19-nor-5α-androst-4-en-3-one(27), which is lacking a 17β-substituent, demonstrates potent anabolic activity[35]. Whereas 15β,16β-cyclopropano-17β-acetoxy-5α-androst-4-en-3-one-17-ol(28) demonstrates progestational activity[36]. This concept is further concreted by the observation that 16α,17α-cyclopropano-17β-acetoxy-19-nor-5α-androstan-3-one(29) demonstrates potent progestational activity[37].

(b) The effect of a 1α,2α-cyclopropane ring

Ethisterone acetate, 17α-ethynyl-17β-hydroxy-19-nor-5α-androst-4-en-3-one 17-acetate(30) demonstrates enhanced progestational potency relative to progesterone standard. The 1α,2α-cyclopropano analogue of 30, 1α,2α-cyclopropano-17α-ethynyl-17β-hydroxy-19-nor-5α-androst-4-en-3-one 17-acetate(31), demonstrates increased progestational potency relative to progesterone standard but exhibits only 0.25X the progestational activity of 30. The 1α,2α-cyclopropano substituent therefore diminishes the progestational potency of ethisterone acetate(30)[38].

(i) Alteration of the partition coefficient.

Progesterone is extensively absorbed by adipose tissue and other biological lipophilic compartments[13]. The partition coefficient of progesterone is greater than that one of 30 or 31 (Table IX). Furthermore, progesterone is the least potent of the progestational agents shown in Table IX. Therefore, high lipophilic character appears to correlate with low progestational potency, e.g. progesterone. Conversly, diminished lipophilic character appears to correlate with potent progestational activity, e.g. 30. The data of Table IX suggests that there is an inverse relationship between the biological potency and the partition coefficient of these substances.

The hydrophobic character of 31 enhances the vascular penetration of this substance. The net effect is to diminish the plasma concentration of 31. Similarly, and relative to progesterone, the decrease in lipophilic character may cause enhanced affinity for progestin receptors or target tissue[25]. A concomitant enhancement in the effectiveness of 31 to bind with and maintain the progestin-receptor complex may also account for the enhanced progestational activity relative to progesterone.

The further decrease in the partition coefficient of 30 is compatable with an even greater diminished vascular penetration of this substance. The diminished

vascular penetration reflects itself as an increase in the plasma concentration of this substance. This may, in part, also contribute to enhance the progestational potency of this substance.

The partition coefficients of 30 and 31 may alter the rate of elimination by altering the rate or the pathway of metabolic detoxification.

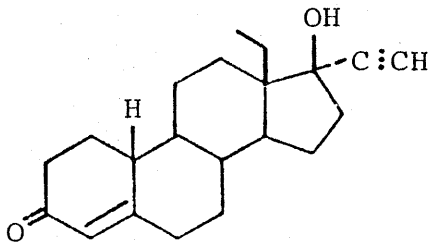
TABLE IX

Progestational activity and partition coefficient of a series of related progestins.

Compound	Progestational Potency	Partition Coefficient
progesterone	1	1.73
30	20	1.00
31	5	1.21

(ii) Isosteric and isoelectric nature of the C-1 bond of cyclic fusion.

The C-1(2) bond of 30 is isosteric and isoelectric on the β-surface with a C-1 site of unsaturation. A C-1 site of unsaturation is known to diminish the rate of A-ring reduction. Therefore, as the C-1 bond of 31 is isoelectric with a C-1 site of unsaturation, the rate of A-ring reduction may be expected to be diminished in



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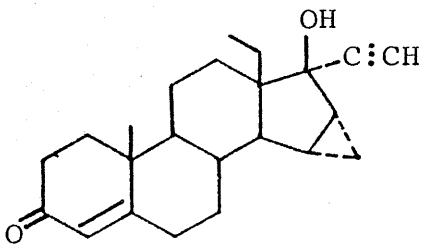

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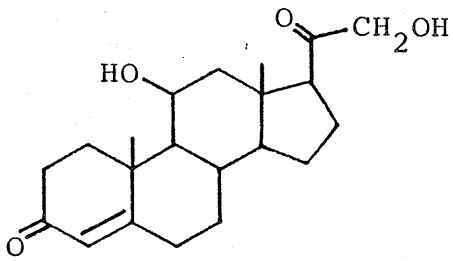

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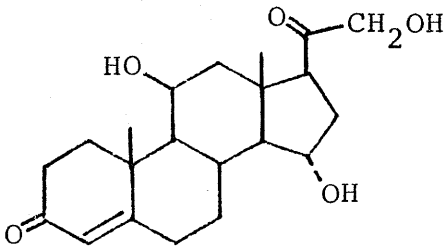

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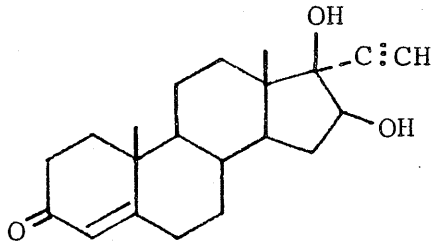

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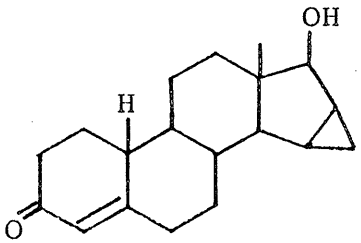

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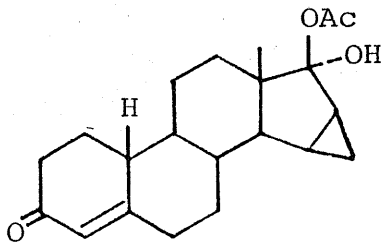

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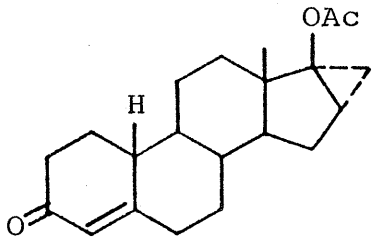

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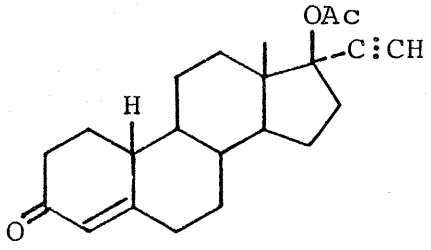

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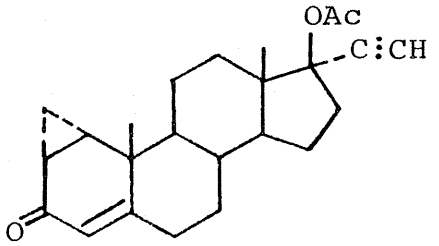

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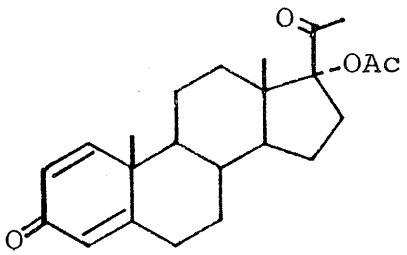

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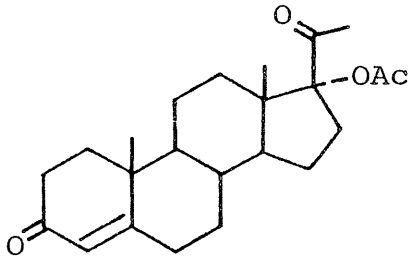


33

this substance.

The progestational activity of progesterone has been implicated with A-ring reduced metabolites[30]. As the reduction of the A-ring in 31 may be impaired by the presence of the isoelectric 1α,2α-cyclopropano substituent, the diminished rate of A-ring metabolite formation may impair the observed biological response of 30. This conclusion is consistent with the observation that 17α-acetoxy-pregna-1,4-diene-3,20-dione(32) demonstrates 0.4X the progestational activity of 17α-acetoxy-pregn-4-ene-3,20-dione(33) relative to progesterone standard[38].

C. ANTI-ANDROGENS

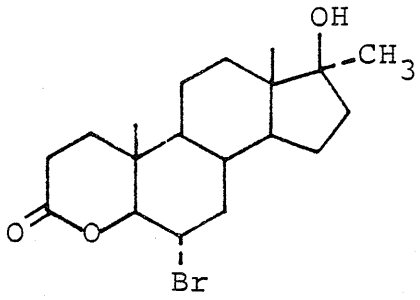
(i) Pregnane derivatives of the 5α-series

(a) Biomolecular bases for anti-androgenic activity.

A variety of substances demonstrate anti-androgenic activity. Androstane derivatives in the 5α-series demonstrate anti-androgenic activity. For example, 6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one(34),BOMT, and 17α-methyl-B-nor-androst-4-en-3-one-17-ol(35) demonstrate anti-androgenic activity[39,40,41]. Similarly, 3,20-dione derivatives of 17α-acetoxy-pregna-4,6-diene-3,20-dione(36) are potent anti-androgens[38]. Non-steroidal substances also demonstrate anti-androgenic activity. For example, N-(4-nitro-3-trifluoromethyl phenyl)-2-methylpropionamide (37), flutamide, demonstrates potent anti-androgenic activity[40].

In the secondary sex glands, androgenic activity is mediated by 5α-dihydrotestosterone[42,43]. The secondary sex organs demonstrate high 5α-reductase activity[42]. In the secondary sex glands, these 5α-reductases reduce testosterone to 5α-dihydrostestosterone. Anti-androgens do not mediate anti-androgenic activity through inhibition of the 5α-reductase system in the secondary sex glands. A reduction in biologically normal 5α-dihydrotestosterone levels is not observed[40].

Anti-androgens have been shown to inhibit the



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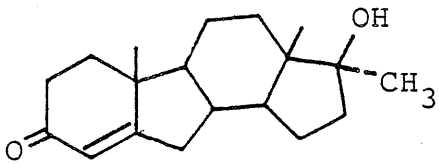

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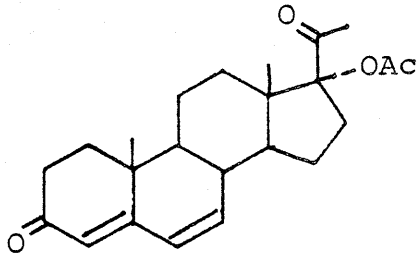

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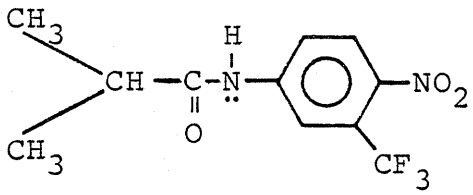

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(prostatic cell) nuclear retention of the 5α-dihydro-testosterone-receptor complex and to diminish the *in vivo* uptake of tritium labelled 5α-dihydrotestosterone by the ventral prostate[40]. Anti-androgens have also been shown to antagonize the formation of the androgen-receptor complex[40,44]. These observations suggest that anti-androgenic substances mediate anti-androgenic activity through one or both of the following mechanisms; (i) alteration of the androgen receptor protein or (ii) competitive antagonism of the natural androgens for the androgen receptor. Experimental evidence strongly suggests that the biomolecular basis for anti-androgenic activity is a competitive antagonism of the natural androgens for the androgen receptor[40,42,45,46].

(b) The effect of unsaturation.

The cross-conjugated pregnane derivative 6-chloro-17α-acetoxy-pregna-1,4,6-triene-3,20-dione(38). demonstrates 0.64X the anti-androgenic potency of 6-chloro-17α-acetoxy-pregna-4,6-diene-3,20-dione(39)[19]. The C-1 site of unsaturation therefore diminishes the anti-androgenic potency of these substances.

(i) Alteration of the partition coefficient.

The carbon-carbon double bond enhances the hydrophilic properties of a substance[5]. The partition coefficient of 38 is less than that one of 39 (Table X). The diminished lipophilic character of 38 enhances the interaction of this substance with aqueous phase biological compartments[29] and concomitantly diminishes the vascular penetration of this substance[13]. Alterations in the lipophilic/hydrophilic balance of a substance may alter the metabolism of that substance[24]. The altered vascular penetration and modified metabolism may account for the diminished biological potency of 38 relative to 39.

Anti-androgenic activity appears to be mediated through competitive antagonism of the androgen receptor. Substances with diminished lipophilic character demonstrate decreased hydrophobic interactions with macromolecules relative to their more lipophilic counterparts[29]. As

hydrophobic interactions are important in the formation and the maintenance of the androgen receptor[47a], the diminished biological potency of 38 relative to the C-1 saturated derivative 39 may arise through a diminished affinity for the androgen receptor.

TABLE X

Biological potency[19] and partition coefficients of some steroidal androgens.

Compound	Biological Potency	Partition Coefficient
38	0.64	2.48
39	1.00	2.75

(ii) The effect on metabolism.

The C-1 site of unsaturation is known to decrease the rate of A-ring reduction in C-4 unsaturated-3-ketosteroids. Furthermore, experimental evidence suggests that C-1 unsaturation also decreases the rate of C-17 side chain metabolism in C_{21} steroids[13]. Therefore, the rate of metabolism of 38 may be expected to be diminished realtive to 39. The observation that 38 demonstrates diminished biological potency relative to 39 suggests that reduction of the C-4 site of unsaturation and/or the C-3 carbonyl group may be a prerequisite for potent anti-androgenic activity. It is known that

androgenic activity is mediated by the 5α-reduced metabolite of testosterone in the accessory sex glands[43]. Similarly, it has been suggested that the biological activity of progesterone involves biologically active metabolites of that substance[47b].

(iii) The effect of A-ring conformation.

The configuration of the steroid nucleus has been defined as planar of "slab-like"[13]. Unsaturation enhances the planar configuration of the steroid nucleus and significantly alters the conformation, either through a direct effect or through long range,conformational transmission[48]. Modest changes in the configuration of the steroid nucleus are known to alter the biological activity. For example, the planar estrane derivative 17β-hydroxy-17α-methyl-estra-4, 9,11-trien-3-one(40) demonstrates potent androgenic activity[49]. The C-9/C-11 saturated congener of the estrane derivative 40, 17β-hydroxy-estr-4-en-3-one(41), demonstrates potent anabolic and anti-estrogenic activity[50].

Figure XII shows the most stable Dreiding model conformations of the anti-androgens 38 and 39 and the androstane anti-androgen derivative 42[51]. The angle (Ø) between the C-3 carbonyl substituent and the C-4 site of unsaturation was measured. The data of Figure

XII is summarized in Table XI. The data of Table XI suggests that when Ø is small, effective competitive antagonism of the androgen receptor is diminished. Conversely, when Ø is large the effectiveness to competitively antagonize the androgen receptor is restored.

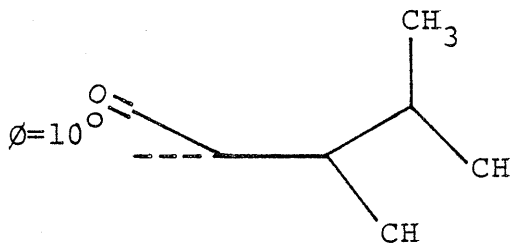
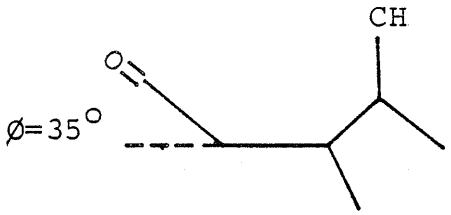
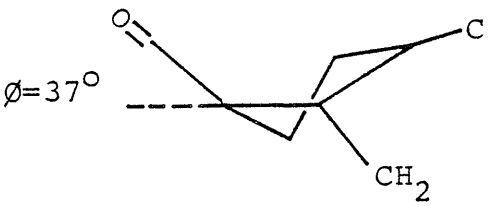
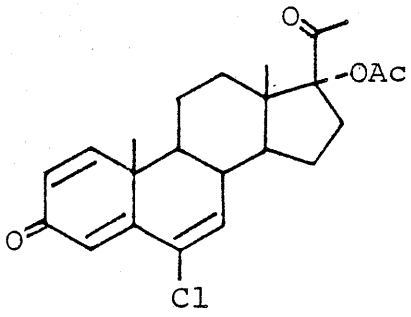
Compound	A-Ring Conformation
38	
39	
40	

Figure XII. The most stable Dreiding model conformation and the angle (Ø) between the C-3 carbonyl substituent and the C-4 site of unsaturation of the anti-androgenic derivatives 38, 39 and 40.

TABLE XI

Biological potencies and the angle (Ø) between the C-3 carbonyl substituent and the C-4 site of unsaturation of two anti-androgenic pregnane derivatives.

Compound	Biological Potency	Ø
38	0.64	10°
39	1.00	35°
40	-	37°



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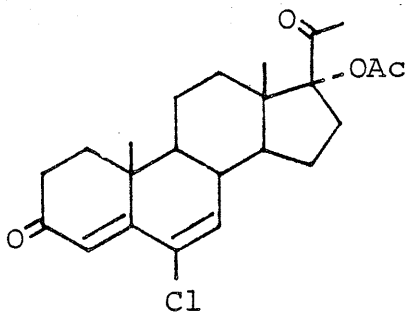

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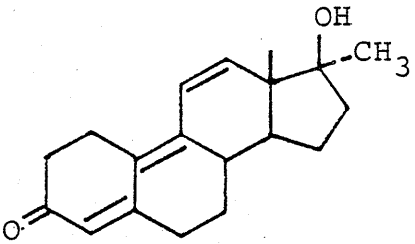

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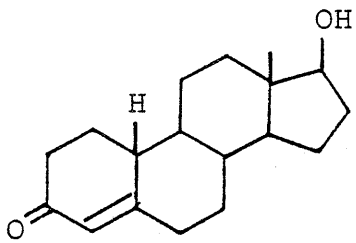

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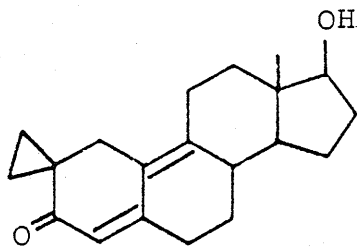


42

(c) The effect of a 1α,2α-cyclopropane ring.

The cyclopropane steroid 1α,2α-cyclopropano-6-chloro-17α-acetoxy-pregna-4,6-diene-3,20-dione(43) is approximately 2X more anti-androgenic than its unsaturated analogue 38[19]. The cyclopropane steroid 1α,2α-cyclopropano-17α-acetoxy-pregna-4,6-diene-3,20-dione(44) demonstrates anti-androgen activity approximately equal to that of 39[38]. Similarly, 1α,2α-cyclopropano-17α-acetoxy-pregna-4-ene-3,20-dione(45) demonstrates diminished anti-androgenic activity relative to 44 but demonstrates enhanced biological potency relative to 38[38]. Table XII summarizes the anti-androgenic potencies of these cyclopropanosteroids.

TABLE XII

Anti-androgenic potencies of a series of related pregnane derivatives.

Compound	Anti-androgenic Potency
38	0.64
39	1.00
43	1.28
44	0.94
45	0.85

(i) Alteration of the partition coefficient.

The cyclopropane ring enhances the lipophilic character[6] of a molecule whereas the carbon-carbon double bond enhances the hydrophilic character of a molecule[5]. Substitution of a carbon-carbon double bond with a fused cyclopropane ring enhances the lipophilic character by 0.48 log units. The partition coefficient of 43 is significantly greater than the partition coefficients of the other pregnane anti-androgens. Table XIII shows the partition coefficients and the biological potencies of these pregnane anti-androgens. The data of Table XIII suggests that high lipophilic character correlates with potent biological activity, e.g. 43, whereas diminished biological response correlates with diminished lipophilic character, e.g. 44.

The manner in which lipophilic character mediates potent biological activity in this series of anti-androgens may be to enhance the affinity of the substance for the androgen receptor and to increase the binding effectiveness between hormone and receptor[49]. The consequences of this change in these two parameters is to ensure a hormone receptor complex which is resistant to competitive displacement by the natural androgen.

The anti-androgen 43, cyproterone acetate, demonstrates enhanced lipophilic character relative to

the other anti-androgenic substances (Table XIII). Cyproterone acetate has been shown to be approximately 0.8X as effective as testosterone and 0.5X as effective as 5α-dihydrotestosterone in competitively antagonizing rat prostate cytosol bound [$^{3}_{H}$] 5α-dihydrotestostorone[52]. The binding of a substance with a biological receptor involves extensive hydrophobic interactions[47b]. The observation that cyproterone acetate demonstrates effective binding with the androgen receptor suggests that extensive hydrophobic interactions[29] are taking place. As hydrophobic character and hence hydrophobic interactions are a reflection of the partition coefficient[5a], the greater the partition coefficient the greater is the biological response[53]. Nevertheless, such linear responses are not infinite[54].

The biological effectiveness of a variety of drug substances has been shown to demonstrate either a parabolic or linear relationship with the partition coefficient[54]. Figure XIII shows a plot of the partition coefficient versus the log of the anti-androgenic potencies (BR) of the anti-androgens discussed above. Figure XIII indicates that a linear relationship may exist between these two parameters.

TABLE XIII

Partition coefficients and anti-androgenic activity[19,38] of a series of related pregnane derivatives.

Compound	Anti-androgenic Potency	Partition Coefficient
38	0.64	2.48
39	1.00	2.75
43	1.28	2.96
44	0.94	2.57
45	0.85	2.94

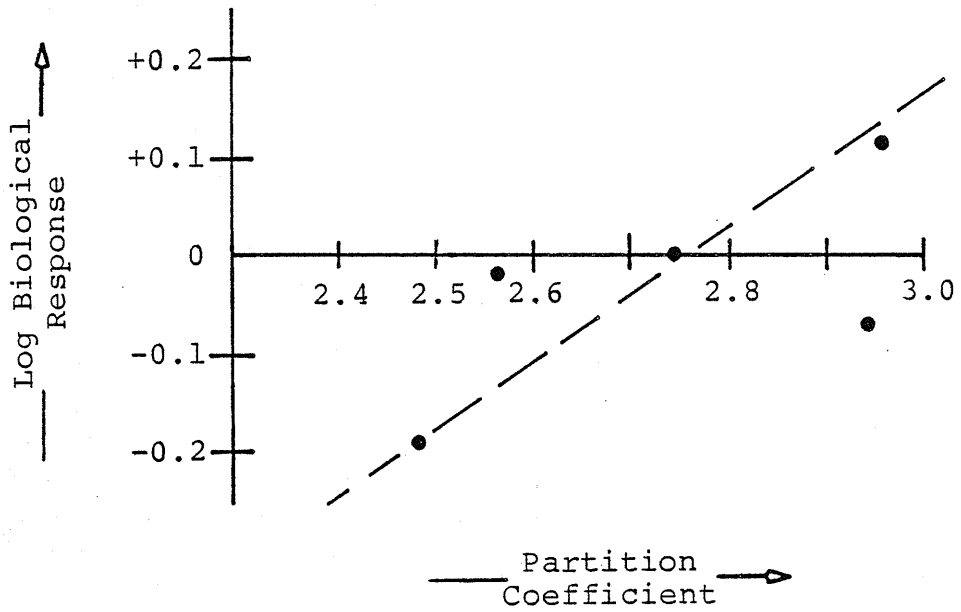


Figure XIII. Plot of the partition coefficient of a series of related anti-androgens versus the log of the biological response (BR).

(ii) Isosteric and isoelectric nature of the 1,2-cyclopropano substituent.

The α-configuration of the cyclopropane ring in the 1,2 cyclopropano substituted anti-androgens, i.e.43, gives rise to a β-face isoelectric and isosteric relationship with a C-1 site of unsaturation. A site of unsaturation may enhance the biological activity of (drug) substances. This effect is believed to be mediated through the interaction of the nucleophilic carbon-carbon double bond with a charged receptor site[26]. Alternatively,this may be related to the effect of a C-1 site of unsaturation on the rate of A-ring catabolism[13]. The observation that the steroisomer of 43, 1β,2β-cyclopropano-6-chloro-17α-acetoxy-pregna-4,6,diene-3,20-dione(46) demonstrates no anti-androgenic activity[19] suggests that there is a β-face interaction between these anti-androgenic substances with the androgen receptor and furthermore, that this interaction with the androgen receptor is maintained by the C-1 pseudo-unsaturated (cyclopropane) bond of 43.

The β-configuration of the 1,2-cyclopropano substituent in 46 gives rise to an α-face isosteric and isoelectric relationship with a C-1 site of unsaturation. The nucleophilic C-1 pseudo-unsaturated bond of cyclic fusion in 46 is now shielded by the hydrophobic

β-methylene atom of the 1,2-cyclopropane ring. Therefore, a charged (electrophilic) receptor site cannot interact with the nucleophilic C-1 bond of the cyclopropane ring. This interaction may be crucial for the effective competitive antagonism of the androgen receptor. Alternatively, the β-methylene atom may, through its physical presence, and therefore for steric reasons, block a hormone-receptor interaction,e.g. with the C-3 carbonyl substituent, which may be crucial for the binding of these anti-androgenic substances to the androgen receptor.

(iii) The effect of A-ring conformation.

Figure XIV represents the most stable Dreiding model conformations of the anti-androgenic agents discussed above. The data of Figure XIV is summarized in Table XIV. The data of Table XIV indicates that an A-ring boat conformation correlates with potent anti-androgenic activity, e.g.43. Similarly, an angle of approximately 30° between the C-3 carbonyl substituent and the plane of the C-4 site of unsaturation is optimal for potent anti-androgenic activity.

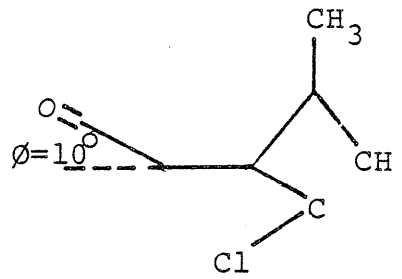
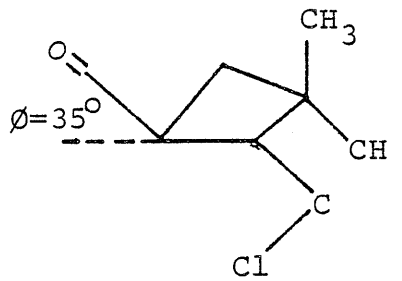
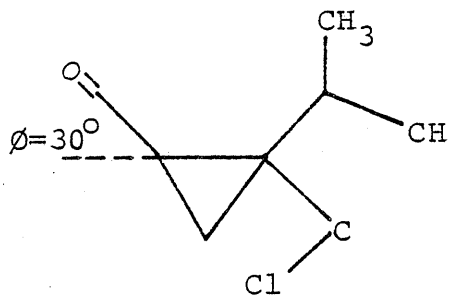
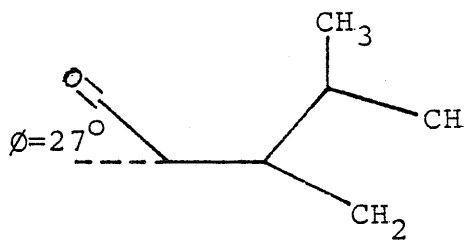
Compound	A-ring conformation
38	
39	
43	
45	

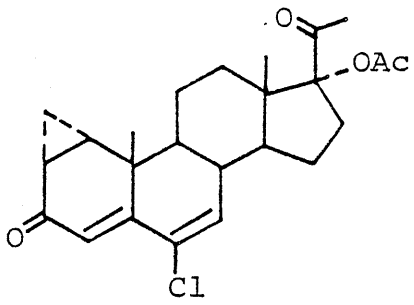
Figure XIV. Most stable Dreiding model conformation of a series of related anti-androgens.

TABLE XIV

Comparison of the anti-androgenic activity of a series of related pregnane derivatives with the A-ring conformation and the angle (Ø) between the C-3 carbonyl substituent and the plane of the C-4 site of unsaturation.

Compound	Biological[a] Potency	A-ring Conformation	Ø
38	50	flattened boat	10°
39	78	boat	35°
43	100	boat	30°
45	66	boat	27°

[a]Relative to the biological potency of 43 which was arbitrarily set at 100%.



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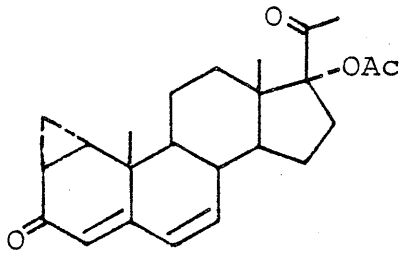

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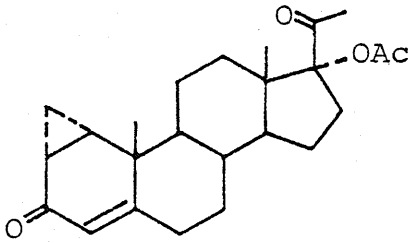

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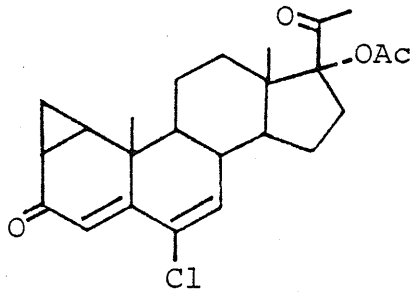

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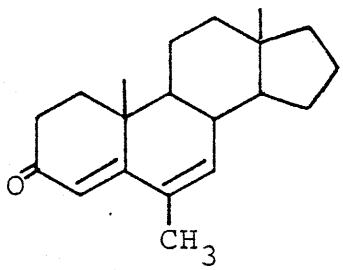
(d) Contribution of the D-ring and D-ring substituents to anti-androgenic potency.

The observation that 6-methyl-androst-4,6-dien-3-one(47) demonstrates anti-androgenic activity[41] equal to that of cyproterone acetate (43) suggests that the C-17 acetyl substituent of the 5α-pregnane series of anti-androgens does not play a major role in effecting potent competitive antagonism of androgens for the androgen receptor. Nevertheless, as 1α,2α-cyclopropano-17α-methyl-17β-acetoxy-D-homo-androst-4,6-diene-3,17a-dione(48) demonstrates only 0.33X the biological activity of cyproterone acetate[19,55], maintenance of the normal conformation of the D-ring is essential for potent anti-androgenic activity.

Pregnane derivatives of cyproterone acetate in which the C-17 substituents are replaced with a 2',3'-tetrahydrofuranyl substituent (49) are also potent anti-androgens. Nevertheless, these substances demonstrate diminished biological activity relative to cyproterone acetate[56].



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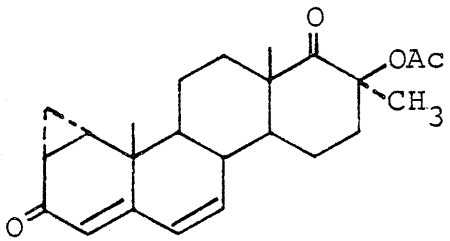

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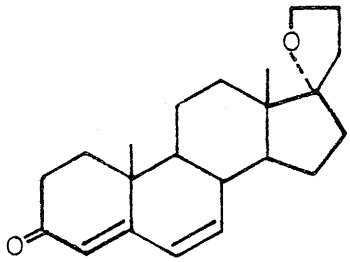

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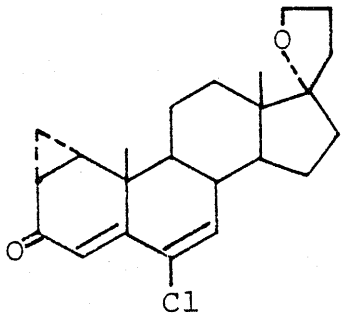
(i) <u>The effect of the C-17 2′,3′-tetrahydrofuranyl substituent on the partition coefficient</u>.

The C-17 tetrahydrofuranyl derivative of cyproterone acetate tetrahydrofuran-2′-spiro-17-(1α,2α-cyclopropano-6-chloro-androsta-4,6-dien-3-one).



50

50, demonstrates 0.33X the anti-androgenic activity of cyproterone acetate[56].

The C-17 tetrahydrofuranyl substituent significantly enhances the lipophilic character of these pregnane derivatives relative to effect of the C-17 substituent on the lipophilic properties of the parent cyproterone acetate series of anti-androgens. Therefore, 50 should demonstrate increased vascular penetration and concomitantly enhanced affinity for adipose tissue[13]. The diminished biological activity of 50 may therefore be mediated through altered distribution and/or metabolism. Alternatively, the bulky C-17 (spiro) tetrahydrofuranyl substituent may diminish the binding effectiveness of 50 to the androgen receptor[13].

(ii) <u>The effect of a 6α,7α-difluorocyclopropano substituent</u>.

The difluorocyclopropanosteroid 51 demonstrates anti-androgenic activity that approximates the anti-androgenic activity of cyproterone acetate(43). The difluorocyclpropano substituent therefore maintains signficant biological activity in these pregnane derivatives[56].

(a) The effect on the partition coefficient.

The non-substitued cyclopropane ring enhances the partition coefficient of a substance. Similarly, halogen atoms, e.g. bromine and chlorine, enhance the lipophilic character of the cyclopropane ring[5b]. The exception is fluorine atom. The substituent partition coefficient of the fluorine atom (π_F) has a value of -0.17 log units[5b]. The difluoro-substituted cyclopropane ring (π=+0.87) therefore diminishes the lipophilic character of the cyclopropane ring. The hydrophilic/lipophilic balance of 51 is now more comparable to that of cyproterone acetate, which demonstrated potent anti-androgenic activity and high affinity for the androgen receptor. As these two substances posses similar hydrophilic/lipophilic ratios, the biological distribution, the metabolism and the affinity for the androgen receptor may be also similar. Thus, 51 may be also expected to demonstrate similar biological activity.

The fluorine atom readily forms hydrogen bonds with appropriately substituted hydrogen atoms, e.g. -OH, $-NH_2$. The binding of 51 to the androgen receptor may involve fluorine-hydrogen bonded interactions. The 1,2-cyclopropanosteroid 50 does not posses the ability to form these hydrogen bonded interactions at C-6. Enhanced affinity for the androgen receptor

mediated through these hydrogen bonded interactions[57] may in part account for the potent anti-androgenic activity of 51.

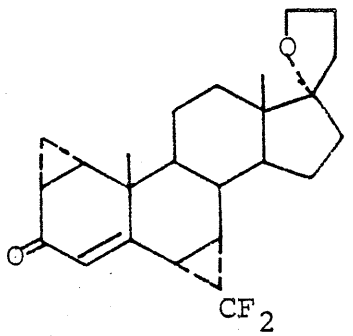
(b) The effect on metabolism.

The 6β-hydroxylation of C-4 unsaturated steroidal C-3 ketones is believed to proceed through an enzyme induced enolization process involving the addition of OH^+ to the C-6 atom (Scheme I)[9]. The 6,7-difluoro-cyclopropano substitutent of 51 may impair and/or block the enolization process and hence C-6 hydroxy-lation and thereby prolong the biological half-life of this substance.

Bulky substituents at C-6, e.g. chloro,methyl diminish the rate of A-ring biotransformation of C-4 unsaturated C-3 ketosteroids[13]. The α-configuration of the 6,7-difluorocyclopropano substituent may function to diminish the rate of A-ring biotransformation as do the C-6 chloro and/or C-6 methyl substituents. This influence on the metabolism of 54 may further protract the biological half-life of this substance.

(c) Isosteric and isoelectric nature of the 6,7-difluorocyclopropano substituent.

The α-configuration of the 6,7-difluorocyclopropano substitutent gives rise to a β-face isoelectric and isosteric relationship between a C-6 site of unsaturation and the 6,7-difluorocyclopropane bond of cyclic fusion. As C-6 unsaturation enhances the biological activity of these 5α-pregnane derivatives, e.g. compare 44 and 45, the biological activity attributable to the C-6 site of unsaturation is maintained through the presence of the isoelectric and isosteric, pseudo-unsaturated C-6(7) cyclopropane bond of cyclic fusion.



51

(ii) B-Nor-5α-androstane derivatives.

B-nor-5α-androstane derivatives, for example 17β-hydroxy-17α-methyl-B-nor-androst-4-en-3-one(52), demonstrate potent anti-androgenic activity[58,59]. The anti-androgenic activity of these substances is, nevertheless, diminished relative to the anti-androgenic activity of the pregnane derivative cyproterone acetate(43).

(a) The effect of a 4α,5-cyclopropano substituent.

The 4α,5-cyclopropano analogue of 52, 4α,5-cyclopropano-17β-hydroxy-17α-methyl-B-nor-5α-androstan-3-one(53) demonstrates diminished biological potency relative to cyproterone acetate and 52[60]. Furthermore, the ability of 53 to competitively antagonize rat prostate cytosol bound [^{3}H] 5α-dihydrotestosterone is diminished by a factor of approximately 1/2000X relative to 52 and approximately 1/400X relative to cyproterone acetate(43)[61].

(i) The effect on the partition coefficient.

The biomolecular mechanism of anti-androgenic activity is belived to be mediated through the competitive antagonism of the natural androgens for the androgen receptor[49]. The significantly diminished ability of 53 to competitively antagonize the binding of the natural

androgens to the androgen receptor may therefore account for the diminished anti-androgenic activity of this substance. The 4,5-cyclopropano substituent of 53 enhances the lipophilic character[6] of this substance relative to a C-4 site of unsaturation by 0.48 log units. The diminished effectiveness of 53 to competitively antagonize [^{3}H] 5α-dihydrotestosterone in the rat prostate cytosol preparation less effectively than testosterone may be a consequence of the preferred interaction of 53 with lipophilic cellular components of the rat prostate cytosol preparation[60]. These lipophilic cellular constituents have a high affinity for lipophilic substances. The affinity of 53 for cellular lipophilic constituents, e.g. phospholipids, cholesterol, may be greater than the affinity of this substance for the androgen receptor.

The diminished biological activity of 53 may also, in part, be explained in terms of the lipophilic character of this molecule. The elevated partition coefficient of 53 will favor interactions with lipophilic biological components, e.g. adipose tissue[13]. The altered distribution may therefore immobilize this substance from the plasma and rapidly diminish blood levels. Similarly, the partition coefficient of 53 may elevate the rate of metabolic detoxification of this substance 24, by enhancing the tendency to partition into lipophilic

biological compartments, e.g. the liver relative to the more hydrophilic congeners of this substance.

The biological activity of a series of related (drug) substances has been shown to demonstrate a parabolic relationship with the partition coefficient (Figure XV)[54]. Implicit in the parabolic relationship are the facts that there is a maximum biological effect and that the maximum biological effect correlates with a maximum partition coefficient value. Substances that demonstrate a lipophilic/hydrophilic balance that lies on either side of the maximum value for the parabola, exhibit diminished biological activity. As the lipophilic/hydrophilic balance of 53 is greater than that of cyproterone acetate (Table X), the lipophilic/hydrophilic balance of 53 may not correlate with the partition coefficient which is associated with a maximum biological response (the maximum biological response and hence the partition coefficient which correlates with the maximum biological response may not necessarily be that one of cyproterone acetate).

(ii) <u>The effect on metabolism</u>.

The presence of the 4,5-cyclopropano substituent may impair hydroxylation of the B-ring. B-ring hydroxylation of 53 may proceed by an enzyme induced

enolization process of the C-3 carbonyl substituent and may be a requirement for the hydroxylation of the B-ring. Furthermore, as the biological reduction of the C-3 carbonyl substitutent of α,β-unsaturated steroidal C-3 ketones is proceeded by the biological reduction of the C-4 site of unsaturation[13], the presence of the 4,5-cyclopropano pseudo-unsaturated bond may impair the reduction of the C-3 carbonyl substituent.

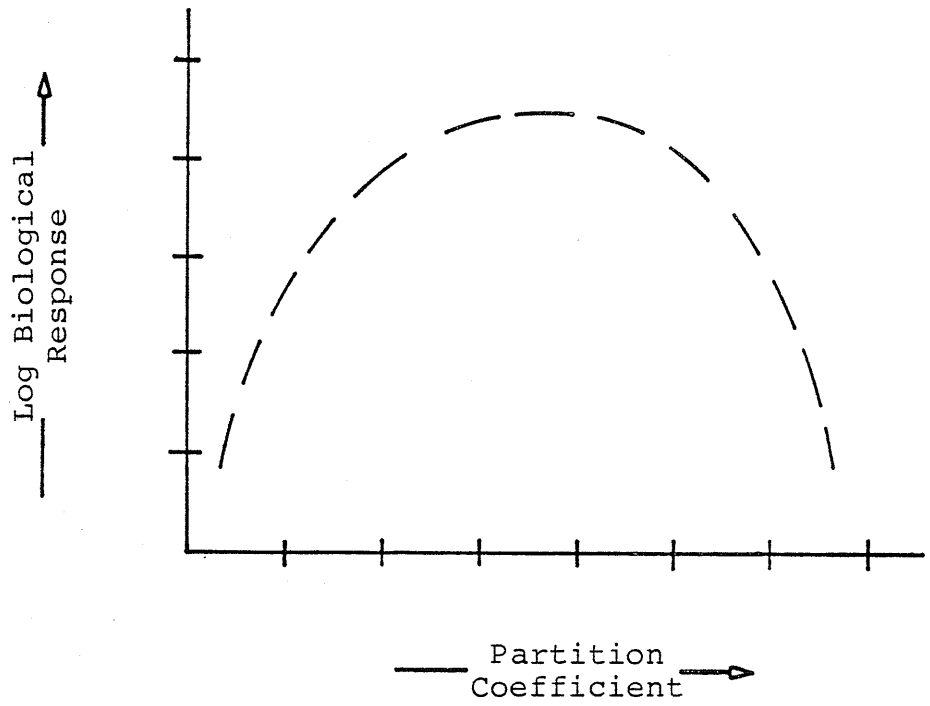
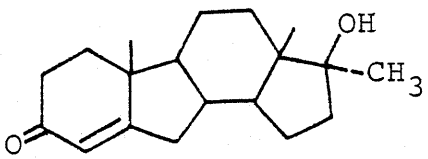


Figure XV. Representative sample in which the partition coefficient of a (drug) substance demonstrates a parabolic relationship with the biological response.

These observations suggest that A-ring metabolism of these B-nor-5α-androstane derivatives is a prerequisite for anti-androgenic activity. There is no conclusive evidence to suggest that this hypothesis may be valid, but, the suggestion that the biological activity of progesterone is mediated by active metabolites gives the hypothesis some credence[47b].

(iii) Isosteric and isoelectric nature of the 4,5-cyclopropano substituent.

The α-configuration of the 4,5-cyclopropano substitutent in 53 generates an isoelectric and isosteric relationship with the β-surface of the C-4 site of



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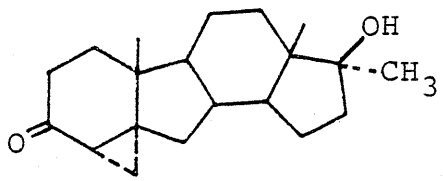


53

unsaturation in substance 52. Therefore, any contribution to the anti-androgenic activity of the C-4 site of unsaturation is maintained in 53. This contribution appears to be important as the majority of potent anti-androgenic steroids possess the C-4 site of unsaturation.

(b) The effect of C-1 unsaturation.

The C-1 unsaturated analogue of 53, 4α,5-cyclopropano-17β-hydroxy-17α-methyl-B-nor-androst-1-en-3-one(54), demonstrates anti-androgenic activity which is equivalent to the anti-androgenic activity of cyproterone acetate(43)[60]. Furthermore, the ability of 54 to displace and competitively antagonize rat prostrate cytosol bound [^{3}H]5α-dihydrotestosterone is 1500X greater than that of 53[59]. The C-1 site of unsaturation therefore enhances the affinity of these B-nor-5α-androstane derivatives for the androgen receptor and thereby potentiates the biological activity of these substances.

(i) The effect of unsaturation on the partition coefficient.

The carbon-carbon double bond diminishes the lipophilic character of a substance by decreasing the partition coefficient by 0.3 log units[56]. Table XV shows the partition coefficients and the apparent inhibition constants* of a series of related B-nor-androstane derivatives. The data of Table XV shows that substances with parition coefficients similar to that of testesterone demonstrate apparent inhibition constants similar to that one of testosterone, e.g.52 and 54. Furthermore, B-nor-androstane derivatives with apparent inhibition constants approximating the apparent inhibition constant of testosterone demonstrate potent anti-androgenic activity, e.g. 52 and 54. The diminished apparent inhibition constant displayed by 54 relative to the other substances suggests that 54

*The apparent inhibition constant is that concentration of inhibitor which yields 50% of inhibition of the original [^{3}H] 5α-dihydrotestosterone binding in rat prostate cytosol[60].

possesses an enhanced affinity for the androgen receptor and a diminished affinity for other lipophilic cellular components of the rat prostate cytosol preparation[60].

The enhanced biological activity of 54 relative to the other B-nor derivatives may be attributed to diminished vascular penetration[13] and/or metabolism[24]. An enhanced affinity for target tissue recognition sites may also in part account for the potent anti-androgenic activity of 54. Alternatively, the C-1 site of unsaturation may function as a nucleophilic binding site for charged (electrophilic) receptor binding sites[26].

TABLE XV

Partition coefficients and apparent inhibition constants of a series of related anti-androgens in relation to testosterone.

Compound	Partition Coefficient	Apparent Inhibition Constant (nM)[a]
52	2.73	0.7
53	3.21	1500
54	2.94	1.0
Cyproterone Acetate (43)	2.96	3.7
Testosterone	2.73	1.7

[a]The apparent inhibition constant is defined as the nanomolar concentration which yields 50% inhibition of rat prostate cytosal bound [^{3}H] 5α-dihydrotestosterone.

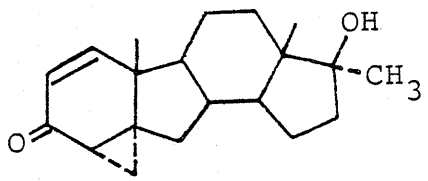
(ii) The effect on the metabolic reduction of the A-ring.

It is well known that C-1 unsaturation diminishes the rate of A-ring biological reduction of C-4 unsaturated C-3 ketosteroids and thereby enhances the biological half-life of these substances[13]. By analogy, the potent anti-androgenic activity of 54 may in part be attributed to the diminished rate of A-ring reduction and a protracted biological half-life.

(c) The effect of A-ring conformation on the anti-androgenic activity.

The most stable Dreiding model conformation of 52,53,54 and testosterone demonstrate no major differences in the A-ring conformation (Figure XVI). Furthermore, the angle between the C-3 carbonyl substituent (Ø) and the plane of the C-4 bond of these most stable Dreiding model conformations are, for all intents and purposes, identical. Table XVI summarizes the data of Figure XVI. The data of Table XVI suggests that there is no correlation between Ø and biological potency. Nevertheless, Figure XVI and the data of Table XVI suggest that there exists a correlation between the A-ring conformation and biological potency, of these substances. For example, the potent anti-androgen 54 possesses an A-ring boat conformation whereas the weakly anti-androgenic substances 52 and 53 possess an A-ring skewed boat

conformation similar to that one of testosterone. Furthermore, 54 demonstrates an enhanced affinity for the androgen receptor relative to 53. This is evidenced by the value of the apparent inhibition constant of 54 which is 1/1500X relative to that one of 53 (Table XV). As the A-ring conformation of testosterone is a skewed boat and testosterone demonstrates potent androgenic activity, substances in this anti-androgenic series of steroids possessing a skewed-boat, A-ring conformation may demonstrate weak or no anti-androgenic activity, e.g. 53. Conversely, substances possessing the A-ring boat conformation may be expected to demonstrate anti-androgenic activity, e.g. 54. More data would be required to verify these observations.



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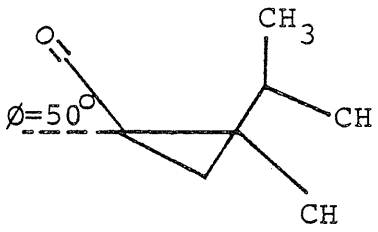
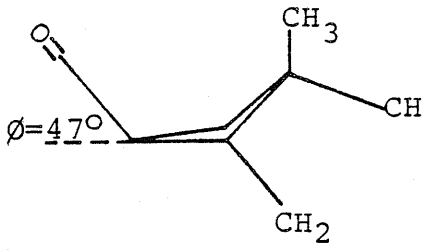
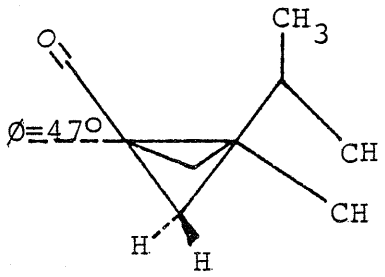
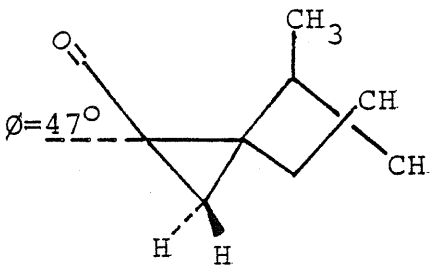
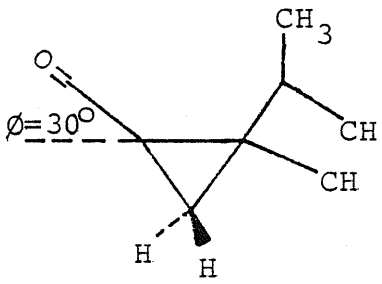
Compound	A-Ring Conformation
Testosterone	
52	
53	
54	
Cyproterone Acetate (43)	

Figure XVI. The most stable Dreiding model, A-ring conformations of a series of related anti-androgens and testosterone.

TABLE XVI

Comparison of the A-ring conformations and the angle (Ø) between the C-3 carbonyl substituent and the C-4 bond with the biological potency[59] of a series of related anti-androgens in relation to the natural androgen prehormone testosterone.

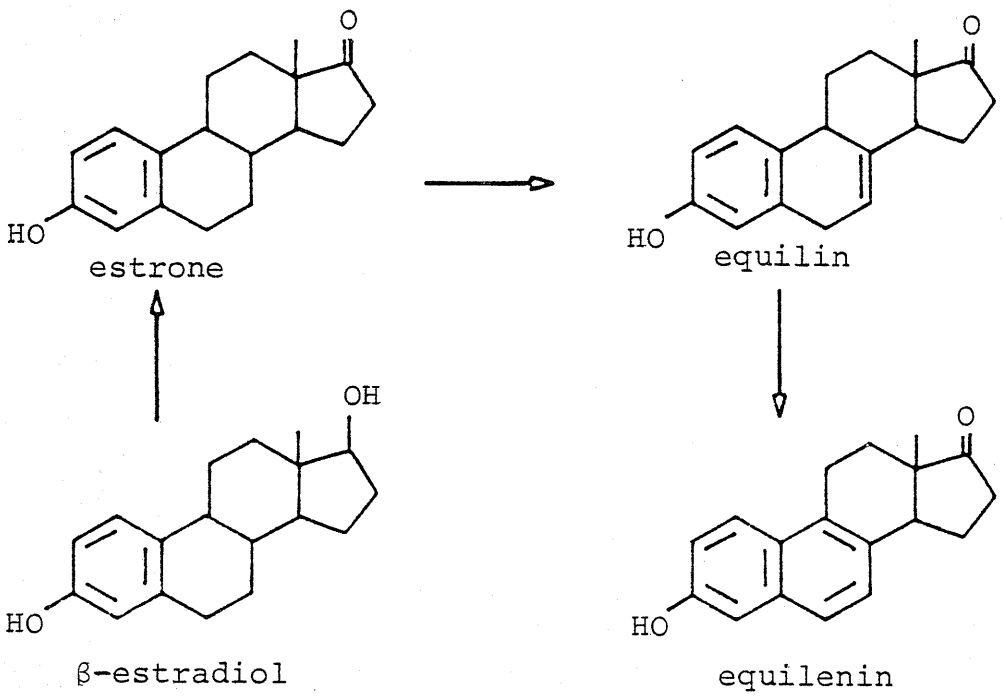
Compound	Ø	Seminal Vesicles (mg)	A-ring Conformation
Testosterone	50°	androgenic	skewed-boat
52	47°	-	boat
53	47°	380	skewed-boat
54	47°	233	boat
Cyproterone Acetate (43)	30°	207	boat

D. ESTROGENS

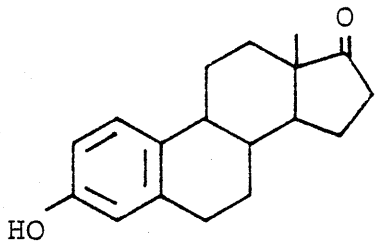
(i) β-Estradiol metabolites

Estrone, 3-hydroxy-estra-1,3,5(10)-triene-17-one (55), is a major metabolite of β-estradiol, 3,17β-dihydroxy-estra-1,3,5(10)-triene(56), in man[61,62]. Estrone is further metabolized to equilenin, 3-hydroxy-estra-1,3,5(10),6,8(11)-pentaen-17-one(57)[47c,61,62,63].

Equilin, 3-hydroxy-estra-1,3,5(10),7-tetraen-17-one(58) is the in vitro precursor to the formation of equilenin by rat liver microsomal enzymes[63]. Equilin is also a metabolite of β-estradiol in man[61]. Scheme II shows a possible metabolic pathway that accounts for the appearance of equilin in human urine. The in vitro



metabolic transformation of equilin to equilenin has been shown to proceed by what appears to be a one-step proton-hydride abstraction occurring at C-6 and C-9, respectively. The process is catalysed by a flavin-linked dehydrogenase[63].



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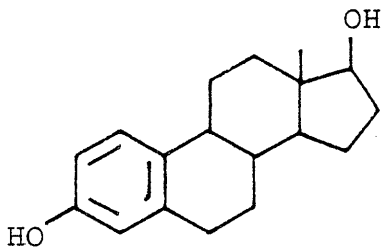

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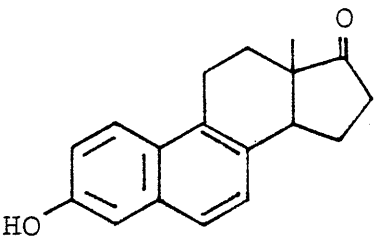

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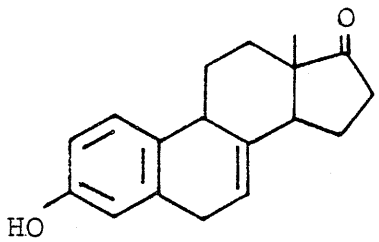

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(ii) The effect of B-ring unsaturation on the biological activity of β-estradiol metabolites

Table XVII shows the uterotrophic activity and the affinity for rabbit uterine cytosol of a series of β-estradiol metabolites[64,65]. The data of Table XVII indicates that B-ring unsaturation diminishes both the affinity for rabbit uterine cytosol and the estrogenic potency of these metabolites. Similar relationships have been shown with other estrogenic substances[64,65].

TABLE XVII

Estrogenic potency and affinity for rabbit uterine cytosol of a series of related β-estradiol metabolites[64,65].

Compound	Binding Affinity	Uterotrophic Activity
estradiol	100	341
estrone	66	100
equilin	24	74
equilenin	8	7

(a) Alteration of the partition coefficient

Table XVIII shows the partition coefficients and the log of the binding affinity for rabbit uterine cytosol of a series of related β-estradiol metabolites. The data of Table XVIII indicates that a diminished partition coefficient correlates with a diminished value in the log of the binding affinity. Figure XVII

suggests that there exists (a) a linear correlation between the partition coefficient and the log of the binding affinity, and (b) that a similar relationship exists between the partition coefficient and the log of the biological response [54].

Estrone demonstrates enhanced metabolic clearance relative to β-estradiol [47d]. For the many substances, the rate of metabolic clearance has been shown to be in part related to the partition coefficient [24]. Substances with elevated partition coefficients would demonstrate diminished biological clearance whereas substances with diminished partition coefficients would demonstrate enhanced biological clearance. The partition coefficients of equilin and equilenin are, respectively, 0.27 and 0.64 log units lower than that one of estrone. The diminished biological potency of these substances may therefore be related, in part to an enhanced rate of biological clearance.

Hydrophobic interactions between steroid and receptor are essential for the formation and the maintenance of the steroid-receptor complex [29]. Substances with elevated partition coefficients demonstrate enhanced hydrophobic interactions [5]. The converse is true of substances with diminished partition coefficients. This is consistent with the diminished binding affinity which

equilin and equilenin display for rabbit uterine cytosol relative to estrone. The diminished hydrophobicity of equilin and equilenin, may therefore compromise the hydrophobic interactions necessary for the formation and maintenance of the steroid-receptor complex [66]. It has been shown that the binding affinity of estrogenic steroids paralells the biological potency of these substances [64].

TABLE XVIII

The partition coefficients and the log of the binding affinity of a series of β-estradiol metabolites.

Compound	Partition Coefficient	Log Binding Affinity
estrone	2.00	0.82
equilin	1.73	0.38
equilenin	1.36	-1.90

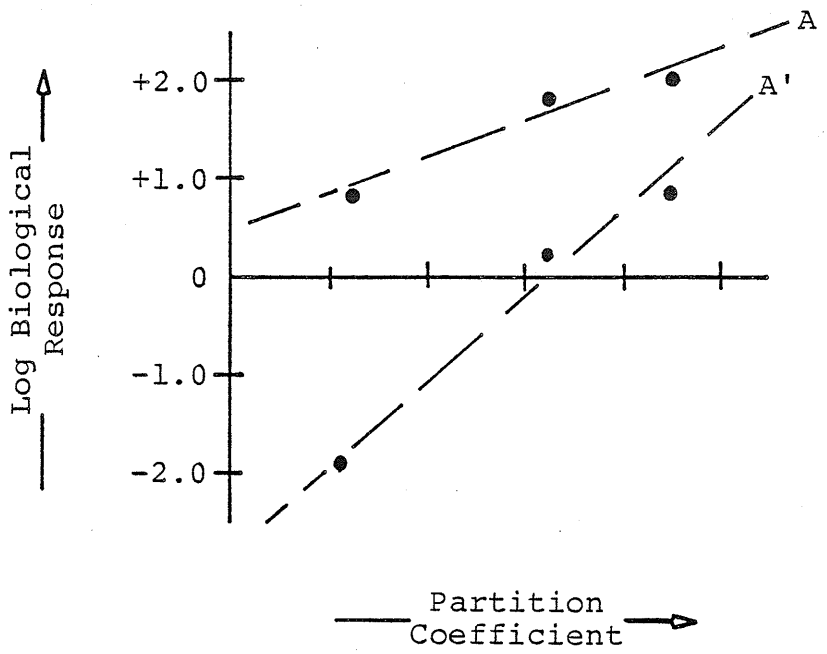


Figure XVII. Plot of the partition coefficient versus the log of the binding affinity (A) and the log of the biological response (A').

(b) Miscellaneous factors

The phenol, the non-polar portion of the steroid nucleus and the A-ring substituents have all been shown necessary in the binding of estrogens to the estrogen receptor.[64] The diminished estrogen receptor affinity of equilin and equilenin may be in part attributable to repulsive forces that exist between the B-ring site(s) of unsaturation and an electron dense region present in the estrogen receptor.[47e] Alternatively, B-ring unsaturation may alter the configuration of D-ring substituents through conformational transmission.[48]

D-ring substituents of estrogenic agents are essential for the maintenance of a viable receptor-steroid complex[64,66].

(iii) The effect of B-ring unsaturation on the estrogenic potency of some synthetic estrane derivatives.

Mestranol, 17α-ethynyl-3-methoxyestra-1,3,5(10) trien -17-ol(59), is a potent estrogenic agent[67]. The C-7 unsaturated analogue of mestranol, 17α-ethynyl-3-methoxyestra-1,3,5(10),7-tetraen -17-ol(60) exhibits 8X the estrogenic potency of mestranol in orally dosed mice[67]. The biological potency of these estrane derivatives is therefore significantly enhanced by a C-7 site of unsaturation.

(a) Alteration of the partition coefficient.

The biological activity of mestranol has been shown to be mediated exclusively by the C-3,0-demethylated metabolite 61[64]. The binding affinity of 61 is 24X greater than that one of mestranol[64]. By analogy, the biological activity of 60 may also be mediated by the C-3, 0-demethylated metabolite 62. As potent estrogenic activity may paralell both the partition coefficient and the binding affinity of estrogenic substances to the estrogen receptor, the binding affinity of 62, and therefore the hydrophobic interactions, may be greater

than those of 61.

The rate of the metabolic transformation of (drug) substances has been shown to be related to the partition coefficient of these substances.[24] Protracted half-lives and diminished rates of biotransformation were demonstrated by substances with enhanced lipophilic character.[24,54] The diminished lipophilic character of mestranol is mediated through the hydrophilic nature of the C-7 site of unsaturation. The diminished lipophilic character of 60 may therefore enhance the rate of the C-3, O-demethylation process of 60 and thereby elevate the plasma levels of the estrogenic metabolite 62.

The observation that equilin demonstrates diminished affinity for the estrogen receptor relative to estrone (Table XVII), suggests that hydrophobic interactions between steroid and the estrogen receptor have been compromised. This effect may be mediated through the diminished partition coefficient of equilin. The C-7 site of unsaturation of 62 diminishes the partition coefficient of this substance by 0.28 log units relative to the saturated analogue 61. Nevertheless, the C-17 ethylynic substituent of 62 enhances the partition coefficient by 0.44 log units (Appendix I). There is therefore a net increase in the partition coefficient of 62 relative to β-estradiol and estrone.

Hydrophoblic interactions between 62 and the estrogen receptor may therefore be expected to be maximal. Table XIX shows the partition coefficients of these estrogenic estrane derivatives.

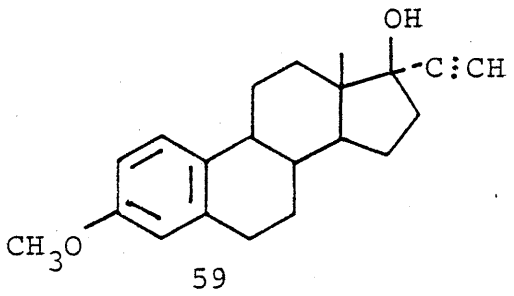
TABLE XIX

Partition coefficients and binding affinity[64] of a series of estrane derivatives.

Compound	Partition[a] Coefficient	Binding[b] Affinity
β-estradiol	0	100
61	0.44	191
62	0.16	-

[a]The partition coefficient of β-estradiol has been arbitrarily set to zero.

[b]Rabbit uterine cytosol.



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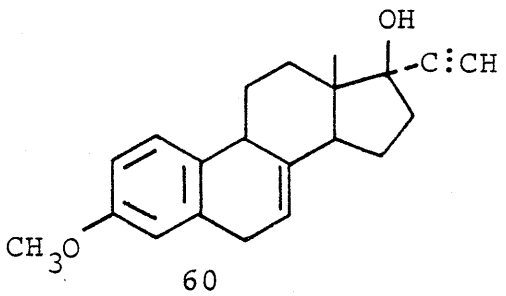

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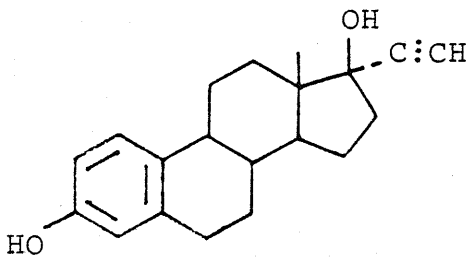

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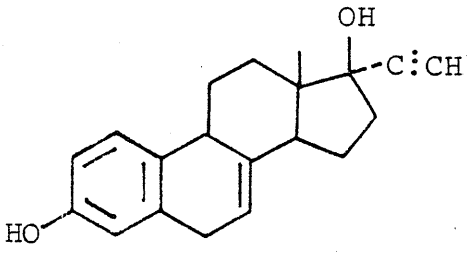


62

(b) Specificity of B-ring unsaturation

The unsaturated estrane derivative 3-hydroxy-estra-1,3,5(10),6-tetraen-17-one(63) demonstrates diminished estrogenic potency and diminished *in vitro* binding affinity for the estrogen receptor (Table XX) relative to equilin (58), the C-7 unsaturated isomer of 63[64]. The partition coefficients of 63 and 58 are identical and the significant differences in estrogenic potency and binding affinity cannot be attributable to differences in the hydrophilic/lipophilic balance between these two substances. In the non-synthetic series of estrane derivatives, C-7 unsaturation diminished both estrogenic potency and estrogen receptor binding affinity (compare estrone and equilin). However, C-6 unsaturation has a significantly more dramatic effect on the binding affinity and the estrogenic potency of these substances. This suggests that the C-7 site of unsaturation may play an important role in the interactions of the steroid with estrogenic receptor[27]. As close association of the receptor and the steroid are essential to the formation and maintenance of a viable receptor-steroid complex, the enhanced biological potency of 60 relative to mestranol (59) may therefore be impart related to the ability of the C-7 site of unsaturation to interact with a charged (electrophilic) receptor site. Mestranol which does not possess the C-7 site of unsaturation may

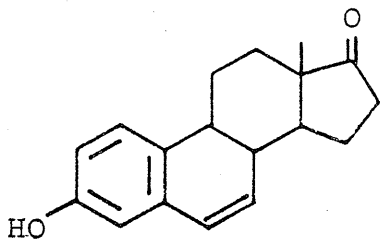
not be expected to display this interaction. Furthermore, the C-6 site of unsaturation of 63 may not be positioned so as to interact with the electrophilic site on the estrogen receptor. This is consistent with the observation that 60 is a potent estrogenic agent and demonstrates high affinity for the estrogen receptor. Alternatively, the effect of the C-6 double bond on the biological potency and receptor affinity may be mediated by a conformational change[47f]. It has been shown that the non-polar portion of the steroid nucleus, the D-ring substituents and the phenolic A-ring, all participate in the binding of estrogens to the estrogen receptor[47g,64]. Conformational changes may compromise the effectiveness of anyone of these important binding sites to interact with the estrogen receptors and therefore diminish binding affinity and biological potency[47f].

TABLE XX

Estrogenic potency and binding affinity of three related estrane derivatives[64].

Compound	Binding[a] Affinity	Estrogenic Potency
estrone	66	100
equilin	24	74
63	8	5

[a]Rabbit uterine cytosol



63

(iv) The effect of a 7α,8α-cyclopropane ring.

The estrane derivative 3-hydroxy-7α,8α-cyclopropano-17α-ethynyl-estra-1,3,5(10)-trien-17-ol(64) demonstrates 1/128X the oral estrogenic potency of mestranol and 1/1000X the oral estrogenic potency of 60[67,68]. The 7α,8α-cyclopropano substituent therefore diminishes the oral estrogenic potency of these estrane derivatives.

(a) Alteration of the partition coefficient.

The partition coefficient of 64 is greater than that one of 61 and significantly elevated relative to that one of 62. As potent estrogenic activity in these substances is demonstrated by 62, the C-3, 0-demethylated metabolite of 60, deviations from the partition coefficient value of this substance (Table XXI) may precipitate alterations in the biological potency[53,54].

The enhanced lipophilic character of 64 may alter

the biological distribution and/or metabolism of this substance.[24,54] Enhanced affinity for adipose tissue and emulsified circulating fats (chylomicrons) may therefore be expected[13]. The lipophilic/hydrophilic balance of 64 may therefore not favor partitioning onto the estrogen receptor or alternatively, the estrogen target tissue.

TABLE XXI

Partition coefficients of a series of related estrogenic estrane derivatives.

Compound	Partition Coefficient
61	1.0
62	0.73
64	1.21

(b) Effect on metabolism.

The presence of the 7,8-cyclopropano substituent may prevent the formation equilenin-like metabolites, which have been shown to demonstrate diminished biological potency relative to equilin.[63,64,65] The effect may be mediated through impairment of the proton-hydride abstraction and isomerization process which is necessary to form the C-6/C-8 sites of unsaturation (Scheme II).

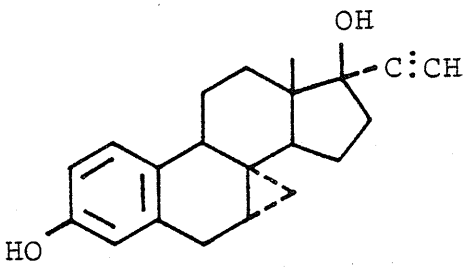
This potentially favorable effect of the cyclopropane ring does not appear to influence the biological activity of this substance.

(c) Isosteric and isoelectric nature of the cyclopropane ring.

The α-configuration of the 7,8-cyclopropano substituent renders the β-surface of the C-7 bond isosteric and isoelectric with the C-7 carbon-carbon double bond of 60. It has been suggested that α-substituents of steroidal estrogens prevent a close association of the steroid and the estrogen receptor[47h]. If the necessary close association between the estrogen receptor and the substrate is compromised, the binding affinity of α-substituted estrogenic compounds for the estrogen receptor should be diminished relative to their β-substituted isomers. Thus, 3,16β,17β-trihydroxy-estra-1,3,5(10)-triene(65) demonstrates enhanced affinity for the estrogen receptor whereas the 16α-hydroxy isomer,3,16α,17β-trihydroxy-estra-1,3,5(10)-triene(66) demonstrates diminished affinity for the estrogen receptor.

The α-methylene atom of 64 may function as other α-substituents and impair or prevent a close association of the steroid and the estrogen receptor[47h]. The close association of steroid and receptor is critical in so far as the formation and maintenance of a steroid-

receptor complex is concerned.



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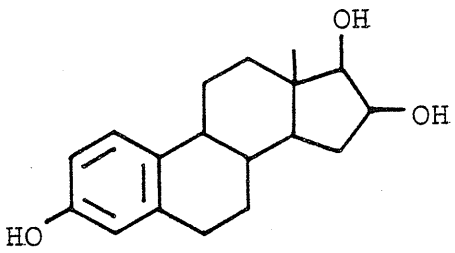

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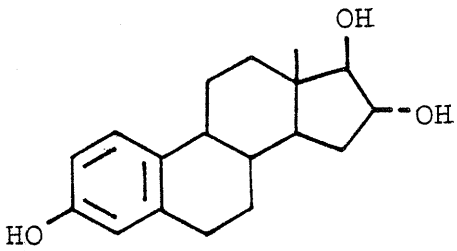


66

E. ANTI-MINERALOCORTICOIDS

(i) C-17 substituted propionic acid γ-lactone derivatives of 5α-androstan-3-one.

(a) Bases for anti-mineralocorticoid activity

The naturally occurring adrenocortical hormones associated with the maintenance of physiological electrolyte balance are aldosterone (67) and desoxycorticosterone(68)[69]. These substances are thought to activate nuclear synthesis of a messenger RNA specific for the coding of an enzyme involved in the active transport of sodium in the distal convoluted tubule of the (kidney) nephron[69]. Steroid hormones are believed to mediate biological activity through messenger RNA induced protein synthesis[70,71,72,73,74]. The active transport of sodium ions from the distal convoluted tubule of the nephron is the mechanism whereby normal electrolyte balance is maintained. Antimineralocorticoid activity may therefore be mediated by at least three mechanism: (a) interference with the normal synthesis of the enzyme responsible for sodium transport in the distal convoluted tubule, (b) interference, at the genone level, in the transcription of enzyme specific messenger RNA and (c) competitive antogonism of the mineralocorticoid receptor. Evidence suggests strongly that competitive antagonism of the mineralocorticoid receptor is the basis for anti-mineralocorticoid

activity[69,75,76,77].

(b) The effect of unsaturation.

The anti-mineralocorticoid 3-(17β-hydroxy-5α-androstan-3-one-17α-yl) propionic acid γ-lactone(69) demonstrates 0.3X the anti-mineralocorticoid activity of 3-(17β-hydroxy-5α-androst-4-en-3-one-17α-yl) propionic acid γ-lactone(70)[78]. The anti-mineralocorticoid 3-(17β-hdroxy-5α-androst-4,6-dien-3-one-17α-yl) propionic acid γ-lactone(71) demonstrates 20X the biological activity of 70[79]. The biological potency of these C-17 substituted propionic acid γ-lactone derivatives can therefore be significantly enhanced by specific A and B-ring unsaturation.

(i) Alteration of the partition coefficient.

Table XX shows the partition coefficients and the biological potencies of a series of related anti-mineralocorticoids. The data of Table XX shows that as the lipophilic character of these substances diminishes, there is a corresponding increase in the biological potency. A plot of the partition coefficient versus the log of the biological response (BR) (Figure XVIII) suggests that there is a linear relationship between the partition coefficient and the log BR values[54]. A diminished partition coefficient in these substances

correlates with potent biological effectiveness (Table XX). As competitive antagonism of the mineralocorticoid receptor is the bases for anti-mineralocorticoid activity, both receptor affinity and competitive antagonism appear to diminish as the lipophilic character of these substances increases, e.g. 69, that is, the binding affinity of these substances is inversely proportional to the partition coefficient.

Alterations in the hydrophilic/lipophilic balance of (drug) substances have been shown to modify the biological distribution and/or the metabolism of these substances[24,54]. The biological potency of 71 may be attributable to diminished vascular penetration[13] and enhanced affinity for target tissue recognition sites[29,54]. Conversely, the diminished biological potency of 69 may be attributed to enhanced vascular penetration and enhanced interactions with lipophilic macromolecules other than the mineralocorticoid receptor.

TABLE XX

Partition coefficients and the biological potency[9,78] of a series of related anti-mineralocorticoids.

Compound	Partition Coefficient	Biological Potency [a]
69	2.00	0.3
70	1.73	1.0
71	1.36	20

[a]The biological potency was obtained by comparing the M.E.D. values of these substances relative to that one of 70 which was arbitrarily set, at 1.0. The median effective does (M.E.D.) is defined as the amount of substance required to block 50% of the mineralocorticoid activity of desoxycorticosterone in adrenalectomized rats.

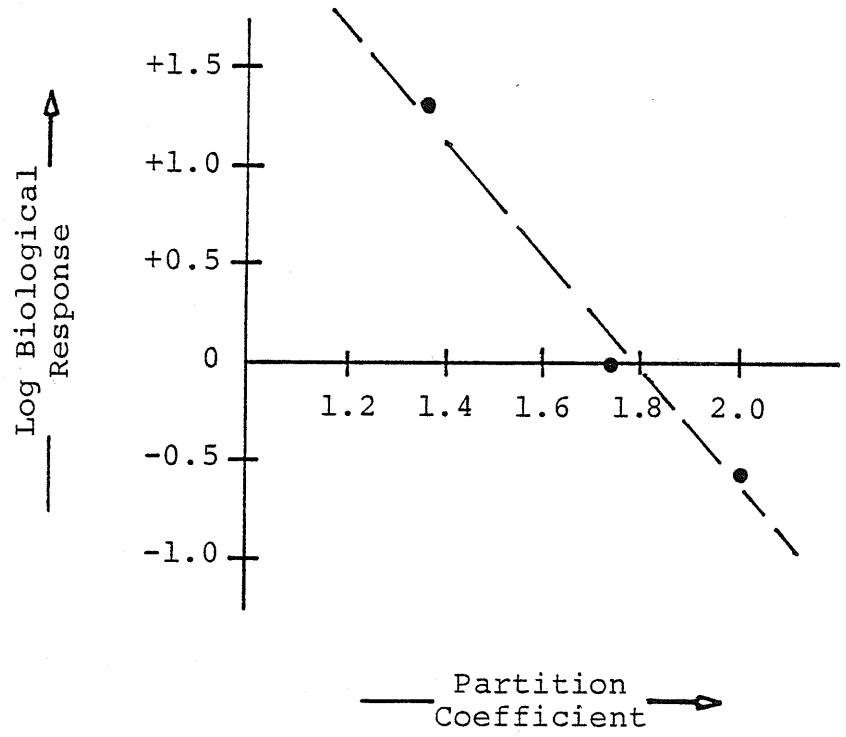


Figure XVIII. Plot of the partition coefficient versus the log of the biological response.

(ii) The effect on metabolism.

The A-ring reduction of C-4 unsaturated 3-ketosteroids proceeds through the initial reduction of the C-4 site of unsaturation. The reduction of the C-3 carbonyl group proceeds only after the reduction of the A-ring C-4 olefinic bond has taken place[13]. The A-ring reduction of 70 may proceed as discussed above and would yield a biologically active metabolite (69) as an intermediate. The A-ring reduction of 69 proceeds directly to yield C-3 hydroxylated metabolites. The C-3 hydroxylated metabolites, by analogy to C-3 hydroxy metabolites of other 3-ketosteroids, may be expected to demonstrate minimal or diminished biological activity[42,78]. The enhanced biological potency of 70 relative to 69 may therefore be attributed to a diminished rate in the formation of biologically inactive C-3 hydroxylated metabolites and the presence of a biologically active metabolic intermediate.

The biotransformation of C-4 unsaturated, 3-ketosteroids has been shown to involve C-6 hydroxylation [9]. This oxidation process is believed to proceed through the mechanism outlined in Scheme I. The biotransformation of 70 may involve hydroxylation at C-6. The presence of the C-6 unsaturated site may impair the enzyme induced isomerization mechanism (Scheme I) which is a necessary step in the hydroxylation process. Nevertheless, hydroxylation through a C-6(7) epoxide intermediate may still take place.[80] The potent

biological activity of 71 may therefore be in part explained on the bases of a diminished rate of the appearance of C-6 hydroxylated metabolites.

(b) The effect of a 6β,7β-cyclopropane ring.

The anti-mineralocorticoid 3-(6β,7β-cyclopropano-17β,hydroxy-5α-androst-4-en-3-one-17α-yl) propionic acid γ-lactone(72) is 2.5X more effective than 71 in eliciting an anti-mineralocorticoid response [76,77]. The 6β,7β-cyclopropano substitutent has therefore a potentiating effect on the biological potency of these substances.

(i) Alteration of the partition coefficient.

The 6,7-cyclopropane ring enhances the lipophilic character of 71 [6]. The partition coefficient of 72 is comparable to that one of 69. The partition coefficient of 69 correlated with weak biological activity. The partition coefficient of some (drug) substances has been shown to demonstrate a linear correlation with the log of the biological response. Nevertheless, many of these straightline corrleations between partition coefficients and log BR have been found in fact to be parabolic when a statistically valid sample of data has been made available. Therefore, although Figure XVIII suggests that a linear correlation between these two

parameters exists, the correlation may in fact only represent the linear portion of the parabola and thus 72 represents a near maximum or maximum value for the parabola.

The lipophilic properties of 72 enhances the vascular penetration of this substance [13]. Similarly changes in the partition coefficient may modify the metabolism of this substance [24,54]. Altered metabolism and elevated vascular penetration can prolong the biological half-life of these substances [81]. The lipophilic properties of 72 may concomitantly give rise to enhanced hydrophobic interactions with macromolecules [29]. Formation and maintenance of the receptor complex are essential for effective anti-mineralocorticoid activity [47a,h]. Hydrophobic interactions between steroid and the receptor are important for the formation and maintenance of the receptor-steroid complex [47h].

(ii) The effect on metabolism.

The 6,7-cyclopropane ring may prevent hydroxylation at C-6 by (a) obstructing the enzyme induced isomerization of the C-4 site of unsaturation (Scheme I) [9], and (b) impairing the formation of a C-6(7) epoxide intermediate [80]. The potent biological activity of 72 may in part be attributable to the impairment of C-6

hydroxylation as mediated by the 6,7-cyclopropane ring.

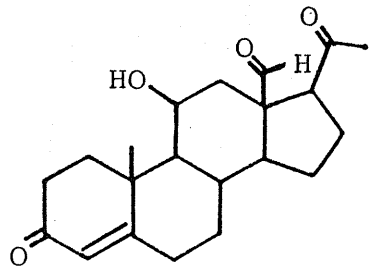
(iii) Isosteric and isoelectric nature of the 6,7-cyclopropane ring.

The β-configuration of the 6,7-cyclopropane ring in 72, renders the α-surface of the C-6 bond isoelectric and isosteric with a C-6 carbon-carbon double bond. The carbon-carbon double bond demonstrates hydrophilic properties and participation through hydrophobic interactions with macromolecules is therefore diminished. The cyclopropyl-methylene atom, by virtue of the enhanced lipophilic character of the cyclopropane ring, should demonstrate a quantitatively greater number of hydrophobic interactions with macromolecules relative to the carbon-carbon double bond. These hydrophobic interactions ensure the formation and maintenance of the receptor-steroid complex.

(ii) 15-ketopregnane derivatives

(a) The effect of unsaturation

The biologically active substance pregn-4-ene-3, 15,20-trione(73) demonstrates anti-mineralocorticoid activity equivalent to that of pregna-4,6-diene-3,15,20-trione(74)[82]. The cross-conjugated triene pregna-1,4, 6-triene-3,15,20-trione(75) demonstrates 0.35X the



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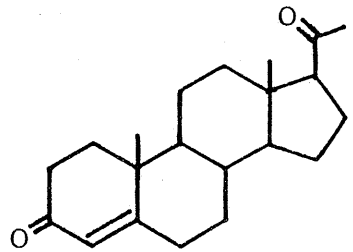

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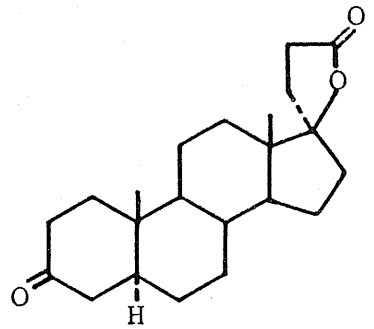

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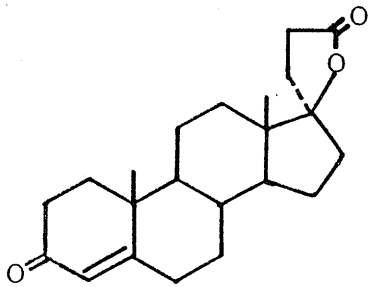

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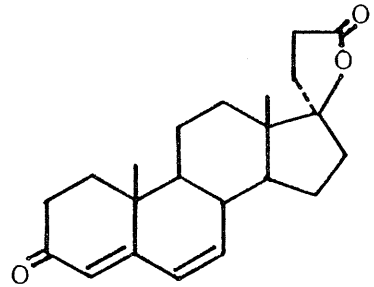

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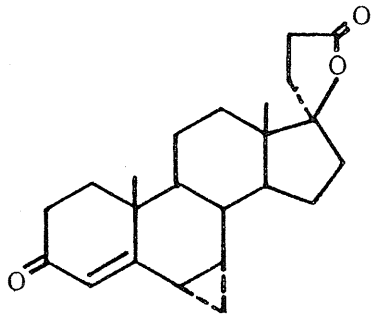


72

biological potency of 73 and 74 [82] Unsaturation has therefore a variable effect on the anti-mineralocorticoid activity of these 15-ketopregnane derivatives.

(i) Alteration of the partition coefficient.

Table XXI shows the biological potencies and the partition coefficients of a series of unsaturated 15-ketopregnane anti-mineralocorticoids. That unsaturation diminishes the lipophilic character is reflected in the partition coefficient values of these substances. A diminuation in the partition coefficient reflects itself as an increased tendency to interact with aqueous phase biological compartments [54] The altered distribution diverts the biological substance from lipophilic compartments, for example target tissues. As competitive antagonism is the bases for anti-mineralocorticoid activity, and hydrophobic interactions are essential to the formation and maintenance of the receptor-steroid complex,[29,47h] substances demonstrating diminished lipophilic character, e.g. 75, will demonstrate weak biological activity. The weak biological activity may be attributed to diminished hydrophobic interactions with macromolecules and a concomitent decrease in the effectiveness of these substances to competitively antagonize the mineralocorticoid receptor.

The partition coefficient of many substances has been shown to be parabolically related to the log of the biological response (BR) [54] Accordingly, 74 may be expected to be less potent than 73, but as these two substances demonstrate identical biological potencies, it must be assumed that the partition coefficients of 73 and 74 lie in similar regions of the parabolic loci in which a maximum biological response is observed. Thus, the partition coefficient of 75 may be expected to lie outside the maximum biological response region of the parabolic loci. The substance (75) should demonstrate diminished biological potency relative to its more lipophilic congeners. This is experimentally observed. Figure XIX is a plot of the partition coefficients versus the log of the biological response (BR). More data would be required to show the relationship between the two parameters.

TABLE XXI

Biological potencies[81] and partition coefficients of a series of unsaturated anti-mineralocorticoids.

Compound	Biological Potency	Partition Coefficient
73	1.0	2.73
74	1.0	2.36
75	0.18	2.09

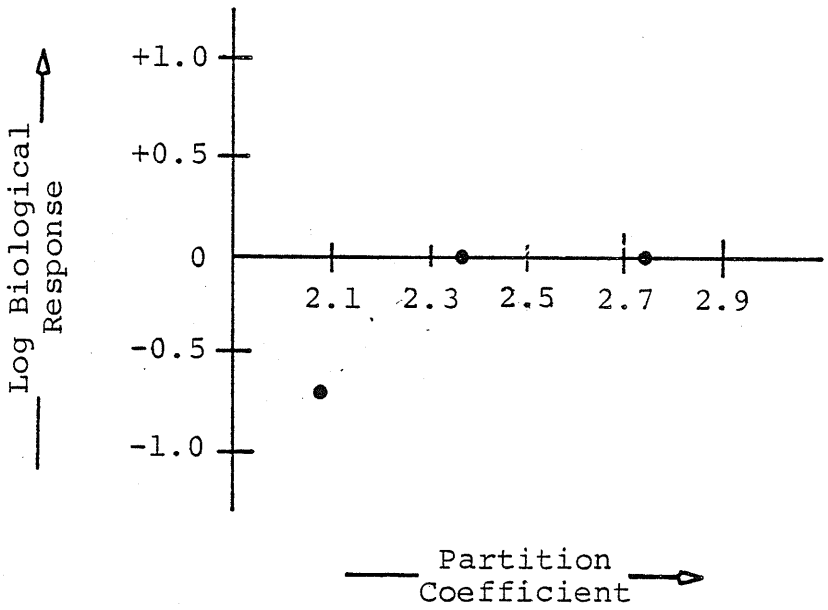


Figure XIX. Plot of the partition coefficient versus the log BR values of a series of related anti-mineralocorticoids.

(b) The effect of a 6β,7β-cyclopropane ring.

Table XXII shows the biological potencies and the partition coefficients of two 15-keto-pregnane derivatives substituted at C-6/C-7 with a cyclopropane ring[83]. The biological potencies and partition coefficients of the unsaturated analogues of these cyclopropanosteroids are also shown for comparison. The data of Table XXII indicates that 6β,7β-cyclopropano-pregna-4-ene-3,15,20-trione(76) demonstrates diminished biological potency relative to its unsaturated analogue 74, whereas 6β,7β-cyclopropano-pregna-1,4-diene-15,20-dione(74) demonstrates

enhanced biological potency relative to its unsaturated analogue 75. The 6β,7β-cyclopropane ring has therefore a variable effect on the biological potencies of these unsaturated anti-mineralocorticoids [83]

TABLE XXII

Biological potencies[82,83] and partition coefficients of some cyclopropane ring substituted 15-ketopregnanes.

Compound	Biological Potency	Partition Coefficient
74	1.0	2.36
75	0.18	2.09
76	0.4	2.94
77	0.4	2.67

(i) Alteration of the partition coefficient.

The partition coefficient associated with optimum biological potency of the unsaturated pregnane anti-mineralocorticoids discussed above is that one of 74 (Table XXII). The substitution of the C-6 double bond in 74 with the 6,7-cyclopropane ring enhances the hydrophobic character of this substance by 0.58 log units [6] The 6,7-cyclopropanosteroid 76 will therefore demonstrate vascular penetration significantly greater than that of 74. Furthermore, 76 will demonstrate enhanced hydrophobic interactions with macromolecules other than the mineralocorticoid receptor relative to 74.

These factors may combine to diminish the biological potency of 76 by a factor of 0.4X relative to 74.

The replacement of the C-6 double bond in 75 with the 6,7-cyclopropane ring enhances the hydrophobic character of this substance by 0.58 log units[6]. The 6,7-cyclopropanosteroid 77 will therefore demonstrate vascular penetration significantly greater than that of 75. Furthermore, 77 will demonstrate enhanced hydrophobic interactions with macromolecules relative to 75. These factors combine to enhance the biological potency of 77 by a factor of 2.2X relative to 75.

Competitive antagonism of the mineralocorticoid receptor is thought to be the basis for anti-mineralocorticoid activity. Enhanced hydrophobic interactions with macromolecules other than the mineralcorticoid receptor diminishes the biological response by compromising effective competitive antagonism for the mineralocorticoid receptor diminishes the effectiveness of this substance to effectively antagonize the minerolocorticoid receptor relative to 77.

The biotransformation of (drug) substances may by modified through alterations in lipophilic/hydrophilic balance[24]. The partition coefficient of the 6,7-cyclopropano steroids 76 and 77 are significantly elevated relative to the partition coefficients of their respective unsaturated analogues 74 and 75. Modifications in the biotransformation

of 76 may account in part, for the diminished biological potency of this substance relative to 74 and similarly, modified biotransformation of 77 may account for the enhanced, biological activity of this substance relative to 75. The partition coefficient of 76 is 0.27 log units greater than that one of 77[5a]. The metabolism of 76 may therefore be significantly modified relative to that of 77[24].

Figure XX is a plot of the partition coefficients versus the log BR values of the anti-mineralocorticoids discussed above. Figure XX does not show a clear relationship between the partition coefficients and the log of the biological response (BR) values for these pregnane derivatives[54].

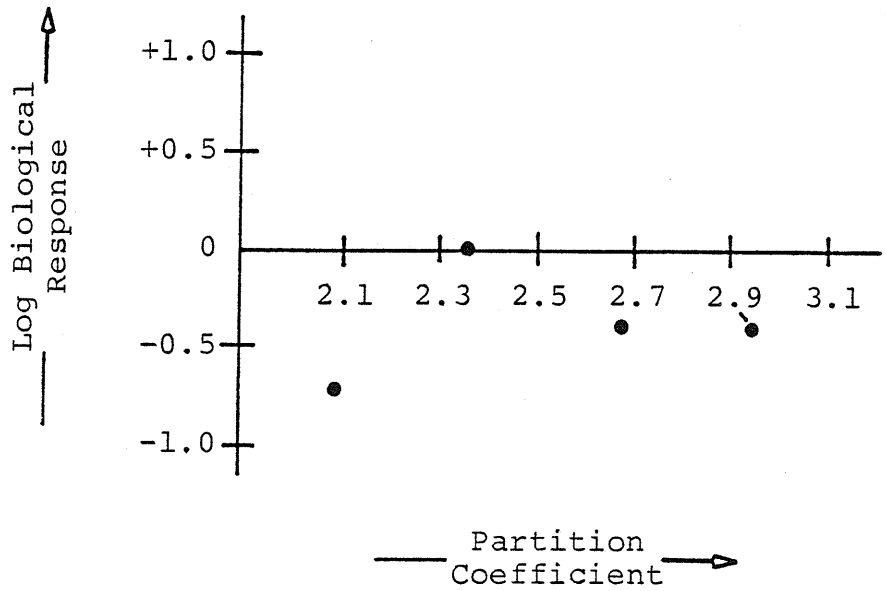


Figure XX. Plot of the partition coefficients versus the log BR values of a series of related anti-mineralocorticoids

(ii) The effect of A-ring conformation.

Figure XXI shows the most stable Dreiding model conformations of the 15-ketopregnane anti-mineralocorticoids disucssed above viewed through the imaginary C-2/C-4 axis. The angle (Ø) between the C-3 carbonyl substituent and the plane of the C-4 double bond was measured. Table XXIII summarizes the data of Figure XXI and includes the biological potencies of these substances for comparison. The data of Table XXIII suggests that potent anti-mineralocorticoid activity is observed for those pregnane derivatives with Ø 35^{o}, e.g. 73 and 74. Furthermore, as Ø becomes progressively smaller the biological potency of these substances is diminished signficantly, e.g. 75 and 76. The correlation between Ø and the biological potency does not apply to 77 nevertheless. If this correlation were operative, 77 should demonstrate similar biological potency as 75. The enhanced biological potency of 77 must be attributed to an enahnced efficiency in competitively antagonizing the mineralocorticoid receptor. The effect of the 6,7-cyclopropane ring on the hydrophobic properties[6] of these substances may account for the deviation in the correlation of Ø with biological potency.

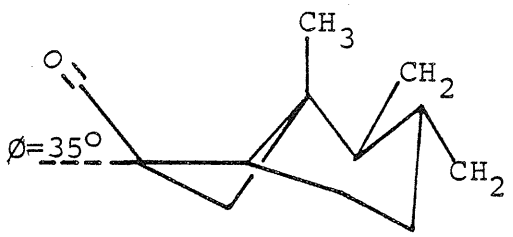
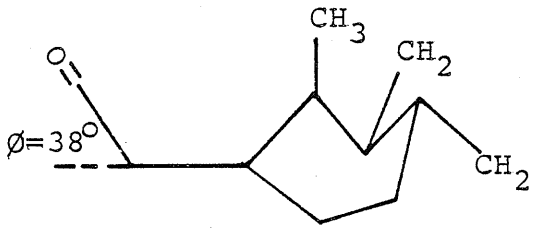
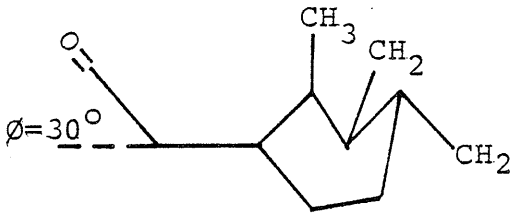
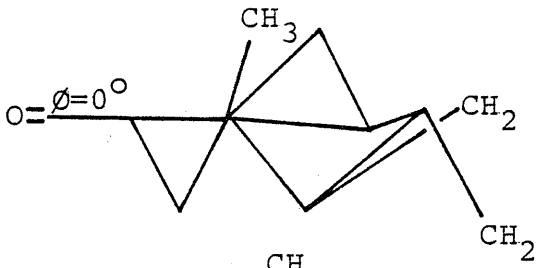
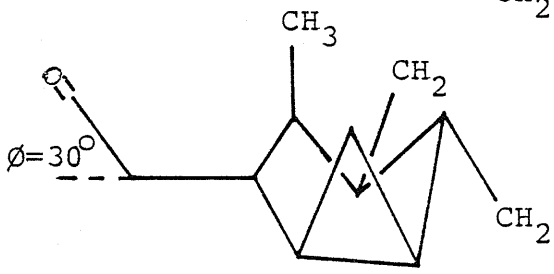
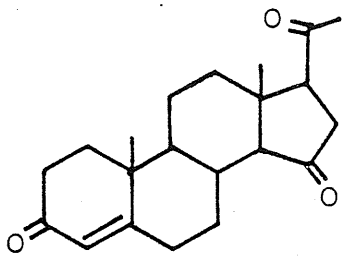
Compound	A-Ring Conformation
73	
74	
75	
76	
77	

Figure XXI. Most stable Dreiding model A-ring conformations of a series of related pregnane derivatives.



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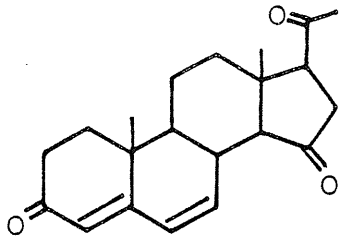

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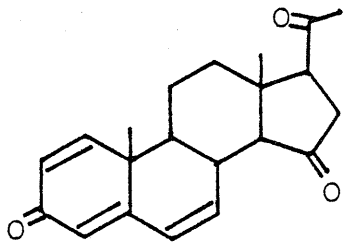

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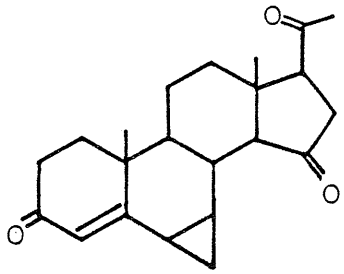

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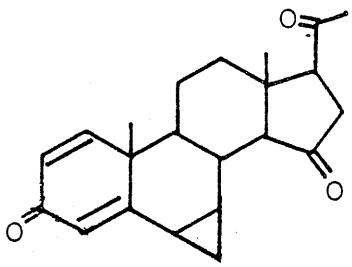

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TABLE XXIII

A-ring conformations, biological potencies and the angle (Ø) between the C-3 carbonyl substituent and the C-4 site of unsaturation of a series of pregnane anti-mineralocorticoids.

Compound	A-ring Conformation	Ø	Biological Potency
73	skeewed-boat	35^{o}	1.0
74	boat	38^{o}	1.0
75	boat	30^{o}	0.18
76	boat	30^{o}	0.4
77	half-chair	-	0.4

F. ANDROGENS

(i) 5α-Androstane derivatives

The androstane derivative, 17β-hydroxy-5α-androstane(78), demonstrates 0.72X the androgenic activity of the natural androgenic substance 17β-hydroxy-5α-androst-4-en-3-one(79), testosterone[42].

(a) The effect of unsaturation

The C-2 unsaturated analogue of 78, 17β-hydroxy-5α-androst-2-ene(80) demonstrates 0.5X the androgenic activity of testosterone[26]. C-2 unsaturation has therefore a diminishing effect on the biological potency of 17β-hydroxy-5α-androstane.

(i) Alteration of the partition coefficient.

The carbon-carbon double bond diminishes the lipophilic character of a substance[5a]. The enhanced hydrophilic character of these unsaturated substances reflects itself as diminished interactions with lipophilic biological compartments and hydrophobic macromolecules[29,54]. The partition coefficient of 78 is 0.27 log units greater than that one of 80. The androgen 80 therefore demonstrates a greater tendency to interact with aqueous phase biological compartments relative to 78[13,24,54]. The modifications in the partition coefficient which give rise to altered

distribution and/or metabolism[24] may account, in part, for the diminished biological potency of 80 relative to the saturated congener 78.

An affinity specific for the androgen receptor is essential for biological activity[49]. The partition coefficient, and hence the lipophilic characeter of a substance, may modify the receptor affinity through alterations in the hydrophobic interactions between a substance and the receptor[29]. Diminished biological activity may arise if hydrophobic interactions are compromised as receptor affinity is similarly compromised. It has been shown that substances that bind to the androgen receptor with enhanced affinity demonstrate potent biological activity[49]. The diminished hydrophobic character of 80 may therefore result in a diminished affinity for the androgen receptor and/or compromise the effectiveness of 80 to form and maintain the steroid-receptor complex.

The biotransformation of (drug) substances may be altered through modifications in the partition coefficient values of substances[24]. Altered and/or accelerated biotransformation of 80 may account for the diminished biological potency of this substance relative to 78.

(ii) The effect on metabolism.

Androgenic activity in man is mediated by the 5α-reduced analogue of testosterone, 17β-hydroxy-5α-androstan-3-one(81), 5α-dihydrotestosterone[47i]. The significance of the C-3 oxygen function for biological activity in these substances has been questioned and because 1,4-seco-17β-hydroxy-5α-androstane(82) demonstrates 0.3X the androgenic potency of testosterone, the C-3 oxygen function cannot be regarded as an essential structural requirement for androgenic activity[84]. Nevertheless, there is evidence to suggest that in fact this may be the exception and, therefore, not necessarily the rule.

The biotransformation of 80 may be anticipated to include the products of allylic oxidation, i.e., C-1 and C-4 oxygenated metabolites. These unsaturated, C-1 and C-4 oxygenated metabolites may undergo further oxidation to yield the C-1 and C-4 ketosteroids, 83 and 84, respectively. The observation that the 17β-acetate of authentic 84[85] demonstrates approximately 20X the androgenic activity of the 17β-acetate derivative of authentic 83[86], suggests that the 4-ketosteroid 84 may be expected to demonstrate enhanced androgenic activity relative to the 1-ketosteroid 83. As the androgenic potency of 80 is comparable to that of 84, the androgenic activity of 80 may arise through the in vivo metabolic conversion of this substance to 84.

The biotransformation of the A-ring non-functionalized steroids such as 78 may, by analogy, owe their androgenic activity to the in vivo conversion of these substances to a C-3 hydroxy metabolite 87. Further oxidation of these hydroxy metabolites[88a] gives rise to the potent androgen 5α-dihydrostestosterone (81). The difference in the androgenic potencies of 80 and 75 may therefore be only a reflection of the androgenic potencies of the A-ring oxygenated metabolites of these two substances.

(b) The effect of a 2α,3α-cyclopropane ring.

The androgenic steroid 2α,3α-cyclpropano-17β-hydroxy-5α-androstane(85) demonstrates 0.3X the androgenic activity of testosterone[30,89,90]. The 2α,3α-cyclopropane ring therefore diminishes the androgenic activity of these 17β-hydroxy-5α-androstane derivatives.

(i) Alteration of the partition coefficient.

Table XXIV shows the androgenic potencies and the partition coefficients of the C-3 de-oxygenated (androgenic) 5α-androstane derivatives discussed above. The data of Table XXIV indicates that a partition coefficient of 2.00, e.g. 78, correlates with potent androgenic activity. Substances with partition

coefficients greater than, e.g. 85, or less than, e.g. 80, that one of 78 demonstrate diminished biological potency.

The partition coefficients of a variety of substances have been shown to demonstrate a parabolic correlation with the log of the biological response (BR)[54]. A plot of the partition coefficient values versus the log BR values of these C-3 de-oxygenated steroids discussed above does not indicate a correlation between the partition coefficients and the log of the biological response values (Figure XXII). More data would be required to show that a correlation between these two parameters does, in fact, exist among these substances.

In this related series of androgenic substances, the compounds which demonstrate enhanced lipophilic character relative to the other members of the series, exhibit both enhanced vascular penetration and enhanced hydrophobic interactions with macromolecules. The 2,3-cyclopropane ring enhances lipophilic character[6] and therefore the hydrophobic properties[29] of substances relative to the effect of a site of unsaturation on lipophilic character. The altered distribution that results from the enhanced affinity for lipophilic biological compartments[13] may account in part for the diminished androgenic potency of 85 relative to 80.

TABLE XXIV

The androgenic potencies[26,88] and partition coefficients of a series of C-3 de-oxygenated 5α-androstane derivatives.

Compound	Androgenic Potency [a]	Partition Coefficient
78	0.72	1.00
80	0.50	0.73
85	0.30	1.21

[a] Relative to testosterone standard (activity=1.0)

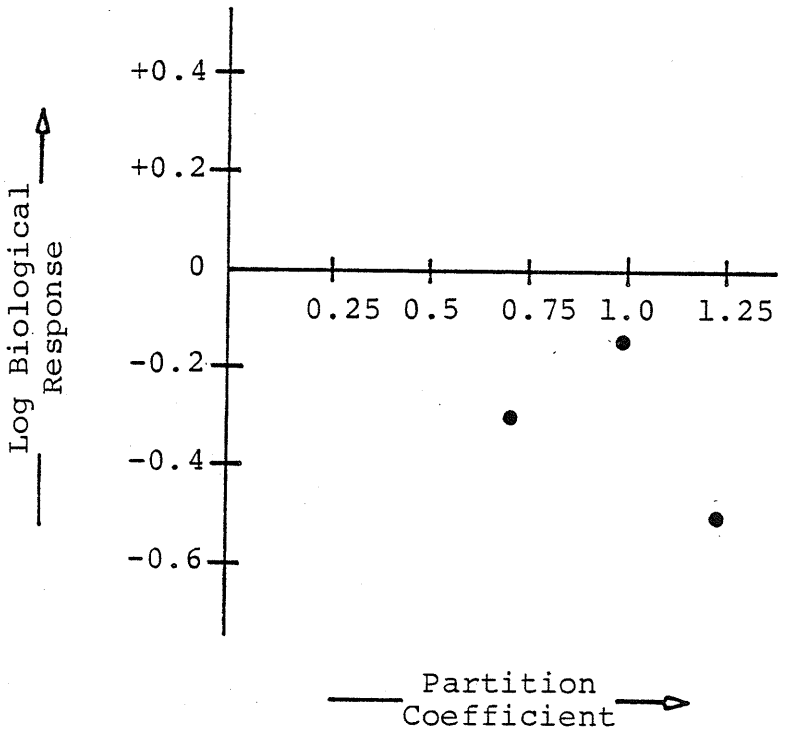
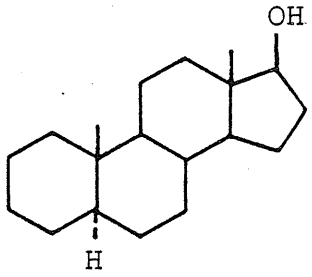


Figure XXII. A plot of the partition coefficients versus the log BR values of a series of C-3 de-oxygenated 5α-androstane derivatives.

(ii) Isosteric and isoelectric nature of the 2,3-cyclopropane ring.

The α-configuration of the 2,3-cyclopropane ring renders the β-surface of the C-2 bond of 85 is isosteric and isoelectric with the C-2 double bond of 80. Evidence suggest that the β-surface of androgenic steroids is involved in receptor interactions[30]. This may account for the lack of androgenic activity in the C-17 hydroxy isomer of testosterone, cis-testosterone(86)[49]. As the C-2 bond of 85 is isosteric with the β-surface of the C-2 double bond of 80, androgenic activity attributable to this function is maintained by the C-2 pseudo-unsaturated cyclopropane bond of 85[88b].

It has been shown that A-ring, α-substituents e.g. 2α- and 3α-methyl substituents, diminish the androgenic activity of C-3 oxygenated 5α-androstane derivatives[13]. This suggests that the formation and the maintenance of the androgen-receptor complex is impaired by the presence of these bulky substituents. The α-configuration of the 2,3-cyclopropane ring in 85 may function to diminish the biological activity of this substance in the same manner in which the 2α- and 3-methyl substituents function to diminish androgenic activity in other androgenic steroids, i.e. by preventing a close association of the steroid and the androgen receptor[13,47h].



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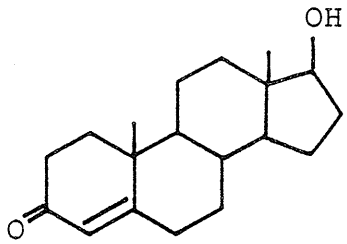

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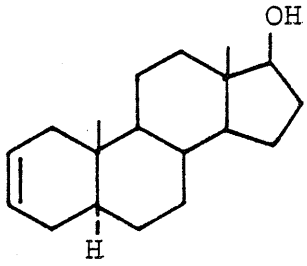

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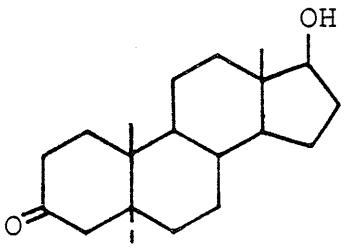

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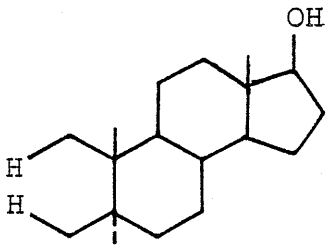

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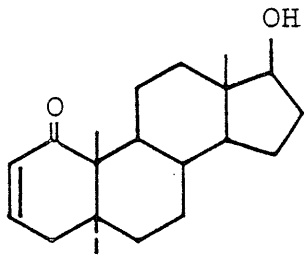

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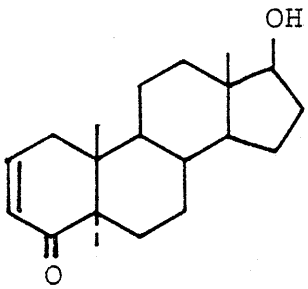

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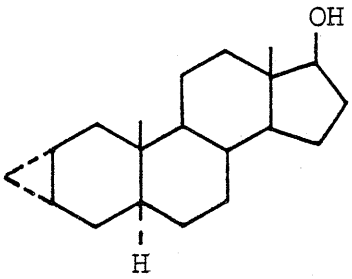

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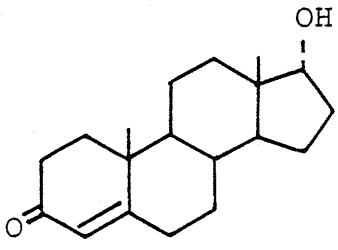

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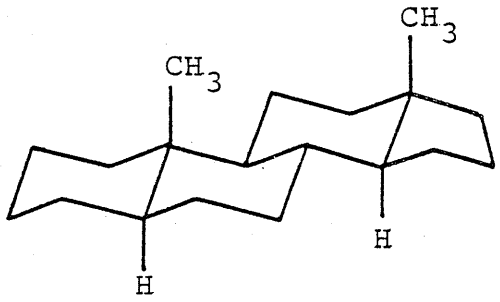
(ii) 5β-Androstane derivatives.

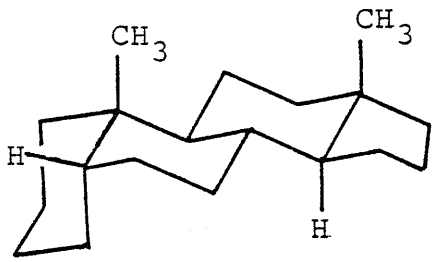
(a) The effect of C-5 configuration on androgenic activity.

Table XXV shows the ventral prostate weights of castrated, mature rats treated with a variety of 5α- and 5β-androstane derivatives[42]. The data of Table XXV indicates that, with few exceptions the 5α-series of androstane derivatives is by far, more effective than the 5β-androstane derivatives in eliciting a weight gain in the ventral prostate of castrated, mature rats. Figure XXIII shows the configurational relationships between the 5α- and the 5β-series of androstane derivatives[92]. The configurational dissimilarity between the 5α- and 5β-series of androstane derivatives (Figure XXIII), the data of Table XXV and the observation that there is a high correlation between androgen receptor affinity and potent androgenic activity[49], suggests that the configuration of the A-ring in the 5β-androstane derivatives does not permit a close association of the steroid with the androgen receptor[47h]. This may impair or prevent the formation and/or maintenance of a viable steroid-androgen receptor complex necessary for the expression of biological activity[91]. The 5β-androstane derivatives do, nevertheless, form

effective steroid-receptor complexes with the progesterone receptor as evidence by the progestational activity of 16α,17α-diflurocyclopropano-5β-androstane-3β,17β-diol diacetate(87)[20].



5α-Androstane Configuration (5α/10β)



5β-Androstane Configuration (5β/10β)

Figure XXIII. The steroid nucleus configuration of the 5α-androstane and 5β-androstane series of androgenic steroids[92].

(b) <u>The effect of a 4α,6-cyclopropane ring on the androgenic potency of 5α-androstane derivatives</u>.

Figure XXIV shows the steroid nucleus configuration of a 4α,6-cyclopropano-5β-androstane[93] derivative. Figure XXIV demonstrates that the A-ring configuration of 5β-androstane[92] is maintained in this 4α,6-cyclopropanosteroid derivative. The androgenic steroid 4α,6-cyclopropano-17β-acetoxy-5β-androstan-3-one(88) demonstrates less than 0.2X the androgenic potency of testosterone(79)[93]. The 4α,6-cyclopropane ring, therefore, diminishes the biological potency of C-3/C-17 oxygenated 5β-androstane derivatives.

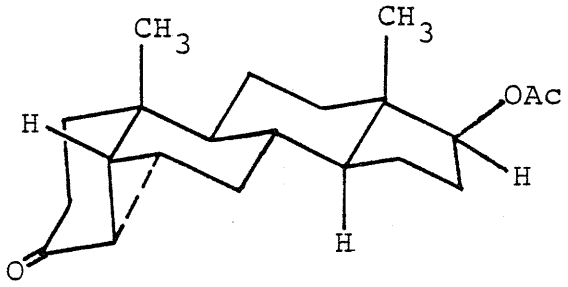


Figure XXIV. Steroid nucleus configuration of 4α,6-cyclopropano-17β-acetoxy-5β-androstan-3-one(88)[93].

TABLE XXV

Ventral prostate weights of castrated, mature rats treated with 5α-androstane and 5β-androstane derivatives[42].

Compound	Ventral Prostate Weights[a]	Relative Potency[b]
5α-Androstane	32.7± 7.2	0.65
5β-Androstane	16.2± 0.6	0.32
3α-Hydroxy-5α-androstane	20.9± 5.2	0.42
3α-Hydroxy-5β-androstane	21.8± 4.4	0.44
3β-Hydroxy-5α-androstan-17-one	43.4± 2.5	0.87
3β-Hydroxy-5β-androstan-17-one	20.2± 4.6	0.40
3α,17β-Dihydroxy-5α-androstane	66.4± 10.2	1.33
3α,17β-Dihydroxy-5β-androstane	22.1± 10.4	0.44
Sesame oil	16.5± 6.4	0.33

[a]The ventral prostate weights are expressed as the the weight, in milligrams, per 100 grams body weight.

[b]Relative to testosterone (activity = 1.0).

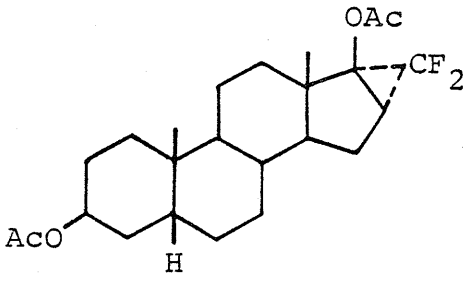
(i) Alteration of the partition coefficient

The partition coefficient of 88 is significantly enhanced relative to that one of 3α,17β-dihydroxy-5β-androstane (89)[42]. The latter substance demonstrates 0.44X the androgenic activity of testosterone (Table XXV). The enhanced lipophilic character of 88 gives rise to enhanced vascular penetration[13] and enhanced hydrophobic interactions with macromolecules[29]. The enhanced hydrophobic interactions with macromolecules other than the androgen receptor may account for the 50% reduction in the androgenic activity of 88 relative to 89. Alterations in the hydrophilic/lipophilic balance of a substance have been shown to alter the metabolism of certain (drug) substances[24]. The enhanced lipophilic character of 88 may therefore result in alterations of the metabolism of this substance to, for example, give rise to 3β-hydroxy metabolites. The 3β-hydroxy derivative of 17β-hydroxy-5β-androstane demonstrates significantly diminished androgenic activity relative to the 3α-isomer 89 (Table XXV)[42]. Furthermore, as a 17-carbonyl substituent diminishes the androgenic activity of 5β-androstane derivatives[42], an enhanced rate in the hydrolysis of the C-17 acetoxy substituent and subsequent oxidation of the hydroxyl substituent to the ketone may also contribute to diminish the androgenic activity of 88.

(ii) The effect of metabolism on the partition coefficient.

The biological activity of some natural steroids, e.g. progesterone, has been suggested to be mediated by active metabolites[47b]. The biologically active metabolite of 88 is most likely 4α,6-17β-hydroxy-5β-androstan-3-one(90). The androgenic activity of both the 3β- and 3α-hydroxy derivatives of 17β-hydroxy-5β-androstane may be mediated through the common 3-ketosteroid 91. The partition coefficient of the C-17 hydroxy metabolite of 88[89], is now diminished relative to that of 91, the C-3 oxidized metabolite of 89 by approximately 0.30 log units. The diminished lipophilic character of 100 may therefore enhance the interactions of this substance with the polar aqueous phases of the biological system and diminish the affinity of this substance for the androgen receptor. The altered distribution may therefore decrease the biological half-life and furthermore, alter the metabolism of this substance[24]. The altered hydrophobic character of 90 may also diminish the hydrophobic interactions[29] of this substance with the androgen receptor and thereby compromise the effective formation and/or maintenance of a viable steroid-receptor complex. It has been shown that potent androgenic activity correlates with enhanced androgen receptor affinity[42]. Similarly,

diminished androgen receptor affinity has been shown to correlate with diminished biological potency[42].



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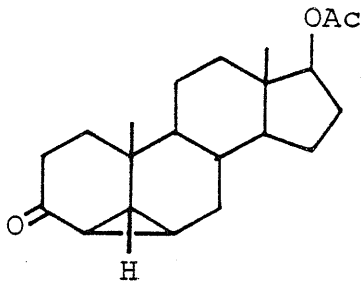

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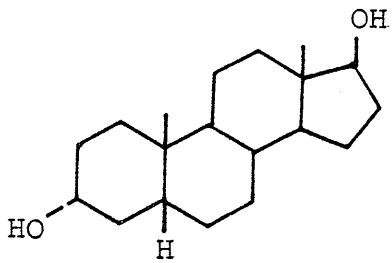

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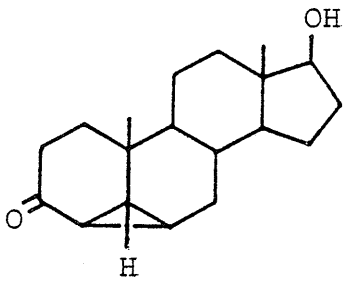

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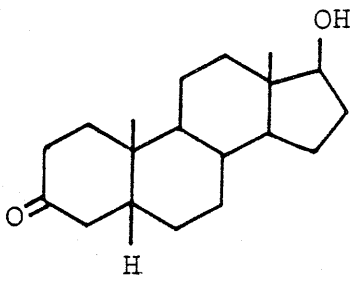

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II. RESULTS AND DISCUSSION

A. Phase Transfer Catalysis

Gibson and DePuy have presented a comprehensive review dealing with the synthesis of cyclopropanols and their derivatives[3b].

Recently, Makosza introduced a method whereby dihalocyclopropane derivatives could be prepared in good yields[94,95,96,97]. Dehmlow utilized this method extensively and prepared some C-3 oxygenated dihalocyclopropanosteroids in excellent yield[99,100]. Phase transfer catalysis, as the method has been termed, has no requirement for anhydrous solvents[100]. Furthermore, dihalocarbene generated by phase transfer catalysis, has been shown to demonstrate enhanced reactivity towards substrate, relative to conventionally prepared dihalocarbene[95,100]. It appeared, therefore, that for the preparation of dihalocyclopropanosteroids, phase transfer catalysis posed no major obstacles and provided the necessary advantages.

The objective at hand was the preparation of pharmacologically active C-3 oxygenated cyclopropanosteroids, i.e. cyclopropanols, demonstrating a favourable anabolic/androgenic ratio. To this end, a C-3 oxygenated A-ring fused dihalocyclopropanosteroids were prepared.

Makosza and others have shown that dihalocarbene generated by phase transfer catalysis readily adds to the carbon-carbon double bond of enol esters[96,99,101]. The preparation of C-3 oxygenated cyclopropano-steroids utilizing the phase transfer method would, nevertheless, necessitate a dehalogenation step whereby the geminal dihalo-substituents could be removed. To circumvent this additional step, an attempt was also made to prepare the desired C-3 oxygenated cyclopropano-steroids directly utilizing the Simmons-Smith methylene transfer reagent[101]. The Simmons-Smith methylene transfer reagent has been shown to add methylene to enol ethers[102] and enol esters[103].

The enol acetate of 17β-acetoxy-5α-androstan-3-one (92) was prepared by the method of Hartshorn and Wallis[104]. Infra-red analysis of the recrystallized product indicated the loss of the carbonyl absorption indicated for six membered ring ketones. The presence of the 17β-acetoxy substituent was confirmed by the acetoxy carbonyl absorption at 1720 cm^{-1}. The appearance of an olefinic absorption (1685 cm^{-1}) and an additional carbonyl absorption at 1740 cm^{-1} indicated the presence of an enol acetate function. On the basis of the infra-red analysis and a sharp melting point, 168-169°C, in agreement with the product obtained by Hartshorn and Wallis, the product was identified as 5α-androst-2-ene-3,17β-diol diacetate (93).

The phase transfer catalysis reaction of 93

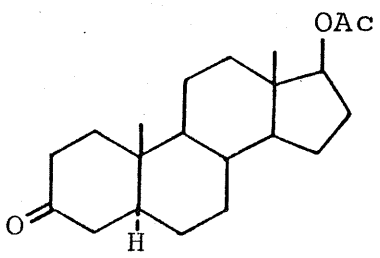
with excess tribromomethane in refluxing aqueous 50% sodium hydroxide yielded a black gummy precipitate which was dissolved in benzene and filtered through an aluminum oxide column. Recrystallization of the combined fractions gave a material with melting point 199-200°C. Infra-red analysis indicated the presence of two carbonyl absorptions at 1763 cm^{-1} and 1735 cm^{-1} and a weak C-H stretch at 3037 cm^{-1}. Proton magnetic resonance showed the presence of two singlet absorptions integrating for three protons each at δ2.082 and δ2.045. These absorptions in the pmr spectrum and the two carbonyl absorptions in the ir were consistent with the presence of the C-3 and 17β-acetoxy substituents, respectively. The absorption in the ir spectrum at 3037 cm^{-1} was assigned to a cyclopropane C-H stretch. Nevertheless, the pmr spectrum did not show a high field absorption consistent with a cyclopropane proton. Instead, the pmr spectrum showed a high field sextet centred at δ0.664 and integrating for one proton. The magnitude of the coupling constants (J=12 Hz, 12 Hz and 4 Hz) was unequivocal evidence to dismiss this sextet as originating from a cyclopropane proton. Furthermore, and regardless as to whether addition of dibromocarbene had taken place on the α- or β-surface of the C-2 bond of 93 there is only one cyclopropane proton (at C-2) and this proton has only two neighbour protons (at C-1). Coupling of this C-2 proton with the C-1 axial and C-1 equatorial protons would result in a triplet or at

best, a quartet. These observations are consistent with those of Bhacca et al. who have shown the presence of a high field (0.64) resonance in the pmr spectra of cyclopropanosteroids which were not assignable to cyclopropane protons[106].

The addition of dibromocarbene to the enol acetate 93 is expected to take place from, and onto, the α-surface of the C-2 site of unsaturation[105,107,108,109]. That addition had taken place onto the α-surface was confirmed by the chemical shift (0.809,s) of the 10β-methyl resonance in the pmr spectrum. A major perturbation of the 10β-methyl proton resonance would be anticipated if addition had taken place onto the α-surface of the C-2 bond of 93[110]. The cyclopropane ring has been shown to demonstrate both diamagnetic and paramagnetic anisotropic effects on the chemical shifts of the 10β- and 13β-methyl substituents[111,112,113,114]. These effects vary with the stereochemistry of the cyclopropane ring. The α-assignment to the configuration of the cyclopropane ring is also consistent with the fact that the 10β-methyl substituent renders the α-surface of the C-2 site of unsaturation sterically less accessible to reagent approach. The mass spectrum of the reaction product of dibromocarbene and 93 showed no molecular ion but was characterized by major ions at m/e 502, 504 and

506. These ions were assigned to the loss of ketene (M^+-ketene). The loss of ketene was consistent with the presence of an acetoxy substituent (3β-acetoxy) that could not be eliminated as acetic acid. Nakata and Tatematsu have shown, by deuterium labelling, that substances in which the acetate substituent cannot be readily eliminated as acetic acid, e.g. phenyl-acetates, eliminate the acetate substituent as ketene[115]. The presence of three major ions at m/e 442, 444, and 446 were consistent with the presence of the 17β- acetate substituent. These peaks were identified as loss of ketene and acetic acid (M^+-ketene-HOAc). On the basis of ir, pmr and ms analysis, the reaction product was identified as 2α,3-dibromocyclopropano-5α-androstane-3β, 17β-diol diacetate (94).

The synthesis of C-3 oxygenated cyclopropano-steroids utilizing 94 as starting material necessitated a dehalogenation step which would yield the desired dehalogenated product and yet not cleave or cause cleavage of the cyclopropane ring. Tribromomethane was utilized in the phase transfer reaction as a carbene source as the carbon-bromine bond can be cleaved with less difficulty than, for example, the carbon-chlorine bond[116,117]. A variety of reagents are available for the reduction of geminal dihalocyclopropane derivatives[118] but no reduction of oxygen substituted dihalocyclopropane derivatives have been reported. This reduction would allow the preparation of steroidal cyclopropanol derivatives. Many of these reagents, e.g. tri-n-butyl tin hydride



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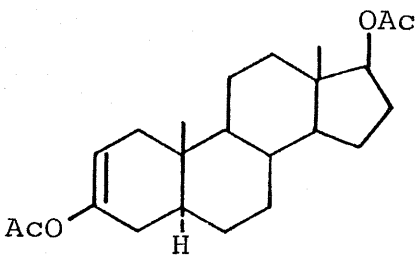

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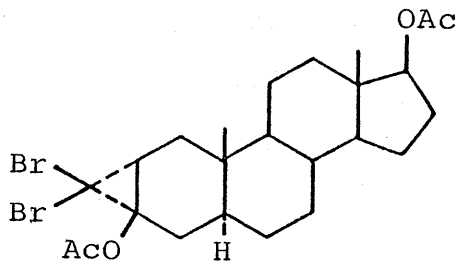

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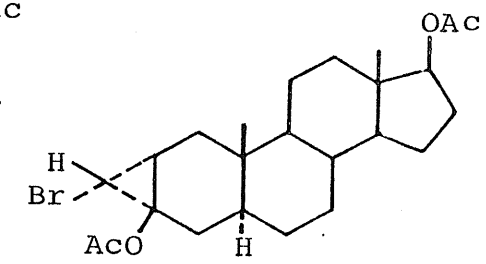

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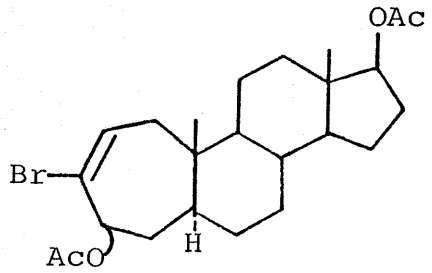

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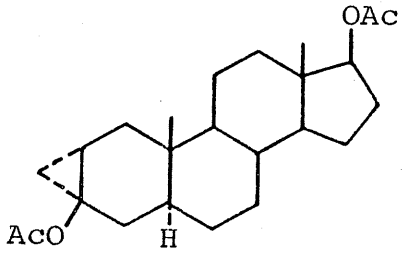

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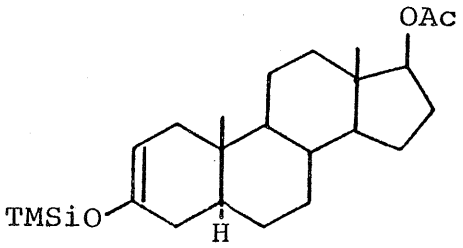

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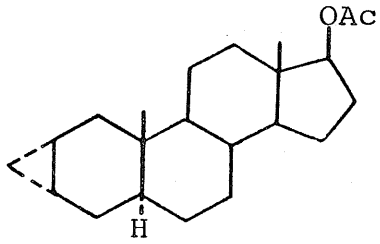

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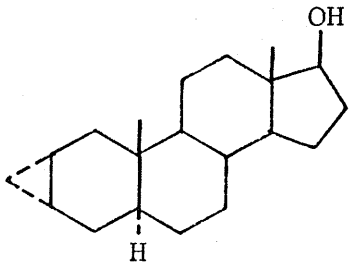

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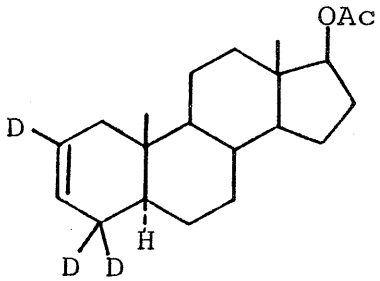

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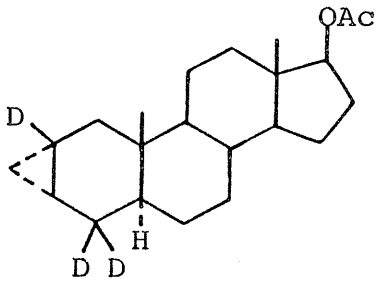

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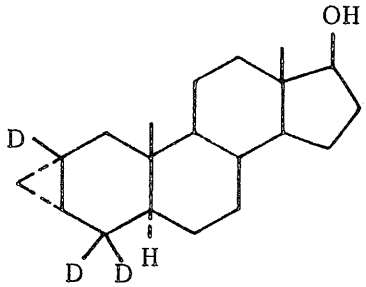

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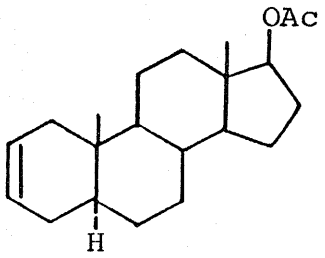

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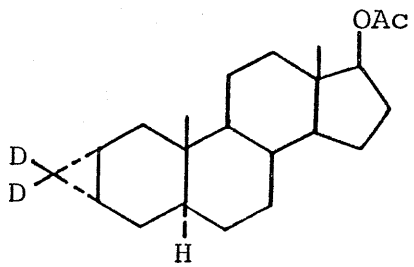

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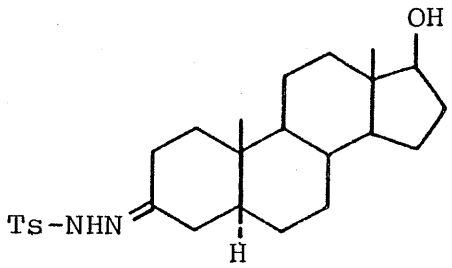

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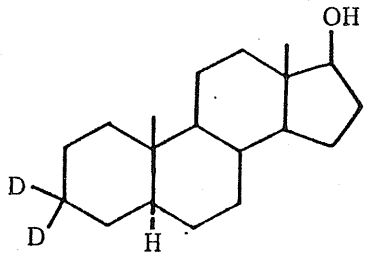

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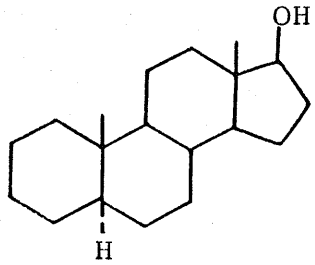

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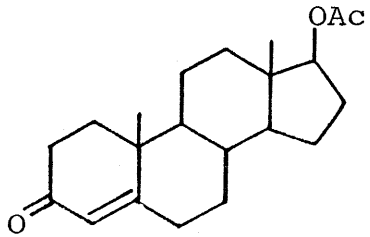

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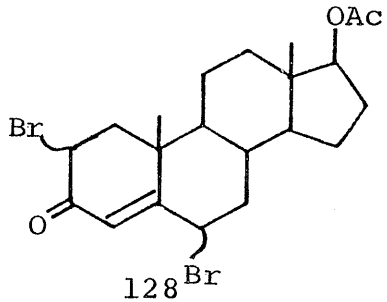

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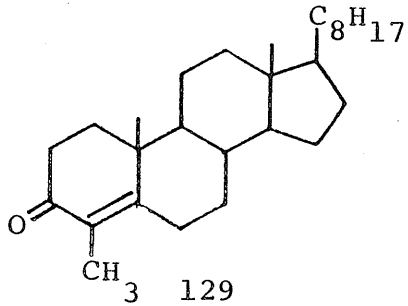

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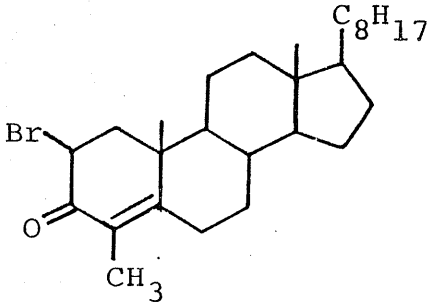

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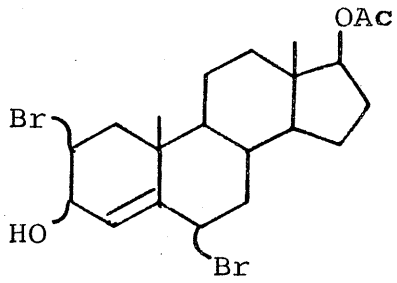

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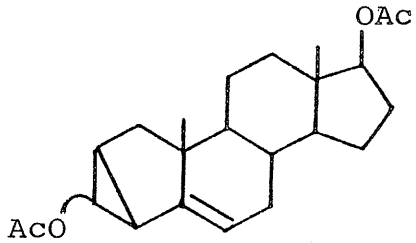

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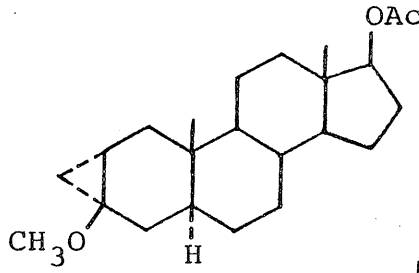


133

134

are so reactive that reduction of functionalized dihalocyclopropane derivatives results in a complex mixture of products[116,119]. Nevertheless, some methods appeared applicable and were attempted. In all cases the removal of both bromine atoms failed, or, the substances were produced in such small quantities that detection was impossible.

B. Attempted reduction of 2α,3-dibromocyclopropano-5α-androstane-3β,17β-diol diacetate (94)

(i) Zn/Cu Couple Reduction of (94)

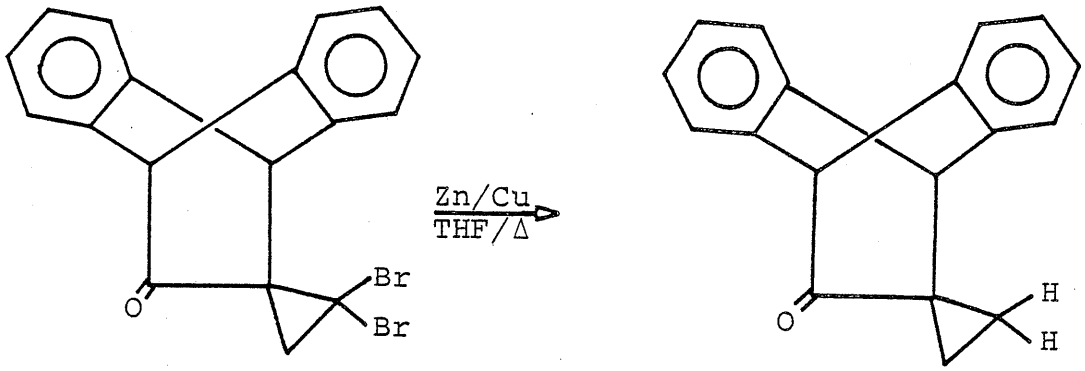
Ripoll utilized the zinc-copper couple to reduce the geminal bromine atoms of (12-oxo-9,10-ethano-9,10-dihydro-anthracene)-11-spiro-1'-(dibromo-2',2'-cyclopropane) (95)[120]. After 18 hr reflux with the zinc-copper couple in tetrahydrofuran, the fully reduced spiro-cyclopropyl-ketone was isolated (Scheme III). Blankenship et al. effected the total reduction of dibromocyclopropano-cyclohexane (96) through reflux with the zinc-copper couple in ether (Scheme IV)[121]. Similarily, Morin et al. utilized the zinc-copper couple to remove the geminal chlorine atoms from 1-phenyl-7,7-dichloro-bicyclo[4.1.0]heptane[122].

The treatment of (94) with the zinc-copper couple in refluxing ether for 4.5 hr (prolonged reaction time did not alter the course of the reaction) gave a crystalline material which was recrystallized to give a sharp

melting point (165-166°C). Infra-red analysis of the pure compound showed two carbonyl absorptions at 1752 cm^{-1} and 1735 cm^{-1}. The presence of two acetoxy methyl absorptions in the pmr spectrum (2.072,1.972), integrating for three protons each and appearing as singlets, were consistent with the presence of the 3β- and 17β-acetoxy substituents, respectively. A downfield doublet in the pmr spectrum (3.364,J=8.8 Hz) was in agreement with the chemical shift of a hydrogen atom bonded to carbon bearing an electronegative substituent (Figure XXV)[123, 124, 125, 126, 127,128]. The doublet nature of this absorption indicated a single adjacent proton. The magnitude of the coupling constant was in the range of values assignable to a cis coupling between cyclopropane protons[123,124,125,126,129,130]. This coupling constant could only arise if the exo-cyclopropane bromine atom had been removed and therefore the exo-cyclopropane proton was coupled with the 2β-cyclopropane proton. The value and nature of the couplings suggested that the zinc-copper reduction of 94 effects the reduction of only one bromine atom. It may be anticipated that the least sterically hindered bromine atom is reduced preferentially, i.e. the exo-bromine atom. The mass spectrum of the Zn/Cu reduction product showed no molecular ion(M^+). Ions appearing at m/e 406 and m/e 409 were consistent with the presence of one bromine atom and loss of acetic acid (M^+-HOAc). On the basis of the ir, pmr and ms data, the

zinc/copper couple reduction product was identified as 2α,3-(endo)-bromocyclopropano-5α-androstane-3β,17β-diol diacetate (97). Reduction of 94 with the zinc/copper couple in refluxing dimethoxyethane gave no clearly defined products.

Scheme III



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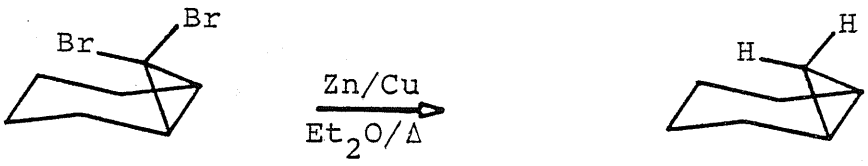
Scheme IV



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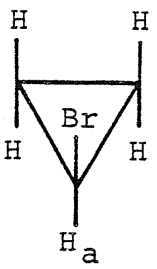
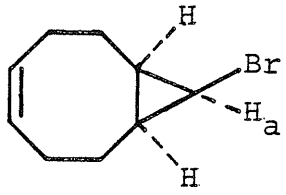
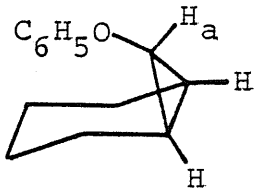


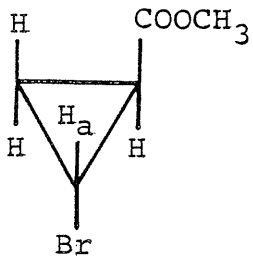
δH_a=2.84 ppm



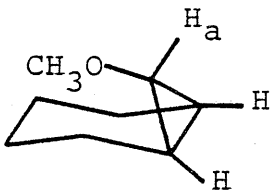
δH_a=3.15 ppm



δH_a=3.52 ppm



δH_a=3.46 ppm



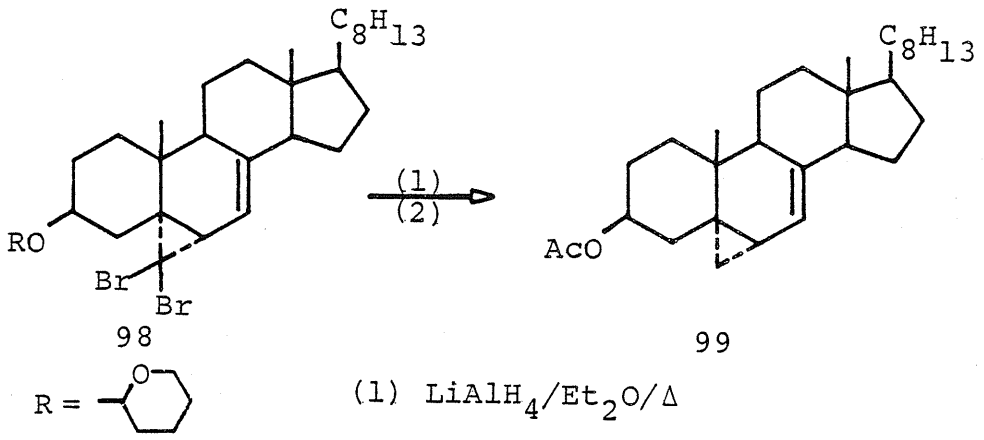
δH_a=2.90 ppm

Figure XXV. Chemical shifts (ppm) of the cyclopropane hydrogen atom (H_a) bonded to carbon bearing and electronegative substitute[124,125,126,127].

(ii) Metal Hydride Reduction of 94.

Nazer has shown that when 3β-(2-tetrahydropyranyl)-5α,6α-dibromocyclopropano-ergosta-7,22-dien-3-ol (98) was refluxed with a 20x excess of lithium aluminum hydride in ether and the resultant reaction product hydrolysed, acetylated and chromatographed over aluminum oxide, a halogen free substance, identified as 3β-acetoxy-5α, 6α-cyclopropano-ergosta-7,22-diene (99) was isolated (Scheme V)[128]. Jefford et al. have shown that when 3,3-dichloro-tricyclo $[4.2.1^{1,6}.0^{2,4}]$ nonane (100) was treated with lithium aluminum hydride, the endo-chlorine atom is preferentially reduced (Scheme VI)[131]. The selectivity (for the endo-halogen atom) and the effectiveness of the metal hydride for the removal of geminal cyclopropane bromine atoms prompted an attempt to utilize this reductive procedure to prepare the desired C-3 oxygenated cyclopropano-steroid from 94. Thus, when 94 was refluxed in ether with a 15x excess of lithium aluminum hydride, a reaction product was obtained, which after acetylation and chromatography over aluminum oxide, gave a crystalline material with m.p. 155-156°C. Infra-red analysis indicated the presence of a carbon-carbon double bond absorption (1638 cm^{-1}) and a poorly resolved, broad carbonyl absorption centered at 1733 cm^{-1}. The pmr spectrum indicated the presence of two singlet absorptions (2.157,2.030) integrating for three protons each. This was consistent with the presence of two

Scheme V



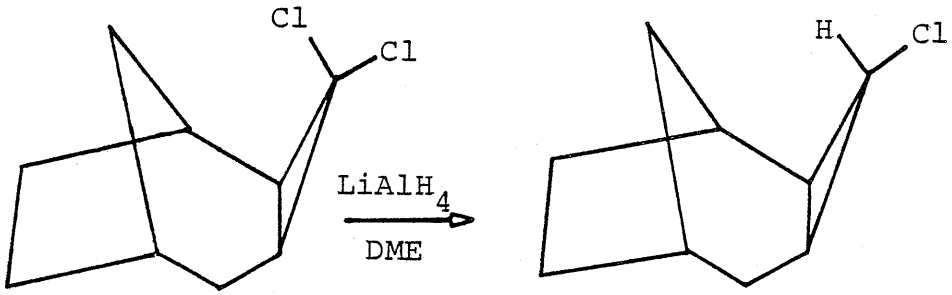
98 99

R =

(1) $LiAlH_4/Et_2O/\Delta$

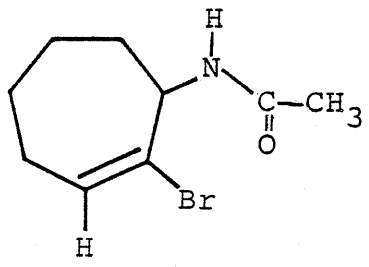
(2) Ac_2O/Pyridine

Scheme VI



100 major isolate

acetoxy substituents. The pmr spectrum also indicated the presence of an olefinic absorption which appeared as a triplet (J=8.0 Hz) and was centred at δ6.132. The chemical shift, coupling constants and the triplet nature of this olefinic absorption was consistent with the chemical shifts, coupling constants and the coupling of the olefinic protons observed in the pmr spectra of N-(2-bromo-1-cyclohepten-3-yl) acetamide (101)[132], cis-3-acetoxy-2-bromo-1-cycloheptene (102)[133], and other related heptene derivatives. Similarly, the chemical shifts of the C-3 methine proton in 101 and 102 (Table XXVI) were consistent with the doublet resonance centred at δ5.484 in the pmr spectrum of the reduction product. The olefinic resonance (6.132) in the pmr spectrum of the lithium aluminum hydride reduction product was furthermore consistent with the C-2 olefinic resonance observed for A-homo-5α-androst-2-ene derivatives[135,136].



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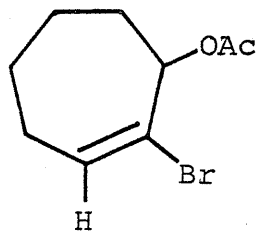

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TABLE XXVI

Proton magnetic resonance data of the methine and olefinic protons of N-(2-bromo-1-cyclohepten-3-yl) acetamide (101)[132], cis-3-acetoxy-2-bromo-1-cycloheptene (102)[133] and the lithium aluminum hydride reduction product of 94.

Compound	Methine (ppm)	Olefinic (ppm)	J (Hz) (olefinic)	Type (olefinic)
101	4.81	6.30	6	t
102	5.50	6.43	7	t
$LiAlH_4$ reduction product	5.484	6.13	8	t

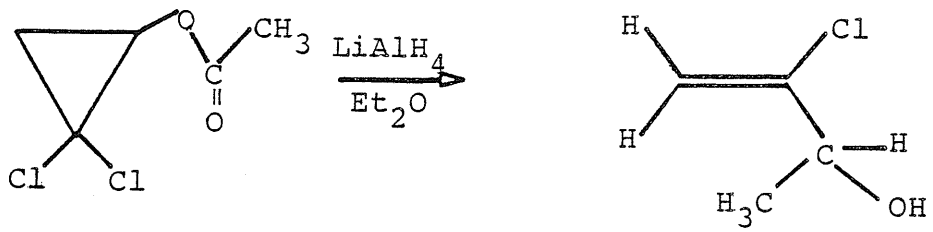
DeSelms has shown that when acetoxy-gem-dichlorocyclopropane is treated with an excess of lithium aluminum hydride, at 25°C., the cyclopropane ring is cleaved and the unsaturated chlorohydrin is exclusively isolated (Scheme VII)[137]. DeSelms has also shown that when 1-acetoxy-6-methyl-7,7-dichlorobicyclo [4.1.0] heptane is treated, at 25°C, with lithium aluminum hydride in ether, the cyclopropane ring is cleaved and an unsaturated heptanol is exclusively isolated (Scheme VIII)[137]. Stork et al. isolated a heptene chlorohydrin in 81% yield by refluxing 1-acetoxy-6-methyl-7,7-dichlorobicyclo [4.1.0] heptane for 2 hours with lithium aluminum hydride (Scheme IX)[138]. These observations suggest

that when functionalized bicyclo[4.1.0]heptane derivatives, such as those discussed above, are treated with the lithium metal hydride at reflux or room temperature, the bicyclic structure homologates to give, exclusively, an unsaturated monocyclic heptane derivative. By analogy, the lithium metal hydride reduction of 94, in refluxing ether, would be expected to give as a major product A-homo-3-bromo-5α-androst-2-ene-4ξ,17β-diol diacetate (103). The mass spectrum of the isolate showed no molecular ions (M^+=466, 468). Major ions were observed at m/e 406 and m/e 408. These ions were consistent with the elimination of the C-17 actexoxy substituent (M^+-HOAc). Major ions at m/e 387, (M^+-Br), 327 (M^+-Br-HOAc) and 267 (M^+-Br-2HOAc) were consistent with the presence of one bromine atom. The ir, pmr and ms data were consistent with the assignment of 103 to the lithium aluminum hydride reduction product. A scheme that accounts for the formation of the A-homo steroid 103 from the treatment of 94 with lithium aluminum hydride is presented in Scheme X.

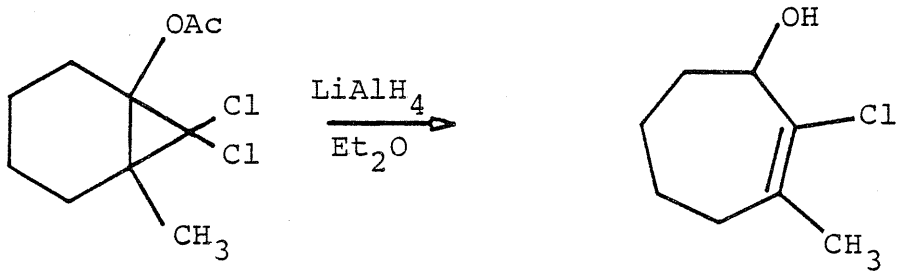
The reduction of alkyl halides, utilizing lithium aluminum hydride as the reductant, proceeds either through a S_N2 or S_N1 mechanism depending on the substrate[131,139]. Substitution-bimolecular (S_N2) mechanisms typically result in the inversion of the configuration at the chiral carbon atom, whereas, substitution unimolecular (S_N1) mechanisms involve carbonium ion intermediates and therefore racemates constitute the product distribution. Jefford *et al.*[140] and

Yamanaka et al.[139] have shown that the reduction of non-functional cyclopropyl dihalides with lithium aluminum hydride proceeds stereospecifically, i.e. with no inversion. These observations rule out the possibility of a S_N2 mechanism (the S_N2 mechanism is geometrically prohibited in these bicyclic substances[131]). Furthermore, the absence of allylic reaction products precludes a S_N1 mechanism as cyclopropane carbonium ions are known to yield allylic derivatives[141,142,143,144]. The carbanion mechanism proposed by Jefford et al.[140] does not appear to be operative in the metal hydride reduction of functionalized dibromocyclopropane derivatives such as 94. Nevertheless, the carbanion mechanism does account for the product distribution of non-functionalized 3,3-dichloro-tricyclo$\boxed{4}$.2.1.$0^{2,4}$ nonane derivatives[131,140]. The reduction of 7,7-dibromobicyclo$\boxed{4}$.1.$\boxed{0}$ heptane (104) with a 4X excess of lithium aluminum hydride in ether proceeds smoothly to yield the bicyclic derivative bicyclo$\boxed{4}$.1.$\boxed{0}$ heptane (105) in 73% yield (Scheme XI)[140]. Neither products of inversion or allylic derivatives are isolated. This suggests that a mechanism which does not involve carbonium ion intermediates nor necessitates the geometry of the S_N2 mechanism is operative. The four-centre mechanism (Scheme XII) is a mechanism which readily accounts for these product distributions. The four-centre mechanism accounts for the lack of both inverted and allylic products in the

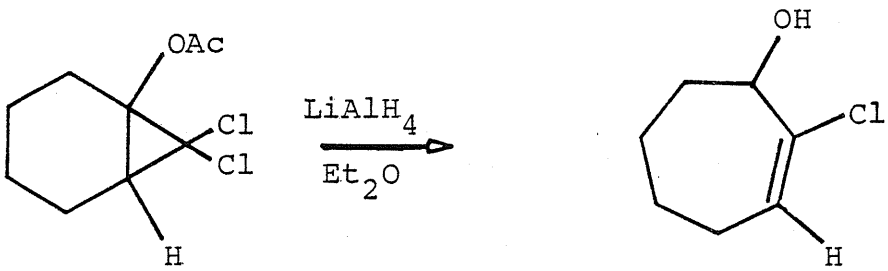
Scheme VII



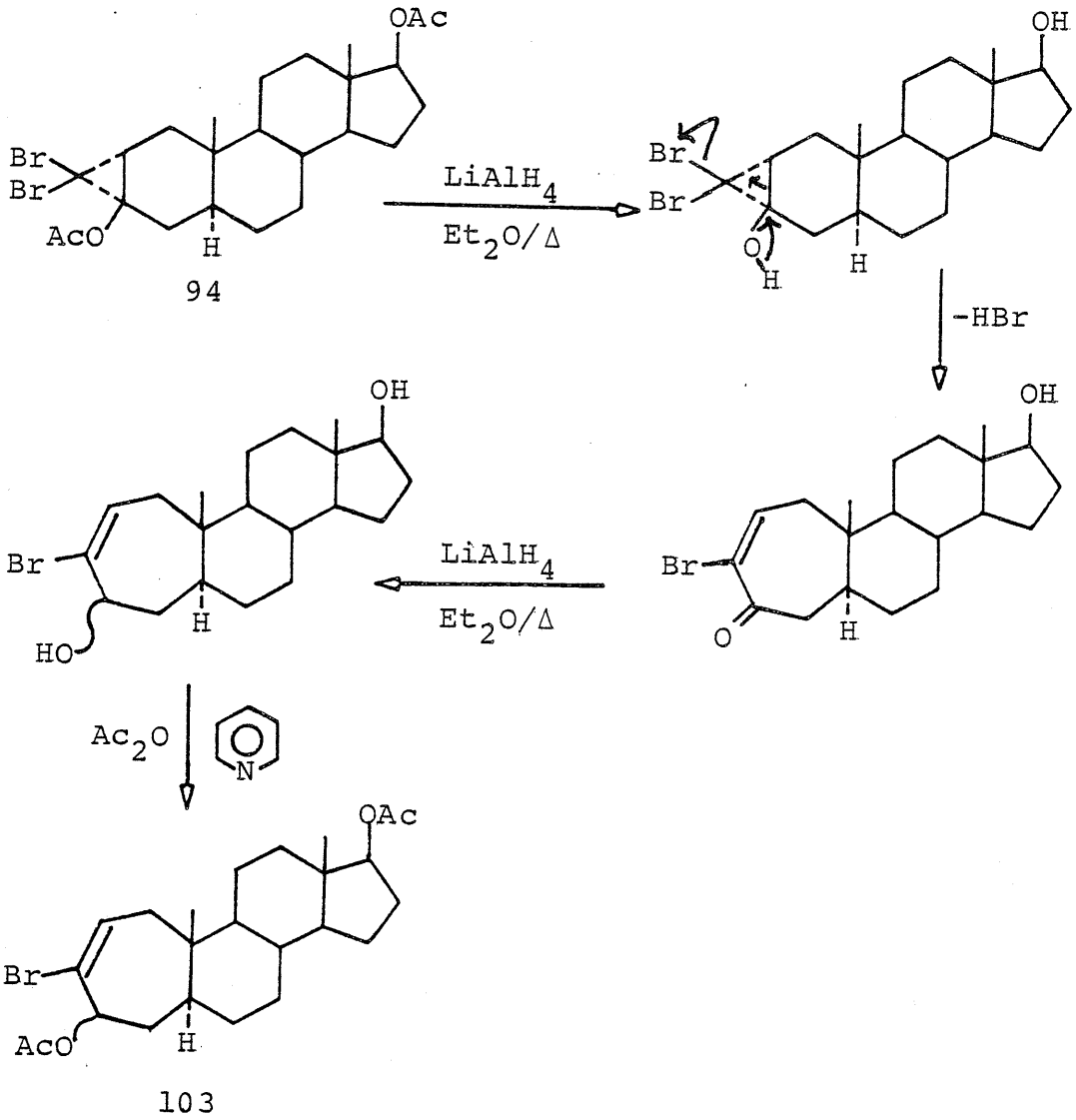
Scheme VIII



Scheme IX

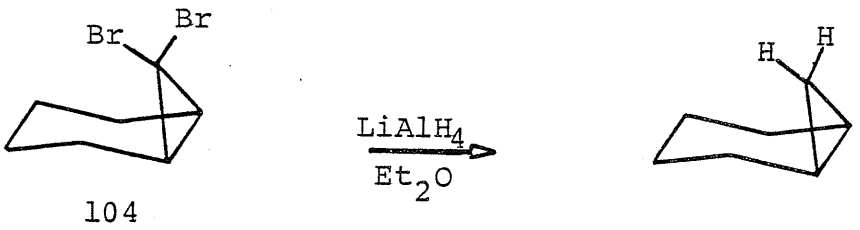


Scheme X


OAc
Br
Br
AcO
H
94
LiAlH4
Et2O/Δ
OH
Br
Br
O
H
H
-HBr
OH
Br
O
H
LiAlH4
Et2O/Δ
OH
Br
HO
H
Ac2O
N
OAc
Br
AcO
H
103

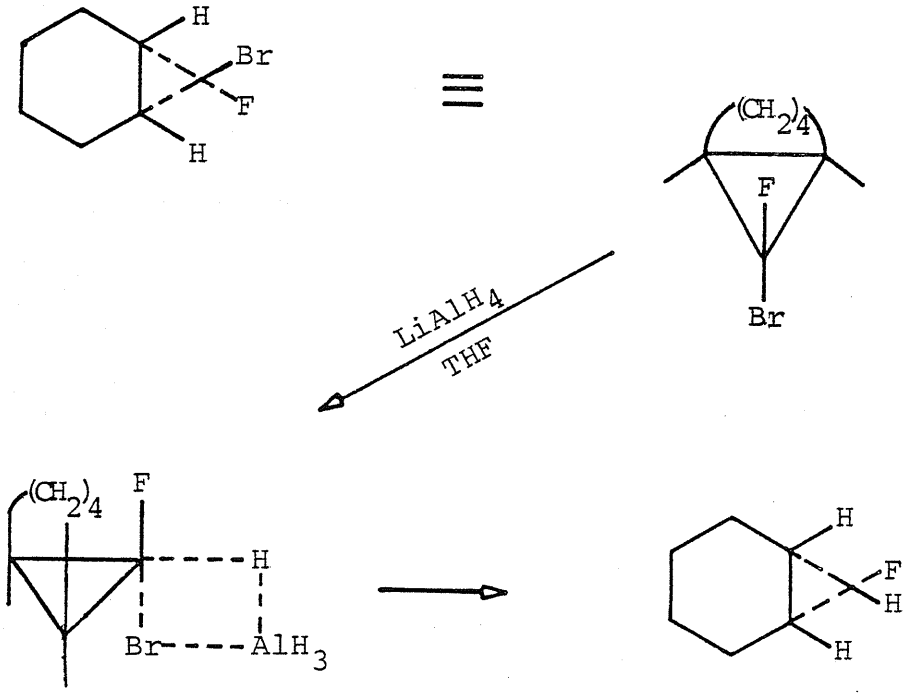
metal hydride reduction of cyclopropyl dihalides[131,139,145]. The lithium aluminum hydride reduction product (103) of the

Scheme XI



104

Scheme XII



C-3 functionalized dibromide 94 is readily accounted for by the initial reduction of the C-3 acetoxy substituent as outlined in Scheme X, in spite of the variety of other possibly mechanisms that may be operative.

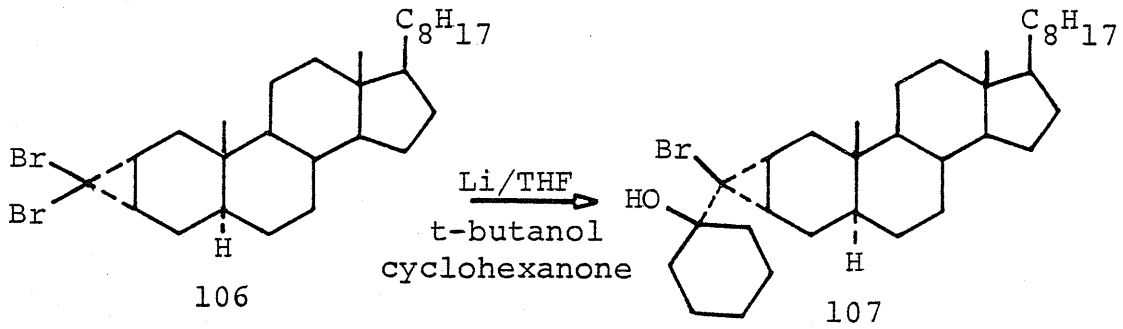
(iii) Lithium Metal/t-butanol Reduction of 94.

Dammann et al. have shown that when the non-functionalized cyclopropano-steroid 2α,3α-dibromocyclopropano-5α-cholestane(106) is treated with lithium metal/t-butanol in tetrahydrofuran, 1-(1-bromo-2β,3β-dihydro-3'H-cyclopropa [2.3]-cholestan-3'-yl) cyclohexanol(107) is isolated, in high yield, when an equimolar ratio of cyclohexanone is added to the reaction mixture (Scheme XIII)[109]. This reagent appears therefore, to selectively reduce the endo-bromine atom. Nazer has shown that when 98 is treated with lithium metal/t-butanol in tetrahydrofuran, and the reaction product is hydrolysed, acetylated and crystallized, a halogen free product, identified as 99 is isolated[128]. Similarly, Fieser and Sachs have shown that when the non-functionalized gem -dichlorocyclopropane derivative 5,5,10,10-tetra-chloro-tricyclo[$7.1.0^{4,6}.0^{1,9}$] decane is treated with lithium metal/t-butanol in tetrahydrofuran, the reduced hydrocarbon is obtained (Scheme XIV)[146]. The reduction of non-functionalized gem -dihalocyclopropanes, utilizing lithium metal/t-butanol in tetrahydrofuran, appears to offer the advantage of stereospecificity, (reduction of the sterically

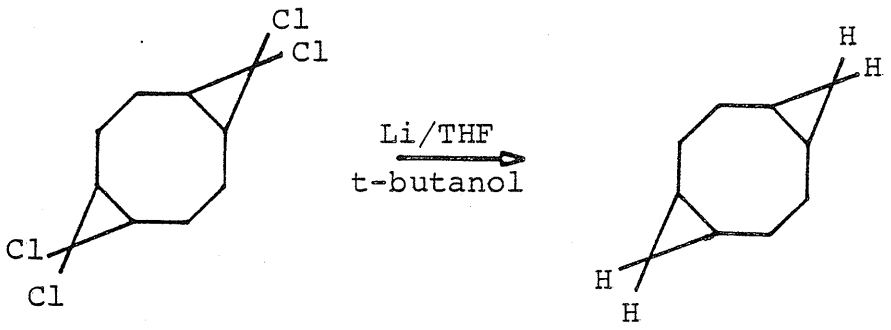
hindered endo-halogen atom) and effecting the total reduction of gem-dichlorocyclopropanes[147].

Treatment of 94 with lithium metal/t-butanol in tetrahydrofuran for 4.5 hours gave a material that showed five components on t.l.c. and which, after acetylation and chromatography over aluminim oxide gave no isolable products or crystalline fractions. Parham et al. have shown that when 1-ethoxy-7,7-dichlorobicyclo [4.1.0] heptane is refluxed with sodium metal/methanol, a complex mixture of products is obtained[148]. This complex mixture included an aromatic isolate (Scheme XV). A similar complex distribution of reduction products of 94, utilizing lithium metal/t-butanol in tetrahydrofuran, can be anticipated.

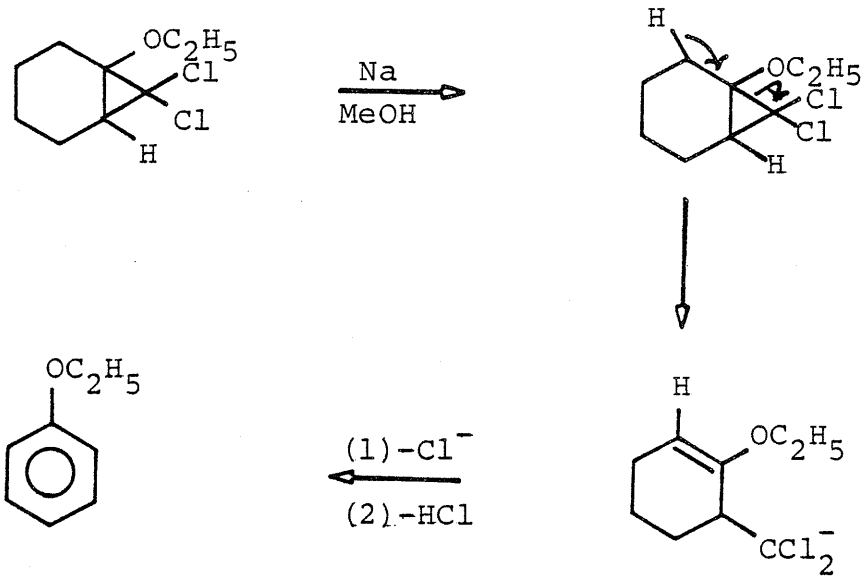
Scheme XIII



Scheme XIV



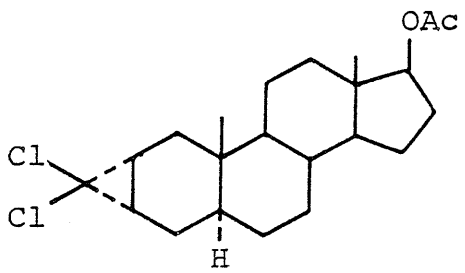
Scheme XV



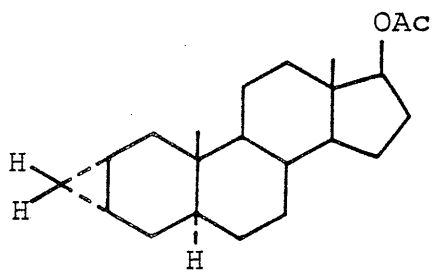
(iv) Birch Reduction of 94.

The reduction of dihalocyclopropano-steroids utilizing sodium metal in liquid ammonia has been successfully applied to several non-functionalized gem-dihalocyclopropano-steroids[128,149,150,151]. Knox et al. obtained 2α,3α-cyclopropano-17β-acetoxy-5α

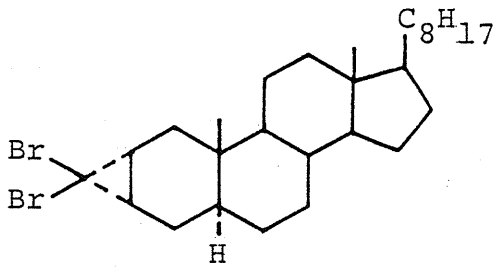
androstane (108) by treating 2α,3α-dichlorocyclopropano-17β-acetoxy-5α-androstane (109) with sodium metal in liquid ammonia[152]. Nazer prepared 2α,3α-cyclopropano-5α-cholestan-22-ene (111) by treating 2α,3α-dibromocyclopropano-5α-cholestan-22-ene (110) with sodium metal in liquid ammonia[128]. Similarly, Bond and Cornelia treated 7α,8α-difluorocyclopropano-5α-cholestan-3β-yl benzoate (112) with sodium metal in liquid ammonia to obtain 7α,8α-cyclopropano-5α-cholestan-3β-yl benzoate (113)[150]. Nevertheless, these methods have not been applied to functionalized cyclopropane derivatives such as 94.



109



108



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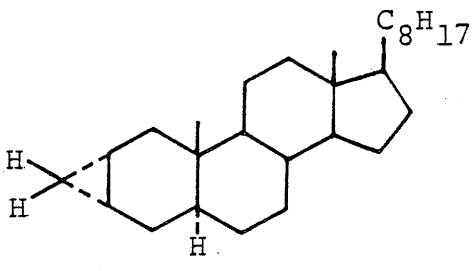
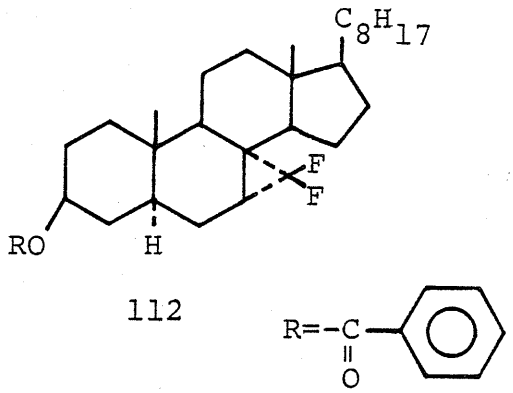


111

The Birch reduction of 94, utilizing sodium metal in liquid ammonia, gave a product, from which, after acetylation and chromatography over aluminum oxide, no definitive products or crystalline fractions were obtained. Metal/liquid ammonia reductions are known to involve radical intermediates. Walborsky and Collins have shown that brominated cyclopropane radicals, unlike their unsubstituted analogues, which may rapidly invert or abstract solvent hydrogen[153], demonstrate a prevalence for the formation of allyl radicals[154]. This is a direct consequence of the known stabilization effect that the bromine atom has on these



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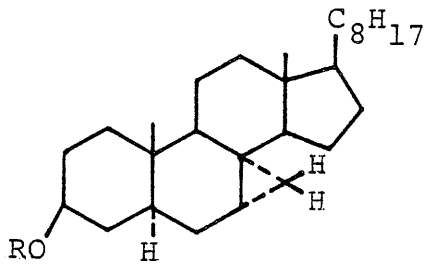


113

allyl radicals. These observations account for the large proportion of allene type products as opposed to (solvent) hydrogen abstracted products in the decomposition of brominated cyclopropane radicals (Figure XXVI).

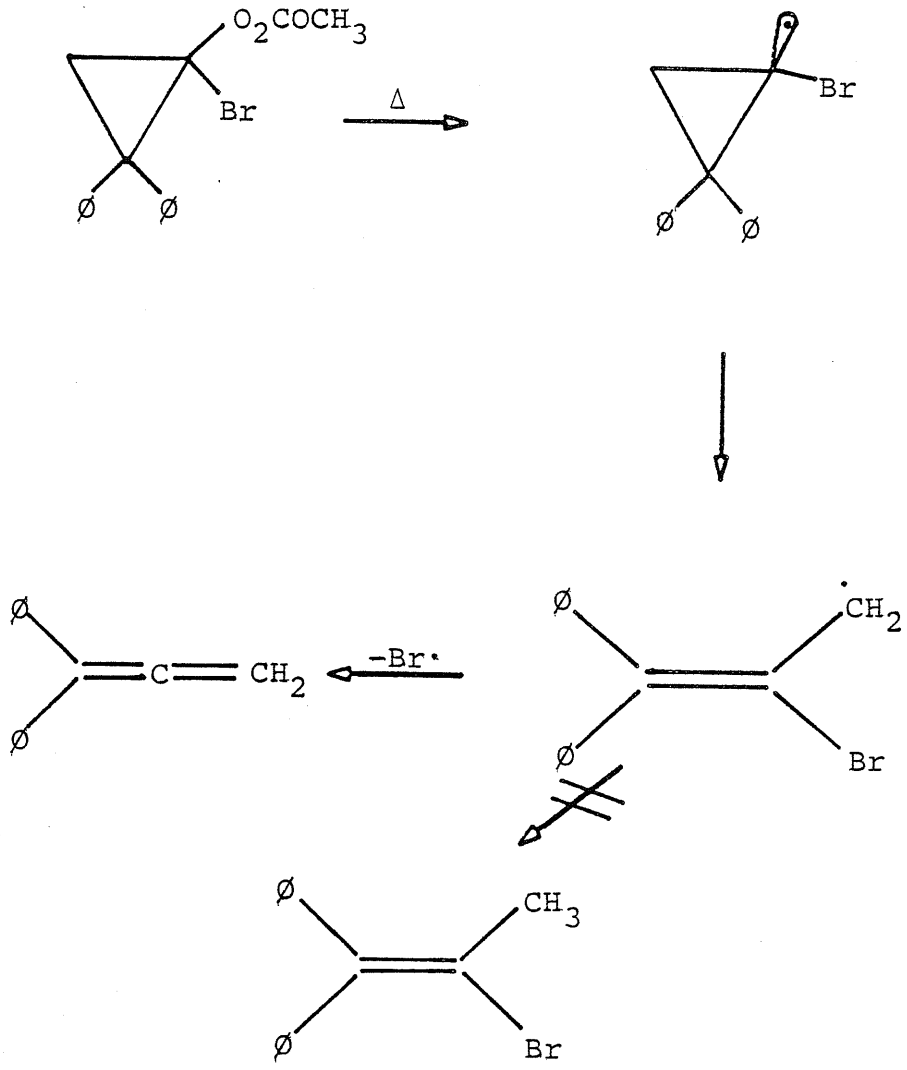


Figure XXVI. Effect of the bromine atom on the product distribution of brominated cyclopropane radicals generated by the decomposition of the corresponding perester.

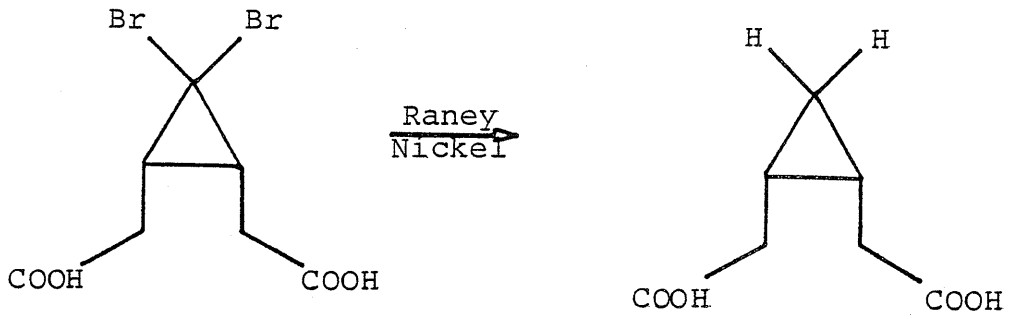
Thus, by analogy with the observations of Walborsky and Collins, a complex mixture of reaction products may be anticipated (the product distribution may be rendered even more complex through the mechanism of radical coupling[155]). The Birch reduction has been successfully applied to the reduction of many non-functionalized geminal dihalocyclopropane compounds. Nevertheless, the reduction of functionalized, e.g. the acetoxy dibromide 94, geminal dihalocyclopropano derivatives appears not to proceed smoothly to yield the desired dehalogenated product(s).

(v) Raney Nickel Reduction of 94.

Hofmann et al. successfully employed the Raney nickel catalyst (Clemmensen reduction had failed) to remove the geminal bromine atoms of dibromocyclopropane-cis diacetic acid (Scheme XVI)[156]. Raney nickel has been extensively used to effect the hydrogenolysis of halogen compounds[157]. Treatment of 94 for 32 hours with Raney nickel catalyst (the Raney nickel catalyst had been stored under ethanol for eight years) gave a product which after recrystallization had a melting point of 160-161°C. Infra-red analysis showed the characteristic C-H stretch of cyclopropane (3070 cm^{-1}) and two carbonyl absorptions at 1740 cm^{-1} and 1734 cm^{-1}. Pmr spectroscopy showed two singlet acetoxy methyl absorptions (1.909,1.1961). A downfield doublet (3.290,J=10 Hz) in the pmr spectrum was consistent with a cyclopropane proton couple cis to another cyclopropane proton (at C-2) and bonded to carbon bearing an electronegative substituent. Mass spectra

showed no molecular ion but ions at m/e 387 (M^+-Br), 345 (M^+-Br-ketene) and 285 (M^+-Br-ketene-HOAc) confirmed the presence of one bromine atom and the 3β- and 17β-acetoxy substituents. The 3β-acetoxy substituent was expected to be eliminated as ketene in accord with the observations of Nakata and Tatemutsu[115]. The 17β-acetoxy substituent was expected to be eliminated as acetic acid.

Scheme XVI



The spectral data confirmed that the Raney nickel reduction of 94 gave exclusively 2α,3-(endo)-bromocyclopropano-5α-androstane-3β,17β-diol diacetate(97).

A high field resonance (0.591,1H) in the pmr spectrum of 97, typical of a cyclopropane proton, and appearing as a sextet could not be assigned to a cyclopropane proton. The 2β-proton of 97 has three neighbour hydrogens (the 1α- and 1β-protons and the exo-cyclopropane proton) and a sextet may arise through three individual splittings. Assignment of the high field resonance (0.591) to the 2β-proton of 97 would not be consistent with the presence of an identical sextet (0.664, 1H) in the pmr spectrum of 94 in which the 2β-proton has only

two neighbour hydrogen atoms and, therefore, may give rise to only a quartet.

The treatment of 94 with freshly prepared Raney nickel W-2 catalyst[158], gave only a complex crystalline mixture which could not be further purified.

C. Treatment of 5α-androst-2-ene-3,17β-diol diacetate (93) with the Simmons-Smith reagent.

The treatment of the enol acetate 93 with a 10 molar excess of the Simmons-Smith reagent for 10 hours gave a reaction mixture that showed two components on t.l.c. (System IV). The slower eluting component showing an R_f value equal to that of the starting material. Treatment of this mixture for an additional 30 hours with an additonal 10 molar excess of the Simmons-Smith reagent[102a] gave a gummy residue which after chromatography over aluminum oxide gave a crystalline material with a sharp melting point (163°-164°C.). Infrared analysis showed the presence of two carbonyl absorptions, 1738 cm^{-1} and 1732 cm^{-1}, and a prominent C-H stretch (3075 cm^{-1}). The presence of two singlet absorptions in the pmr spectrum, δ2.023 and δ1.962, integrating for three protons each, confirmed the presence of the C-3 and 17β-acetoxy substituents. The pmr spectrum also showed a high field sextet (0.514) upon which was observed a partially superimposed triplet (0.462). The resultant heptet integrated for two protons. The high field sextet at δ0.514 could not be assigned to a cyclopropane proton but in light of the known diamagnetic anisotropic properties of the cyclopropane ring[105,106,113,114], it may be expected that the origins of this resonance lie in an up field shift of a B-ring proton, e.g. the 9α,11α- or 12α-proton. In the pmr spectrum of non-cyclopropanated steroids, the 9α,11α and 12α protons normally resonate within

the methylene envelope and are therefore not usually observed. The high field triplet (0.462) could be assigned to the endo-cyclopropane proton and was consistent with the cyclopropane C-H stretch (3075 cm^{-1}) observed in the infra-red spectrum of this substance. The magnitude of the coupling constants (J=6.0Hz) were consistent with a trans and gem coupling of cyclopropane protons[123,124,129,130]. Assignment of the high field triplet (0.462) to the exo-cyclopropane proton could be ruled out on the basis of the known higher chemical shift resonances of endo-cyclopropane protons in bicyclo [4.1.0] heptane derivatives relative to the chemical shift values of the exo-cyclopropane protons in these same derivatives[126,130]. Furthermore, the exo-cyclopropane proton has one cis and one gem coupling constant. The magnitude of the cis coupling constant has been found, in most bicyclo [4.1.0] heptane derivatives, to exceed the magnitude of the cyclopropane trans coupling constant[123,130]. This cis coupling constant most often lies in the range of 7.5Hz to 9.0Hz in these substances[123].

The stereochemistry of the cyclopropane ring was assigned the α-configuration. This assignment was consistent with the known (β-surface) steric inaccessibility of the C-2 unsaturated bond of 93 to the Simmons-Smith reagent in this natural series of 19-methyl containing steroids[108,109]. Similar observations, attesting to the steric sensitivity

of the Simmons-Smith reagent, have also been made in non-steroidal olefins[159]. The lack of major perturbations in the chemical shift of the 10β-methyl substituent (0.914, s) also indicated an α-configuration for the C-2 cyclopropane ring[112, 113,114]. The mass spectrum indicated the addition of 14 amu to the molecular weight of the starting olefin. The ir, pmr and ms data indicated the structure as 2α,3-cyclopropano-5α-androstane-3β,17β-diol diacetate(114).

D. Treatment of 3-trimethylsilyloxy-5α-androst-2-en-17β-yl acetate (115) with the Simmons-Smith reagent.

(i) Preparation of 3-trimethylsilyloxy-5α-androst-2-en-17β-yl acetate(115).

The trimethylsilyloxy enol ether 115 was prepared by the method of House et al. (Method A)[160]. Infra-red analysis of the recrystallized product, melting point 98-103°C. showed the expected ester carbonyl absorption (1733 cm^{-1}) and the olefinic bond absorption (1670 cm^{-1}). The pmr spectrum showed an olefinic absorption (4.44,q) which integrated for one proton and typically demonstrated the expected small allylic coupling constant (J=2.0 Hz). The singlet at 0 ppm, integrating for nine protons, confirmed the presence of the trimethylsilyloxy substitutent[161,162,163,164]. Mass spectra indicated the characteristic ions at m/e 404(M^+), 262(M^+-142) and 202 (M^+-142-HOAc). The loss of 142 amu in the ms spectrum was consistent with the loss of the trimethylsilyloxy substituent as trimethylsilyloxy-butadiene[165]. The ir, pmr and ms data confirmed the structure as 3-trimethylsilyloxy-5α-androst-2-en-17β-yl acetate(115). Attempts to prepare 115 by capturing the sodium hydride generated enolate anion of 92 with trimethylsilylchloride using the method of Stork and Hudrilk[166] consistently failed. This result is in agreement with the observations of House et al.[160]. These workers attempted to prepare trimethylsilyl enol ethers utilizing the sodium hydride method described

above and noted that the results were uniformly unsuccessful.

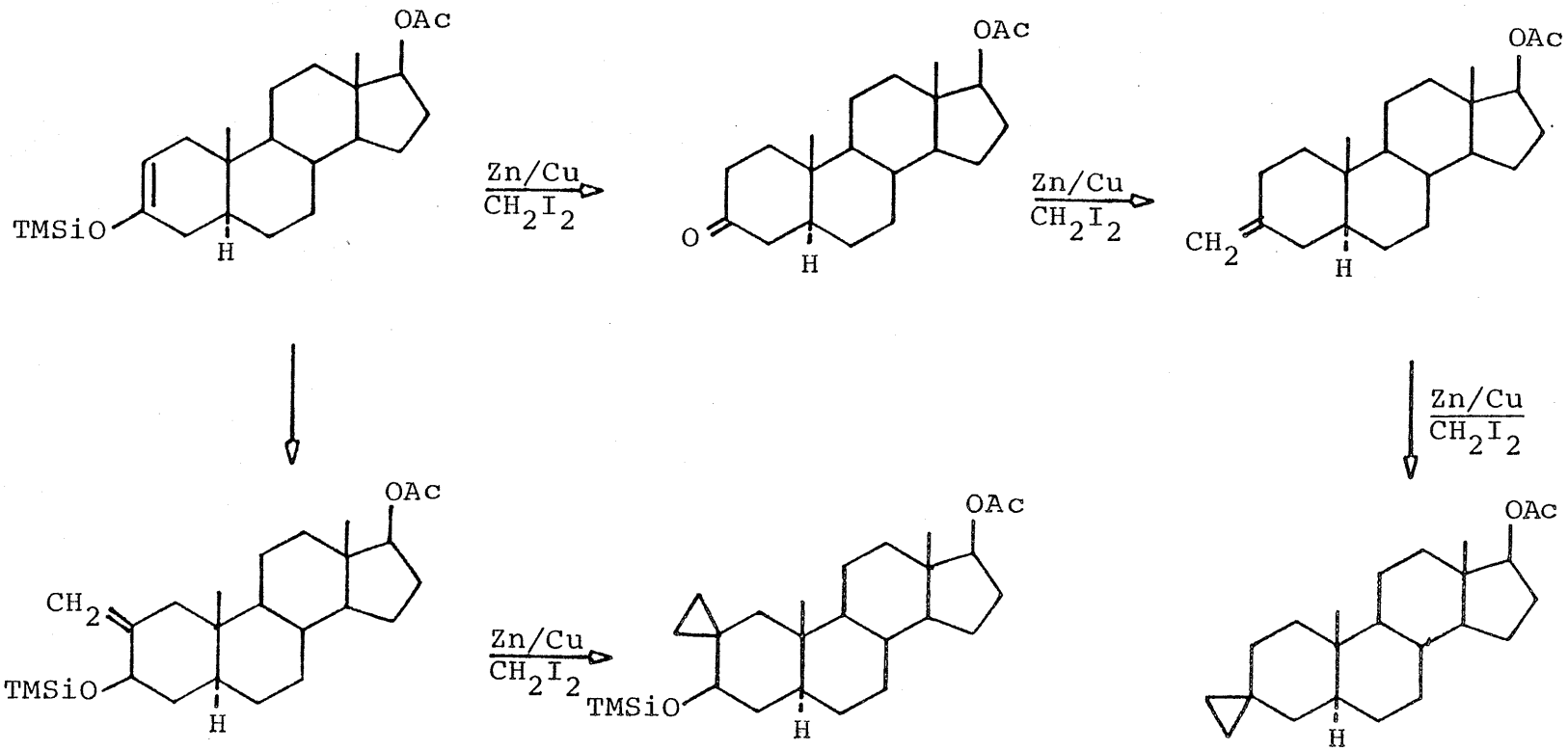
(ii) Simmons-Smith reaction.

The treatment of 115 with a 10 molar excess of Simmons-Smith reagent gave a gummy residue which was not purified further. Infra-red analysis showed a C-H stretch (3063 cm^{-1}) and a carbonyl absorption (1737 cm^{-1}) consistent with the presence of cyclopropane or olefinic protons and the 17β-acetoxy substituent, respectively. The pmr spectrum did not show the presence of an olefinic proton but expansion of the 17α-proton resonance (4.55,q $J_{a,a}$=12 Hz, $J_{a,e}$=7.5 Hz) indicated the presence of what appeared to be a superimposed singlet resonance. The chemical shift (4.56) of this singlet (superimposed upon the 17α-proton resonance) may be a portion of the quartet observed for the C-2 proton resonance (4.44) of the starting material. Alternatively, this singlet may be due to the presence of olefinic material[167]. The pmr spectrum also showed a broad multiplet at δ0.402 integrating for two protons and a high field non-resolved sextet (0.05) integrating for two protons. No singlet resonance integrating for nine protons and corresponding to the trimethylsilyloxy ether substituent were observed. The addition of the Simmons-Smith reagent to the trimethylsilyloxy enol ether 115 thus proceeds to give a complex mixture of products. Nevertheless, the pmr spectrum indicated the presence of cyclopropane protons but, as the trimethylsilyloxy substituent appears not to be present this may be attributed to such reaction

products as indicated in Scheme XVII[102b,167]. Olefinic and cyclopropane derivatives have been isolated from similar reactions although the products still retained the trimethylsilyloxy group[167]. The presence of four cyclopropane protons in this spiro compound (Scheme XVII) may account for the two high field resonance absorptions at δ0.402 and δ0.05 in the crude reaction mixture which integrated for two protons each. There is no indication to suggest that trimethylsilyloxy enol ethers are unstable compounds. These substances have been shown to be stable substances and can be stored if protected from water and aqueous acids[160,168].

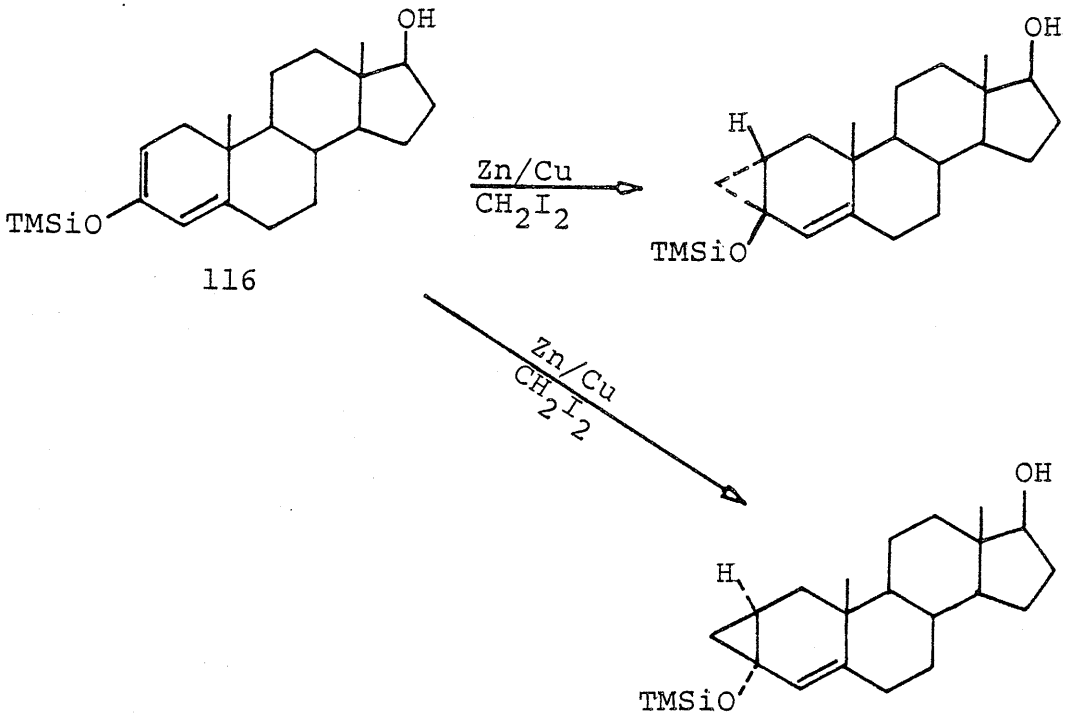
It is of interest to note that although the addition of the Simmons-Smith reagent to the trimethylsilyloxy enol ether gave only spectroscopically detectable quantities of an unidentified cyclopropane containing steroid, the addition of the Simmons-Smith reagent to the alcohol 116, utilizing the zinc/silver couple, proceeds smoothly to yield 85% of a 1:1 mixture of the 3ξ-trimethylsilyloxy-2ξ,3ξ-cyclopropano stereoisomers (Scheme XVIII)[169,170]. The successful outcome of this reaction may be attributed to the enhanced reactivity of the methylene transfer reagent when a zinc/silver couple is utilized[171,172] or alternatively, the enhanced stability of the homoannular trimethylsilyl enol ether 116.

Scheme XVII


OAc
TMSiO
H
Zn/Cu
CH2I2
OAc
O
H
Zn/Cu
CH2I2
OAc
CH2
H
Zn/Cu
CH2I2
OAc
CH2
TMSiO
H
Zn/Cu
CH2I2
OAc
TMSiO
H
Zn/Cu
CH2I2
OAc
H

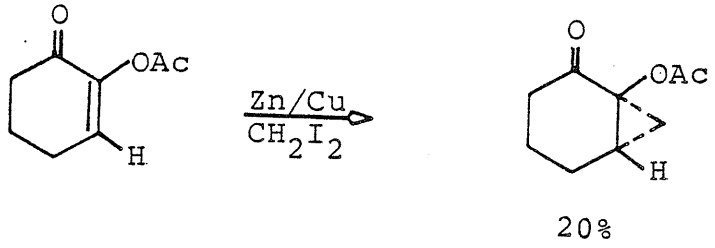
Kohout and Fajkos[112,114] and Joska et al.[113] have shown that the addition of the Simmons-Smith reagent to the isolated carbon-carbon double bond of steroidal olefins proceeds without complications to yield the desired cyclopropano-steroids. However, the addition of the Simmons-Smith reagent to enol acetate derivatives has proven to be less than promising as these reactions invariably resulted in complex reaction mixtures and low yields of the desired oxygenated cyclopropane derivatives (Scheme XIX)[102b, 103]. Furthermore, the required hydrolysis of the acetate substituent necessitates the use of nucleophilic base or electrophilic (protic) reagents. As the cyclopropane ring is known to be base and acid labile, isolation of the desired cyclopropanols was improbable[135,173]. Alternatively, metal hydride may be utilized to reduce the acetate substituent to yield the cyclopropanol directly[174,175,176]. Nevertheless, tertiary cyclopropanols have been prepared from 2-chlorethyl ether derivatives (Scheme XX)[177]. The addition of the Simmons-Smith reagent to olefinic substrate may be impaired through steric crowding of the site of unsaturation[159]. It was, therefore, of great significance when it was observed that the reactivity of the Simmons-Smith reagent to enol acetates could be significantly enhanced through the use of a zinc/silver couple[171,172]. More significant though, was the observation that the Simmons-Smith reagent demonstrated enhanced reactivity towards the electron rich trimethylsilyloxy

Scheme XVIII



enol ethers (Scheme XXI)[178]. The hydrolysis of the silicon-oxygen bond can be effected under essentially neutral conditions[168], thereby diminishing the probability of cleaving the cyclopropane ring. A variety of monocyclic and bicyclic trimethylsilyloxycyclopropano derivatives have been prepared[160,178,179].

Scheme XIX



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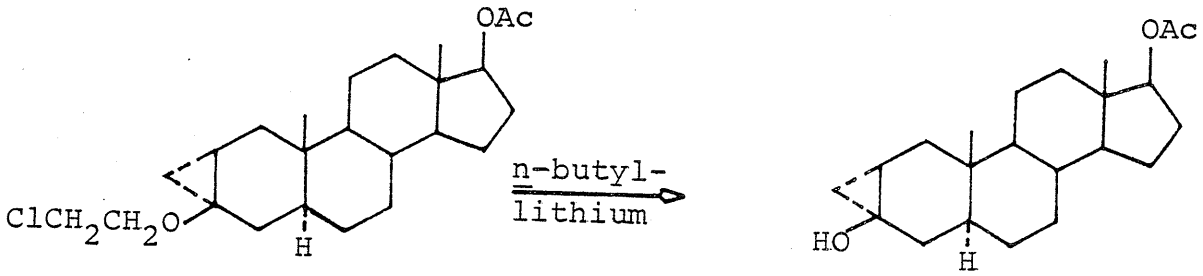
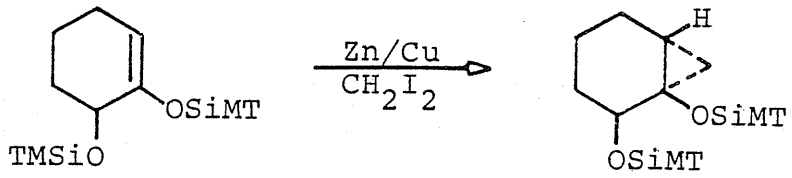
Scheme XX



Scheme XXI



83%

E. Synthesis of deuterated steroidal compounds.

(i) Synthesis of 2α,3α-cyclopropano-2β,4,4-deuterio-17β-acetoxy-5α-androstane-d_3 (120)

The cyclopropano steroid 2α,3α-cyclopropano-17β-acetoxy-5α-androstane (117) was prepared by the method of Templeton and Kim[180]. Treatment of the reaction product (117) with methanolic potassium hydroxide (2% w/v) for one-half hour gave a product which, after recrystallization from warm methanol/ether, gave the known alcohol 2α,3α-cyclopropano-17β-hydroxy-5α-androstane(118). The sharp melting point was in agreement with the literature value[180].

The preparation of the 2,4,4-deuterated analogue of 118 necessitated the preparation of the appropriately deuterated C-2 olefin. To this end, 17β-acetoxy-5α-androstan-3-one (92) was refluxed for 2.5 hours, with deuterium oxide in dry dioxane to which had been previously added sodium metal. The mass spectrum of the crystalline product showed the anticipated molecular ions; m/e 336, 335, 334, 333 and 332 (22%d_4, 43%d_3, 30%d_2, 3%d_1, 2%d_0). The crystalline product was immediately dissolved in methylene chloride and treated with one equivalent of bromine in methylene chloride. The addition of bromine proceeded rapidly. The enolization of the C-3 ketosteroid at 25°C. was expected to favor the thermodynamically more stable C-2 enol intermediate. Therefore, under these thermodynamically controlled conditions, bromination at C-2 was favored. The crude product of the

bromination reaction was immediately treated, for 10 hours, with sodium borohydride in dry dioxane containing deuterium oxide. The pmr spectrum (60 Hz) of the crude mixture of epimeric bromo alcohols showed the expected absorption (3.99, broad) of a proton (the C-3 hydrogen) bonded to carbon substituted with the electronegative substituent[124,125,126]. The presence of the 17β-acetoxy substituent was confirmed by a singlet absorption (2.03) which integrated for three protons. Mass spectroscopy showed the expected multiplicity of molecular ions at m/e 413, 414, 415 and 416 (M^+). The ions appearing at m/e 353, 354, 355 and 356 were consistent with the loss of the 17β-acetate substitutent as acetic acid (M^+ -HOAc). The epimeric mixture of bromo alcohols was not purified further and was immediately treated with the zinc/copper couple in glacial acetic acid for 24 hours (Method II)[181]. The crude reaction product was filtered through ethyl acetate washed aluminum oxide[182] to give a crystalline product which, after recrystallization, had a melting point 95-98°C . Infra-red analysis showed the presence of an olefinic C-H stretch (3024 cm^{-1}) and an olefinic absorption (1642 cm^{-1}). The carbonyl absorption (1735 cm^{-1}) confirmed the presence of the 17β-acetate. The pmr spectrum showed the presence of an olefinic proton (5.90,s,broad) which integrated for one and one-quarter protons. The integration for the olefinic proton in the pmr spectrum indicated that 75% of the deuterium had been

retained at C-2. The mass spectrum showed the anticipated molecular ions at m/e 333, 332, 331 and 330 (35%d_3, 40%d_2, 21%d_1, 4%d_0). The ir, pmr and ms data were consistent with the structure, 2,4,4-deuterio-17β-acetoxy-5α-androst-2-ene-d_3 (119). The treatment of 119 with a 10 molar excess of Simmons-Smith reagent in refluxing ether for 8 hours gave a crystalline product. Recrystallization of the reaction product afforded a material with melting point 102-103°C . The pmr spectrum showed the presence of the 17β-acetoxy substituent (2.039,s) and two high field absorptions centred at δ0.525 and δ-0.175 indicating the presence of cyclopropane protons. The high field absorption at δ0.525 appeared as a heptet and integrated for two protons. This absorption could not be assigned to either the C-3, the endo- or the exo-cyclopropane protons. The high field absorption at δ-0.175 appeared as a non-symmetical triplet and integrated for one proton. The magnitude of the coupling constants ($J_{trans}=J_{gem}$=5 Hz) were consistent with the expected couplings of an endo-cyclopropane proton[123,126]. As the endo-cyclopropane protons of bicyclo [4.1.0] heptane derivatives are known to resonate at higher field than the corresponding exo-cyclopropane protons of bicyclo [4.1.0] heptane derivatives[126], the absorption in the pmr spectrum at δ-0.175 was assigned to, and was consistent with the endo-cyclopropane proton. The presence of the 17β-acetoxy substituent was confirmed by a singlet absorption (2.039) integrating for three protons.

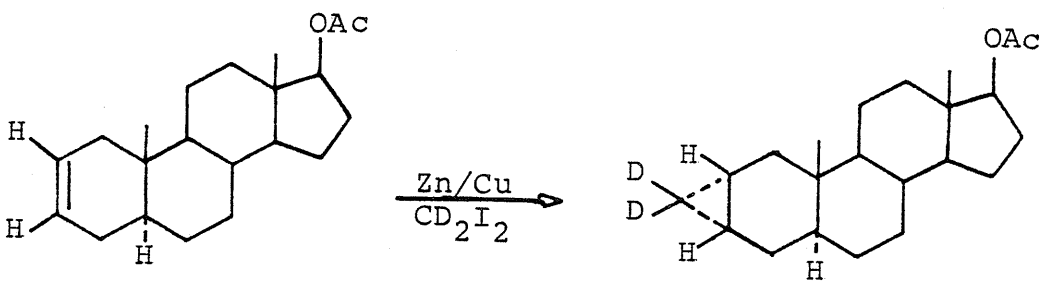
The cyclopropane ring was assigned the α-configuration. This is in accord with the known steric sensitivity of the Simmons-Smith reagent[159], and the absence of any perturbation in the 10β-methyl resonance (0.791,s)[110]. Addition of methylene to the β-surface of the C-2 site of unsaturation would be expected to cause a major alteration in the chemical shift of the 10β-methyl substituent. The ir, pmr and ms data indicated the structure as 2α,3α-cyclopropano-2β,4,4-deuterio-17β-acetoxy-5α-androstane-d_3 (120). Treatment of 120 with methanolic potassium hydroxide (2% w/v) for one-half hour gave 2α,3α-cyclopropano-2β,4,4-deuterio-5α-androstan-17β-ol-d_3 (121).

(ii) Synthesis of 2α,3α-deuteriocyclopropano-5α-androstan-17β-yl acetate-d_2 (123)

The course of reaction outlined in Scheme XXII was employed for the preparation of the deuterated cyclopropanosteroid 123. Treatment of 17β-acetoxy-5α-androst-2-ene (122) with the Simmons-Smith reagent(CD_2I_2) for 21 hours gave a crude reaction product that showed two components on t.l.c. (System II). The slower eluting fraction showed an R_f which was identical to that of the starting material. Ozonization of the crude reaction product in chloroform gave a reaction product, which, after filtration through an aluminum oxide column (7.0 g.) gave a crystalline material. Recrystallization of this material from warm

methanol gave a pure crystalline sample with a sharp melting point (99-101°C)[180]. Infra-red analysis of the crystalline sample showed a cyclopropane C-H stretch (3065 cm^{-1}). The carbonyl absorption in the ir spectrum was assigned to the 17β-acetoxy substituent. Mass-spectroscopy showed a molecular ion (M^+=332) indicating a mass gain of 16 amu. Major ions in the ms appearing at m/e 272 and 257 were consistent with the loss of acetic acid (M^+-HOAc) and a methyl substituent (M^+-HOAc-CH_3). A major ion appearing at m/e 262 was identified as the loss of 70 amu (M^+-HOAc-70) and was consistent with the loss of the A-ring through a retro-Diels-Alder decomposition mechanism. The ir and ms data indicated the structure as 2α,3α-deuteriocyclopropano-5α-androstan-17β-yl acetate-d_2 (123).

Scheme XXII



122 123

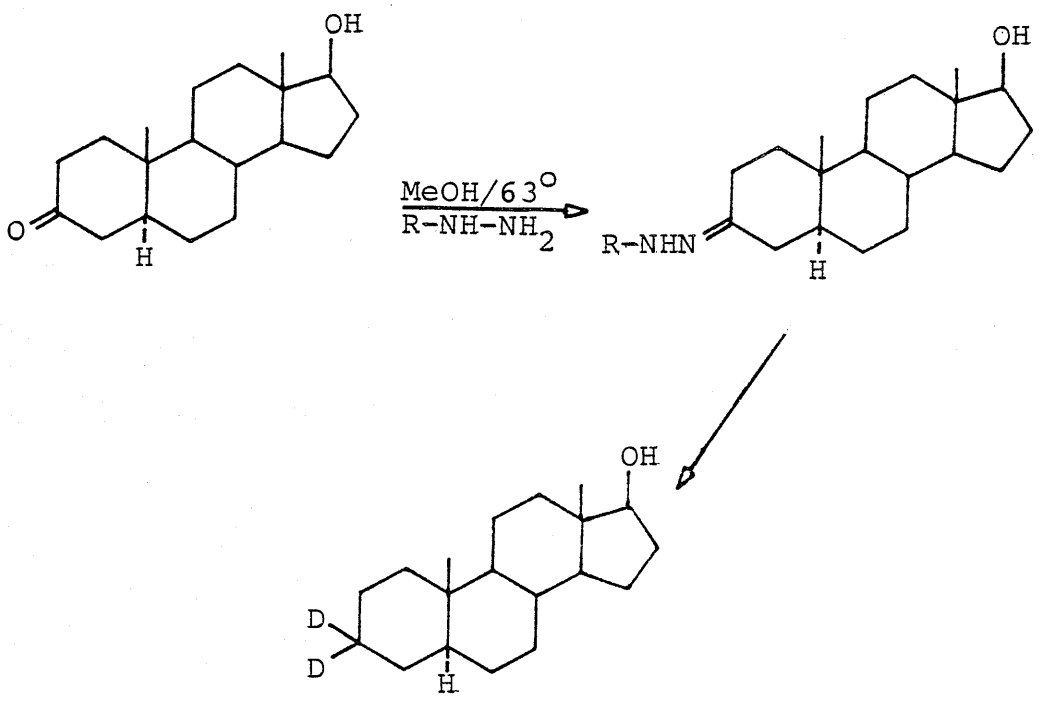
(iii) Synthesis of 3,3-deuterio-17β-hydroxy-5α-androstane-d_2 (125).

The reaction procedure illustrated in Scheme XXIII afforded the deuterated derivative 125 in good yield. The tosylhydrazone of 17β-hydroxy-5α-androstane-3-one (81) was prepared by the method of Djerassi et al.[183] using an equimolar ratio of ketone substrate and p-toluenesulfonylhydrazine reagent. Evaporation of the reaction solvent, at reduced pressure gave a crystalline material (119°C decomposition) that showed one component on t.l.c. (System VI). Infra-red analysis showed a free hydroxyl absorption (3614 cm^{-1}) and a free amine absorption (3294 cm^{-1}) characteristic of the secondary amine of a sulfonylhydrazono substituent. The ir spectrum also showed a characteristic imine absorption (1600 cm^{-1}) and two absorptions (1385 cm^{-1} and 1158 cm^{-1}) indicating the presence of the sulfonyl group. The ir data indicated the structure 3-tosylhydrazono-17β-hydroxy-5α-androstane (124). Treatment of the tosylhydrazone 124 with a 12 molar excess of lithium aluminum deuteride[183,184] in dry dioxane for two hours and decomposition of the metal deuteride complex with deuterium oxide gave a crystalline material which, after filtration through an aluminum oxide column (30 g.), afforded a crystalline product with a sharp melting point (165° - 166° C). Mass spectroscopy showed a molecular ion at m/e 278 (M^+). The major ions in the ms at m/e 263 and m/e 260 were consistent with the loss of a

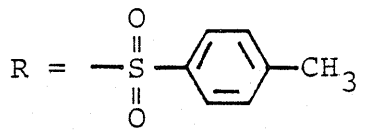
methyl substituent (M^+-CH_3) and the elimination of the 17β-hydroxy substituent as a molecule of water (M^+-H_2O), respectively. The mass spectrum indicated the structure 3,3-deuterio-17β-hydroxy-5α-androstane-d_3 (125).

The C-3 dihydro derivative, 17β-hydroxy-5α-androstane (126), was prepared from 17β-hydroxy-5α-androstan-3-one (81) by the method of Huang[185] using potassium hydroxide (11% w/v) and hydrazine monohydrate. The melting point of the recrystallized product (164.5-165.5°C) was in agreement with the literature value.

Scheme XXIII



125

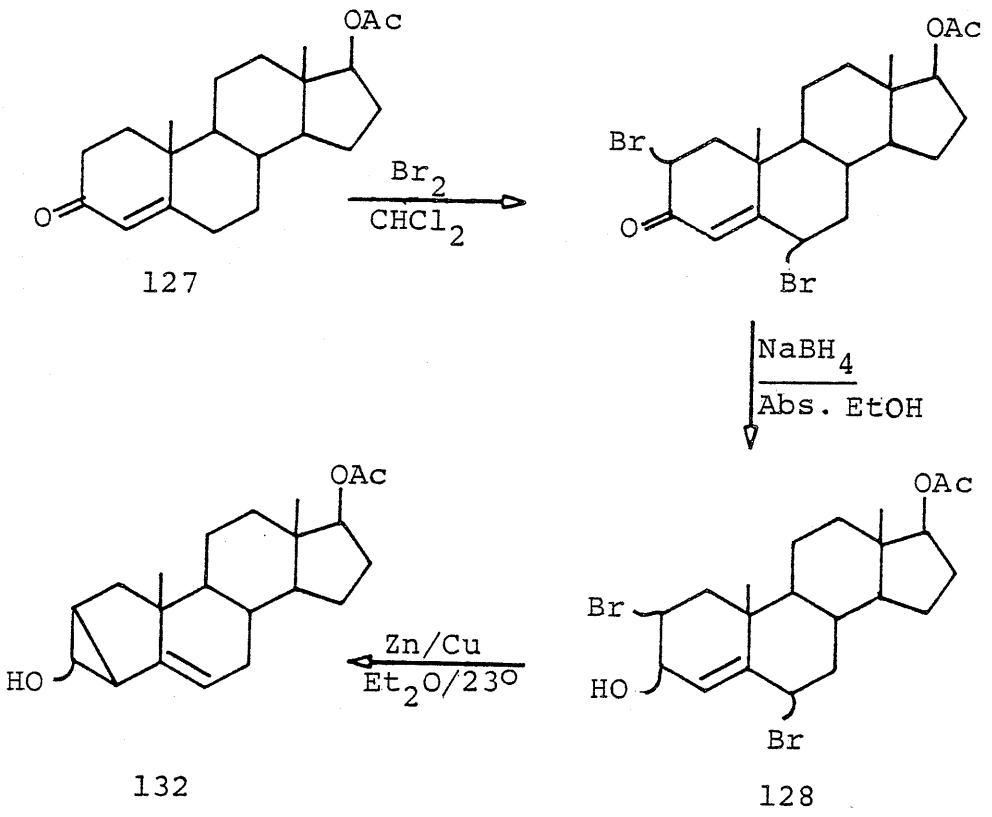


F. Attempted synthesis of 2β,4-cyclo-androst-5-ene-3ξ,17β-diol diacetate (132).

The sequence of reactions outlined in Scheme XXIV were carried out with a view to the preparation of C-5 unsaturated 2,4-cyclo-androstane derivatives. Bromination of 17β-acetoxy-androst-4-en-3-one (127) with two equivalents of bromine in methylene chloride gave rapid decolorization and the product showed two components on t.l.c. Infra-red analysis of the crude reaction product showed the presence of two carbonyl absorptions (1745 cm^{-1} and 1695 cm^{-1}). These carbonyl absorptions in the ir spectrum could be assigned to the 17β-acetoxy substituent and the C-3 conjugated ketone. The absorption in the ir spectrum at 1620 cm^{-1} was consistent with the presence of the C-4 (conjugated) site of unsaturation. Based on the ir data, the bromination product was assigned the structure 2β,6ξ-dibromo-17β-acetoxy-androst-4-en-3-one (128). This assignment was consistent with the observations of Djerassi *et al.* who have shown that the dibromination of C-4 unsaturated 3-ketosteroids proceeds to give the 2,6-dibromo (C-4 unsaturated) 3-ketosteroid derivatives[186]. Shoppee *et al.* have shown that the monobromination of 4-methyl-cholest-4-en-3-one (129) in acetic acid proceeds to give exclusively 2β-bromo-4-methyl-cholest-4-en-3-one (130)[187]. This suggests that dibromination of testosterone acetate (127) proceeds to give the isomeric 2β,6ξ-dibromo derivative(s) as the major product(s)[186].

The proportion of axial and equatorial isomers was not determined and the crude reaction mixture was employed for the next sequence of reactions.

Scheme XXIV



The crude mixture of isomeric dibromides was immediately treated with sodium borohydride in absolute ethanol. The reaction proceeded rapidly and the product gave two major components on t.l.c.. Infra-red analysis showed the presence of a free hydroxyl absorption (3580 cm^{-1}). The carbonyl

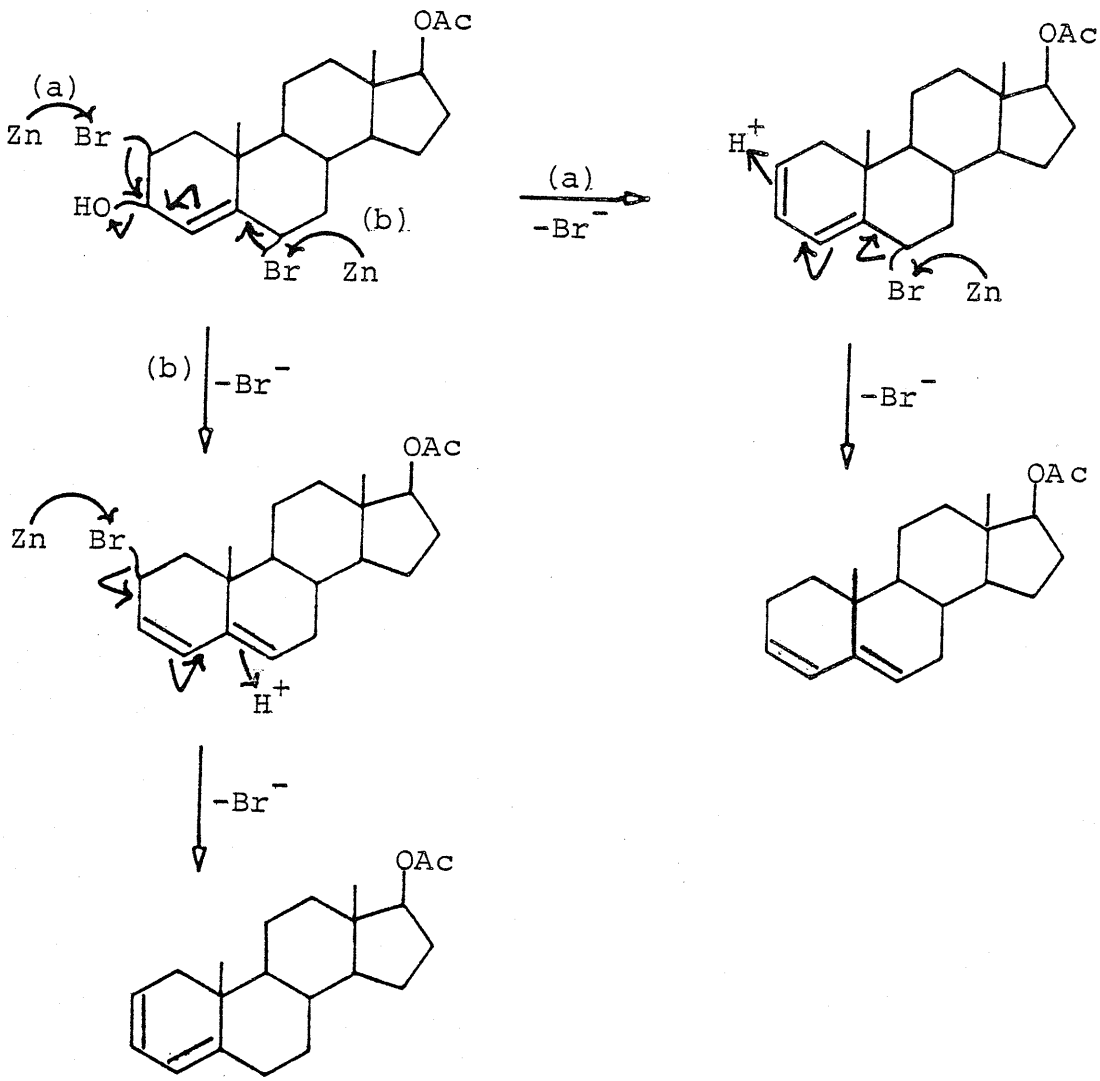
absorption at 1738 cm^{-1} was consistent with the presence of the 17β-acetoxy substituent. A non-conjugated olefinic absorption (1680 cm^{-1}) in the ir spectrum of the reduction product further confirmed that the reduction of the C-3 ketone substituent had taken place. The ir data was consistent with the assignment of 2β,6ξ-dibromo-3ξ,17β-hydroxy-5α-androstan-17β-yl acetate(131) to the structure of the sodium borohydride reduction product.

The mixture of dibromo alcohols was immediately treated with an excess of zinc/copper couple (Method I) for three hours at room temperature. The crude reaction product showed three major components on t.l.c. (System IV). Infra-red analysis showed the carbonyl absorption (1734 cm^{-1}) typical for the 17β-acetoxy substituent. A strong olefinic C-H stretch (3020 cm^{-1}) and two olefinic absorptions (1680 cm^{-1} and 1620 cm^{-1}) indicated the presence of olefinic products. Infra-red analysis of the crude acetylation product was consistent with that observed for the crude reaction product. The pmr spectrum of the crude acetylation product showed singlet resonances (1.186, 3H and 0.836, 3H) which may be assigned to the 10β- and 13β- methyl substituents. The singlet resonance (2.045, 3H) could be assigned to the 17β-acetoxy methyl hydrogens. A series of low field resonances (6.173, 6.109, 5.795, 5.727 and 5.295) in the pmr spectrum of the crude acetylation product indicated the presence of olefinic products. Alternatively, these resonances may be

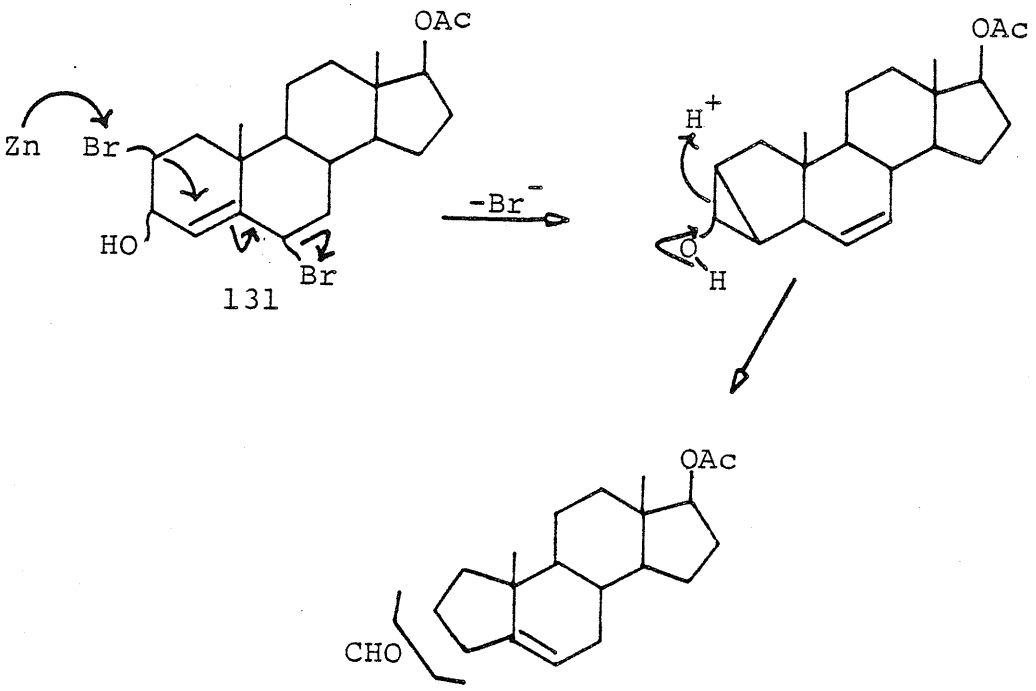
interpreted as two doublets and one singlet (5.295). These low field resonances all integrated for less than one proton and constituted, respectively, 6.5%, 12%, 12%, 2.2% and 2.2% of the total crude acetylation mixture. Chromatography over ethyl acetate washed aluminum oxide gave no clearly defined products or crystalline fractions. A reaction course consistent with the appearance of olefinic products in the zinc/copper couple reduction of the dibromo alcohols is shown in Scheme XXV.

The methine protons of 2β,4β-cyclo-5α-androstane-3β,17β-and 2β,4β-cyclo-5α-androstane-3α,17β-diol diacetate appear, in the pmr spectrum, at δ3.63 and δ4.25, respectively[181]. The C-2 and C-4 cyclopropyl hydrogens of these substances resonate within the methylene envelope and are thus not observed. Therefore, the absence of a high field resonance in the pmr spectrum of the zinc/copper couple reduction product (Scheme XXIV) does not necessarily indicate that no 2,4-cyclo products are present. However, the observed absence of a proton resonance in the region δ2.72 to δ4.62 strongly suggests that no 2,4-cyclo products are present. Nevertheless, a 2,4-cyclo product may have formed through the course of the reaction and subsequently decomposed (Scheme XXVI). The presence of a 2,4-cyclosteroid intermediate may be ascertained by the observance of a down field proton resonance characteristic of aldehydes in the pmr spectrum. The absence of this down field absorbance in the pmr spectrum

Scheme XXV



Scheme XXVI



of the zinc/copper, crude acetylation product also confirmed that a 2,4-cyclo product was not present as a reaction intermediate.

The attempted synthesis of 2β,4β-cyclo-androst-5-ene-3ξ,17β-diol diacetate as discussed above, was postulated to proceed through a concerted mechanism necessitating the overlap of the sp^3 molecular orbital of the C-2 atom with the axially orientated p molecular orbital of the C-4 site of unsaturation. Concomitant rehybridization of these orbitals to give the $sp^{4.12}$-hybridization state of carbon-carbon molecular orbitals of the cyclopropane bond(s) must accompany the overlap of these two orbitals. The formation of the 2β,4β-cyclopropane bond must be accompanied by the expulsion of the C-6 bromine atom. Concomitant rehybridization of the C-5 and C-6 atomic orbitals must accompany the

expulsion of the C-6 bromine atom to establish the C-5 site of unsaturation.

The elimination of a 2β-bromine atom with the zinc/copper couple would be expected to proceed more rapidly than the elimination of a 2α-bromine atom as the former is destabilized through steric crowding by the angular 10β-methyl substituent. Moreover, the 2β-molecular orbital has a 1,3-diaxial orientation or a 1,3-pseudo-axial orientation, depending on the conformation of the A-ring, with the p molecular orbital of the C-4 site of unsaturation. This 1,3-diaxial relationship between the sp^3 molecular orbital at C-2 and the C-4 p molecular orbital at C-4 is compatible with the overlap of these two orbitals and hence the formation of the 2,4-cyclpropane bond. A concerted mechanism for the formation of a 2,4-cyclopropane bond is furthermore compatible with a β-configuration of the C-6 bromine atom. Formation of the 2,4-cyclopropane bond through a concerted mechanism necessitates the formation of a C-5 site of unsaturation and expulsion of the C-6 bromine atom. The β-configuration of the C-6 bromine atom ensures maximum charge separation in the transition state and facilitates the overlap of the C-5 and C-6 molecular orbitals to form the C-5 site of unsaturation. The dibromination of testosterone acetate has been shown to most likely yield a dibromo derivative which is isomeric at C-6, i.e. 2β,6ξ-dibromide of testosterone acetate. If the proportion of C-6 equatorial isomer is

significantly greater than the proportion of C-6 axial isomer, the formation of olefinic products may be anticipated to greatly exceed the formation of 2,4-cyclo derivatives as the overlap of the C-5 molecular orbital with the C-6 equatorial molecular orbital is not compatible in a concerted mechanism (the C-6 equatorial bromine atom lies at approximately right angles to the p molecular orbitals of the C-5 site of unsaturation).

G. Proton magnetic resonance of some 2α,3α-cyclopropano-5α-androstane derivatives.

The dibromocyclopropanosteroid 94 showed a six line resonance at δ0.664 which integrated for one proton (Figure XXVIIa). The coupling constants (J=12, 12 and 4 Hz) and the sextet nature of this resonance were not consistent with it being a cyclopropyl proton. A similar high field sextet (0.591, 1H) which could also not be assigned to a cyclopropane proton was observed in the pmr spectrum of the endo-bromocyclopropanosteroid 97 (Figure XXVIIIa). The coupling constants (J=10, 10 and 5 Hz) were of the same order of magnitude as those observed for 94. Furthermore, evidence attesting to the origins of this high field sextet from non-cyclopropyl protons is gleaned from the appearance of a similar high field sextet (0.47, 1H) in the pmr spectrum of the deuterated cyclopropanosteroid 119 (Figure XXIXa). The coupling constants (J=10, 10 and 5 Hz) are identical with those of compound 97.

The cyclopropanosteroid 114 (Figure XXVIIb) showed the presence of a high field sextet (0.514, 1H) in the pmr spectrum. The coupling constants (J=10, 9 and 4 Hz) and the chemical shift were in good agreement with the observed coupling constants and chemical shift noted for the high field sextet in the cyclopropanosteroid discussed above. A second high field resonance (0.463, t), which integrated for one proton, could be assigned with assurance to the

endo-cyclopropane proton (the 2β-cyclopropane proton which has four adjacent protons would give rise to a more complex coupling pattern). The endo-cyclopropane protons of bicyclo [4.1.0] heptane derivatives are known to resonate at higher field relative to the corresponding exo-cyclopropane protons in bicyclo [4.1.0] heptane derivatives[126]. Furthermore, this assignment was consistent with the coupling constants, which were in agreement with a geminal coupling (J_{gem}=6 Hz) and a trans coupling (J_{trans}= 6 Hz) for cyclopropane protons. The magnitude of the trans and the geminal couplings of protons in cyclopropane are diminished relative to the magnitude of the cis couplings of these cyclopropane protons. The cyclopropyl-methyl-ether 133 (Figure XXIXb) also showed a high field sextet (0.514, 1H) and a high field triplet (0.109,1H) in the pmr spectrum. The high field triplet (0.109) was assigned to the endo-cyclopropane proton. The coupling constants (J_{gem}=J_{trans}=6 Hz) were consistent with the trans relationship of the endo-cyclopropane proton with the 2β-proton and the geminal relationship of the endo-cyclopropane proton with the (down field) exo-cyclopropane proton. The higher chemical shift value of the endo-cyclopropane proton in compound 133 (relative to the chemical shift of the endo-cyclopropane proton in the 3β-acetoxy analogue 114. was consistent with the loss of the C-3 carbonyl substituent. The carbonyl substituent is known to possess paramagnetic anisotropic affects[110b].

The cyclopropyl alcohol 118 showed three high field absorptions in the pmr spectrum (Figure XXIXc). A sextet (0.51, 1H), with coupling constants (J=10, 9 and 4 Hz) of similar magnitude as those of the unidentified sextet observed throughout the above compounds, could similarly not be assigned to a cyclopropyl proton. A second sextet appearing at higher field (0.46, 1H) was identified as the exo-cyclopropane proton of this compound. This sextet could arise from the coupling of the exo-cyclopropane proton with the 2β-, 3β- and geminal protons. The coupling constants of the sextet appearing at δ0.46 in the pmr spectrum, could not be calculated with total accuracy as the sextet appearing at δ0.51 was partially superimposed upon that one appearing at δ0.46. Nevertheless, the pmr spectrum indicated two large couplings (2 x J_{cis}) and one small coupling (J_{gem}). The resultant three splittings gave rise to this partially obscured sextet. The third high field resonance (-0.19, 1H) appeared as a quartet and was assigned to the endo-cyclopropane proton. The two trans couplings ($J_{endo,2}$ = 5 Hz and $J_{endo,3}$ = 5 Hz) and the one geminal coupling (J_{gem} = 5 Hz) were consistent with the observed quartet. Decoupling experiments involving irradiation of the high field quartet (-0.19) showed no change in the sextet pattern.

The pmr spectrum of the deuterated cyclopropanosteroid 120 (Figure XXVIIIb) showed a distorted high field sextet (0.527, 2H) consisting of seven peaks. The integrand indicated two protons within this heptet of peaks. Evidence

gleaned from the above examples suggests that the sextet, which had consistently appeared throughout these cyclopropanosteroids, is present in this heptet and accounts for one of the protons in this integrand. This is consistent with the observation that no other proton in the A-ring of this substance can give rise to a sextet pattern of couplings. The second proton within this heptet may be the exo-cyclopropane proton which has been shown above to resonate at approximately δ0.46. The lack of cyclopropane protons in the pmr spectrum of the deuterated cyclopropanosteroid 123 indicates that this assignment is valid. The 3β-cyclopropane proton of 120 is excluded as the source of the second proton in the heptet (0.527) as it is not present in the pmr spectrum of deuterated 119. The high field resonance (-0.175, 1H) in the pmr spectrum of 120 was assigned to the endo-cyclopropyl proton. The coupling constants ($J_{gem}=J_{trans}$= 5 Hz) were consistent with the triplet nature of this resonance and are in accord with the typical values of J_{gem} and J_{trans} for cyclopropane protons[123,124,126]. The assignment of the endo-cyclopropane proton to the resonance at δ-0.175 is consistent with the known higher field, chemical shift values of endo-cyclopropane protons in bicyclo [4.1.0] heptane derivatives relative to the chemical shift values of the exo-cyclopropane protons in these same derivatives[123,124,126].

The unidentified high field sextet resonating at approximately δ0.58 ± 0.08 and integrating for one proton was

a salient feature of these cyclopropanosteroids (Table XXVII). This sextet could not be assigned to a cyclopropane proton in any of the substances discussed above. Several authors have reported the appearance of high field proton absorptions in the pmr spectrum of cyclopropanosteroid derivatives which could not be assigned to the protons of the cyclopropane ring[105,106]. The observations of these workers are consistent with the observations made in the pmr spectra of this series of cyclopropanosteroids and are a reflection of the known diamagnetic anisotropic effects of the cyclopropane ring. The anisotropic effects of the cyclopropane ring have been investigated and reported by Kohout and Fajkos[112] and Kohout et al.[114]. The differences in the chemical shifts of the observed sextets in the substances discussed above may lie in the distance through which the shielding effect operates. A probable assignment to this high field sextet is the 9α-proton[106], which lies in the conical (shielding) regions extending above and below the cyclopropane ring. The 9α-proton is the most likely assignment to this high field sextet as it lies closest to the cyclopropane ring, relative to, for example, the 11α- or 14α-protons which have also been implicated as the source of these high field resonances[109]. The 9α-proton can give rise to a sextet through three splittings ($J_{9\alpha,8\beta}$, $J_{9\alpha,11\beta}$ and $J_{9\alpha,11\alpha}$) two of which are trans diaxial splittings and one of which is an axial/equatorial splitting. A sextet of couplings is also

to be expected for the 11α- and 14α-protons. The assignment of the sextet to the 9α-proton is also consistent with the observed diminution of the coupling constants in passing from the dibromocyclopropanosteroid 94 to monobromcyclopropanosteroid 97. The magnitude of the geminal and vicinal coupling constants have been shown to be altered by spatial orientations of electronegative substituents[188,189,190]. This effect may well be expected to be related to internuclear distances. Therefore, the most proximal atom, i.e. the 9α-proton, would be expected to be the one most influenced rather than the more distant atoms, e.g. the 11α- or 14α-protons. However, this observation is not consistent with the observed magnitudes of the unidentified sextet coupling constants obtained for the monobromocyclopropanosteroid 97 and the cyclopropanosteroid 114. The coupling constants of the sextet in these two compounds are identical. If this effect were operative then loss of the bromine atom may well be anticipated to alter the magnitude of the coupling constant. This is not observed. Nevertheless, the chemical shift of the sextet in 114 is at higher field (Δ=0.077 ppm) than that one of 97. This is consistent with the loss of the electronegative substituent.

A comprehensive compilation of the pmr data of the 2α,3α-cyclopropano derivatives discussed above is shown in Table XXVIII.

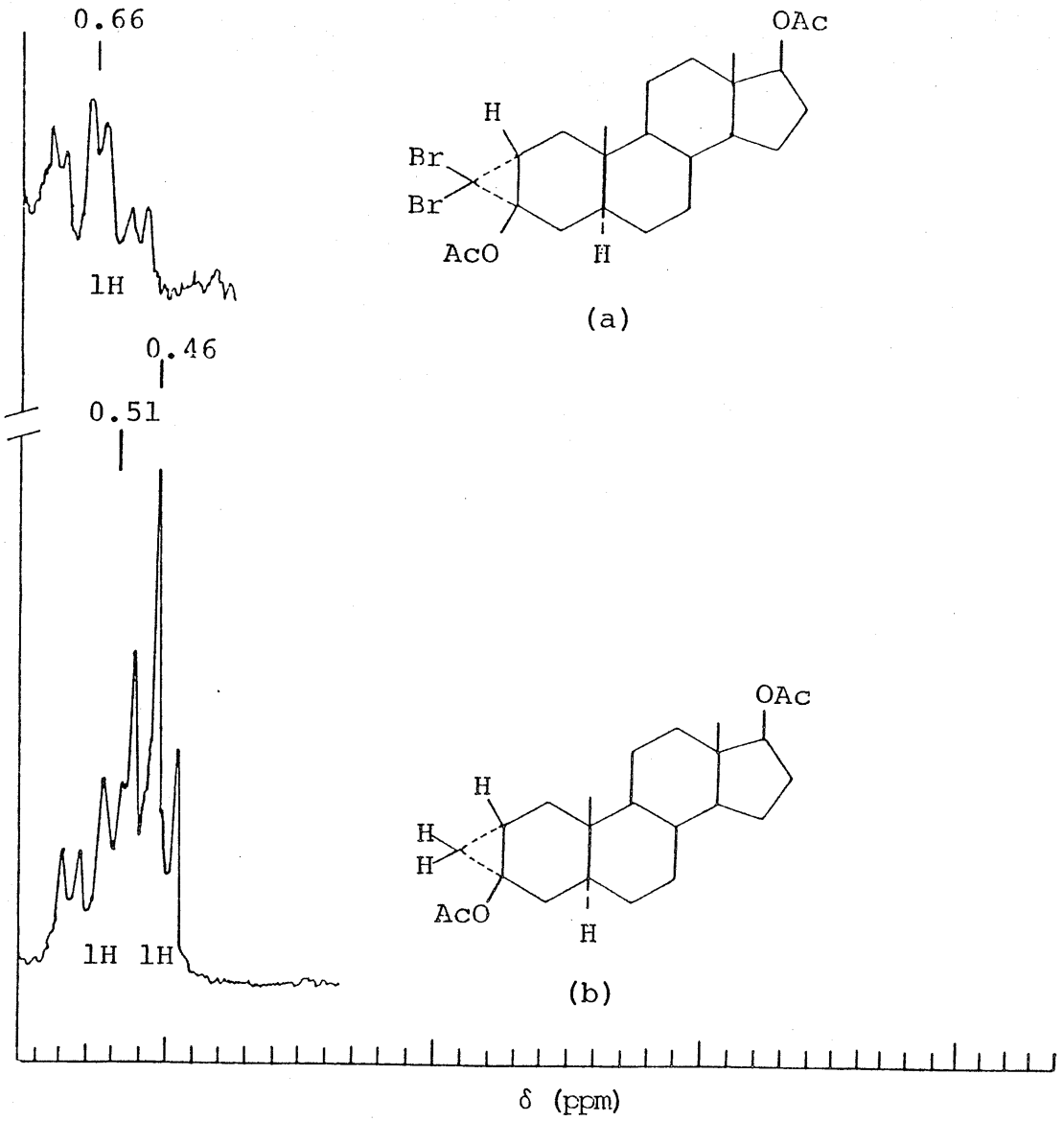


Figure XXVII. High field bands in the 220 mHz pmr spectra of 2α,3α-cyclopropanosteroid derivatives.

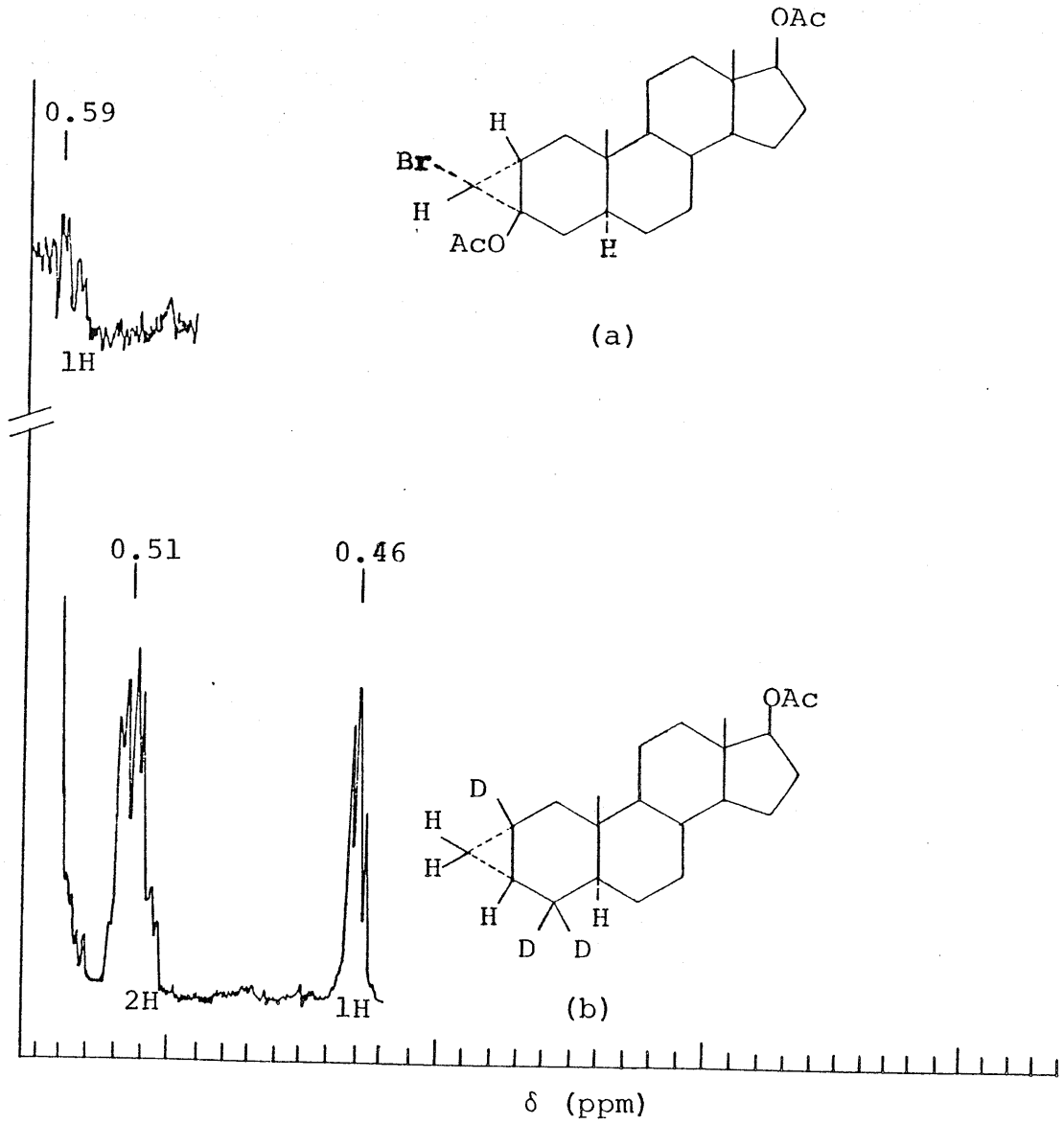


Figure XXVIII. High field bands in the 220 mHz pmr spectra of 2α,3α-cyclopropanosteroid derivatives.

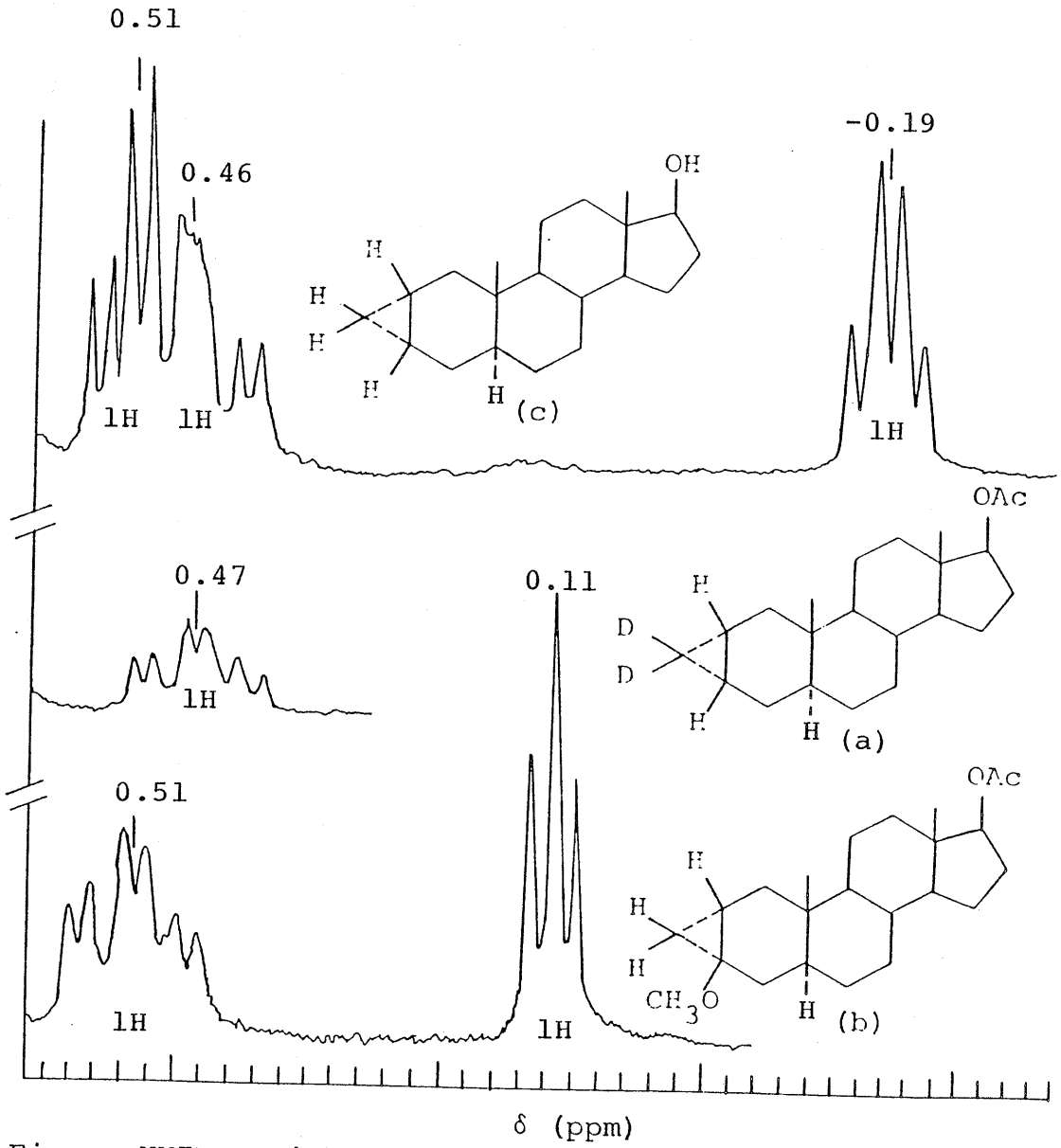


Figure XXIX. High field bands in the 220 mHz pmr spectra of 2α,3α-cyclopropanosteroid derivatives.

TABLE XXVII. High resolution (220 mHz) pmr spectra data of the high field bands of some 2α,3α-cyclopropanosteroid derivatives.

Compound	δ(ppm)	Integration	Type[a]	J(Hz)
94	0.664	1H	sx	J=12,12,4
97	0.591	1H	sx	J=10,10,5
114	0.514	1H	sx	-
	0.462	1H	t	$J_{\underline{gem}}=J_{\underline{trans}}=6$
118	0.510	1H	sx	J=10,9,4
	0.460	1H	sx	-
	-0.190	1H	q	J=5,5,5
120	0.527	2H	h	-
	-0.175	1H	t	$J_{\underline{gem}}=J_{\underline{trans}}=5$
123	0.470	1H	sx	J=10,10,5
133	0.514	1H	sx	J=10,10,5
	0.109	1H	t	$J=_{\underline{gem}}=J_{\underline{trans}}=6$

[a]For the list of abbreviations see Appendix III.

TABLE XXVIII. Pmr data (220 mHz) of some 2α,3α-cyclopropano-5α-androstane derivatives (chemical shift/coupling/coupling constant).

Compound	Protons[a]							
	17α-H	3β-OAc	17β-OAc	10β-CH_3	13β-CH_3	Endo-H	Exo-H	Others
94	4.585/ t/8	2.116	2.050	0.816	0.786	-	-	0.664/ ss/12,12,4
97	4.580/ t/8.8	2.027	1.972	0.882	0.772	-	3.364/ d/10	0.591/ sx/10,10,5
114	4.55/ t/9	1.982	1.921	0.874	0.763	0.462/ t/6	-	0.514/ sx
118	3.570/ t/8	-	-	0.762	0.688	-0.183/ q/4	-	0.510/ sx/10,9,4
120	4.545/ t/8	-	2.039	0.793	0.770	-0.175/ t/5	-	0.527/ h
123	4.535/ t/8	-	2.003	0.753	0.730	-	-	0.47/ sx/10,10,5
133	4.573/ t/8	-	2.036	0.836	0.730	0.109/ t/6	-	0.514/ sx/10,10,5

[a] For the list of abbreviations see Appendix III.

H. Mass spectra of some 2α,3α-cyclopropano-5α-androstane derivatives.

The mass spectra of the brominated cyclopropanosteroids 94 and 97 were characterized by the absence of a molecular (M^+) ion and the presence of a major ion at m/e 201 (Table XXIX). The mass spectra of the A-homo-3-bromosteroid 103, similarly showed no molecular ion and a major ion at m/e 201. A fragmentation pattern consistent with the major ions observed in the ms of 99 is shown in Scheme XXVII. The mass spectra of the brominated cyclopropanosteroids 94 and 97 demonstrate fragmentation patterns similar to the A-homo-3-bromosteroid 103. It is therefore, conceivable that the mass spectral fragmentation of 94 and 97 may proceed through an intermediate A-homo analogue such as 103 (the cyclopropane ring has been shown to cleave under electron impact[191,192]). The m/e 201 ion in the mass spectra of 94 may be readily accounted for through the scheme outlined below (Scheme XXVIII). Similarly, the ion observed at m/e 201 ion in the mass spectra of 97 may be readily accounted for as outlined below (Scheme XXIX).

The fragmentation pattern of the cyclopropanosteroid 114 (Scheme XXX) demonstrated some features which were characteristic of the fragmentation patterns of the brominated cyclopropanosteroids 94 and 97. The loss of ketene (M^+-42), characterized by the ion at m/e 346, and the

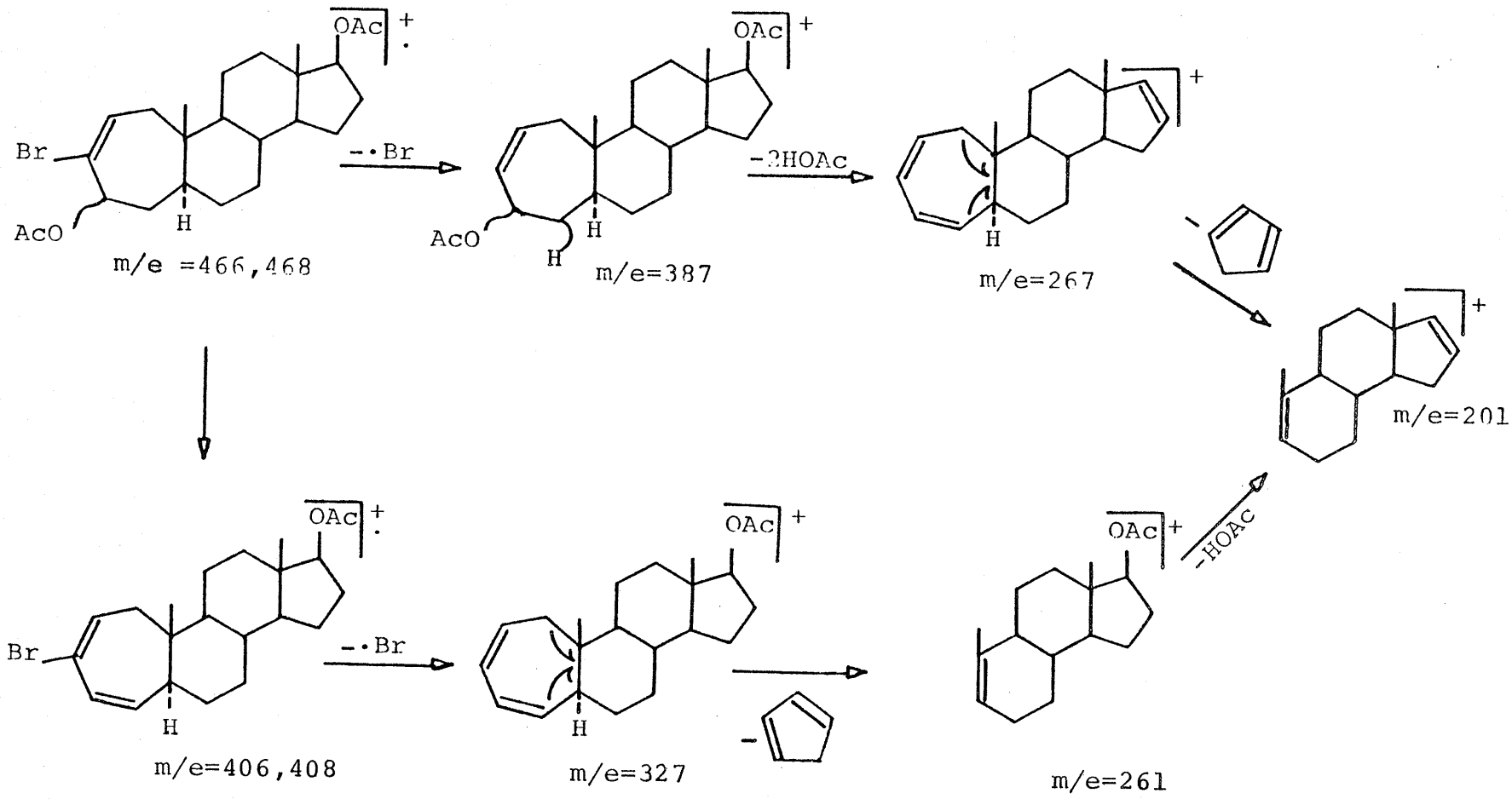
elimination of the A-ring (M^+-42-84), consistent with the ion at m/e 262, were features that were common to the fragmentation pathways of 94, 97 and 114. The prominence of a molecular ion (M^+-388) however, was a contrasting feature in the ms of this substance (M^+ values were not observed in the ms of 94 or 97). Similarly, the loss of a methyl substituent (M^+-CH_3), characterized by the ion at m/e 373 was only observed in the ms of 114. The formation of an m/e 202 ion (M^+-42-84-HOAc), which is the m/e 201 equivalent of 94 and 97, was not a prominent feature in the ms of 114 (Scheme XXX).

The salient feature in the mass spectra of the deuterated cyclopropanosteroid 123 was the absence of complexity (Scheme XXXI). The molecular ion (m/e 332) was observed, but at relatively low intensity (3.3%). The ms of the deuterated cyclopropanosteroid 123 has a fragmentation pattern characteristic of C-2 unsaturated steroidal olefins. The ms of 123 is characterized by the elimination of the A-ring (m/e 262) which may arise through a retro-Diels-Alder reaction. C-2 unsaturated steroidal olefins characteristically fragment, under electron bombardment, through elimination of the A-ring by the retro-Diels-Alder mechanism[165]. The retro-Diels-Alder mechanism may also be operative in the elimination of the A-ring of 114. The ion at m/e 262, in the ms of 114, may arise from the parent ion m/e 346, through loss of 84 amu (M^+-42-84). The fragment constituting 84 amu can be mechanistically shown to

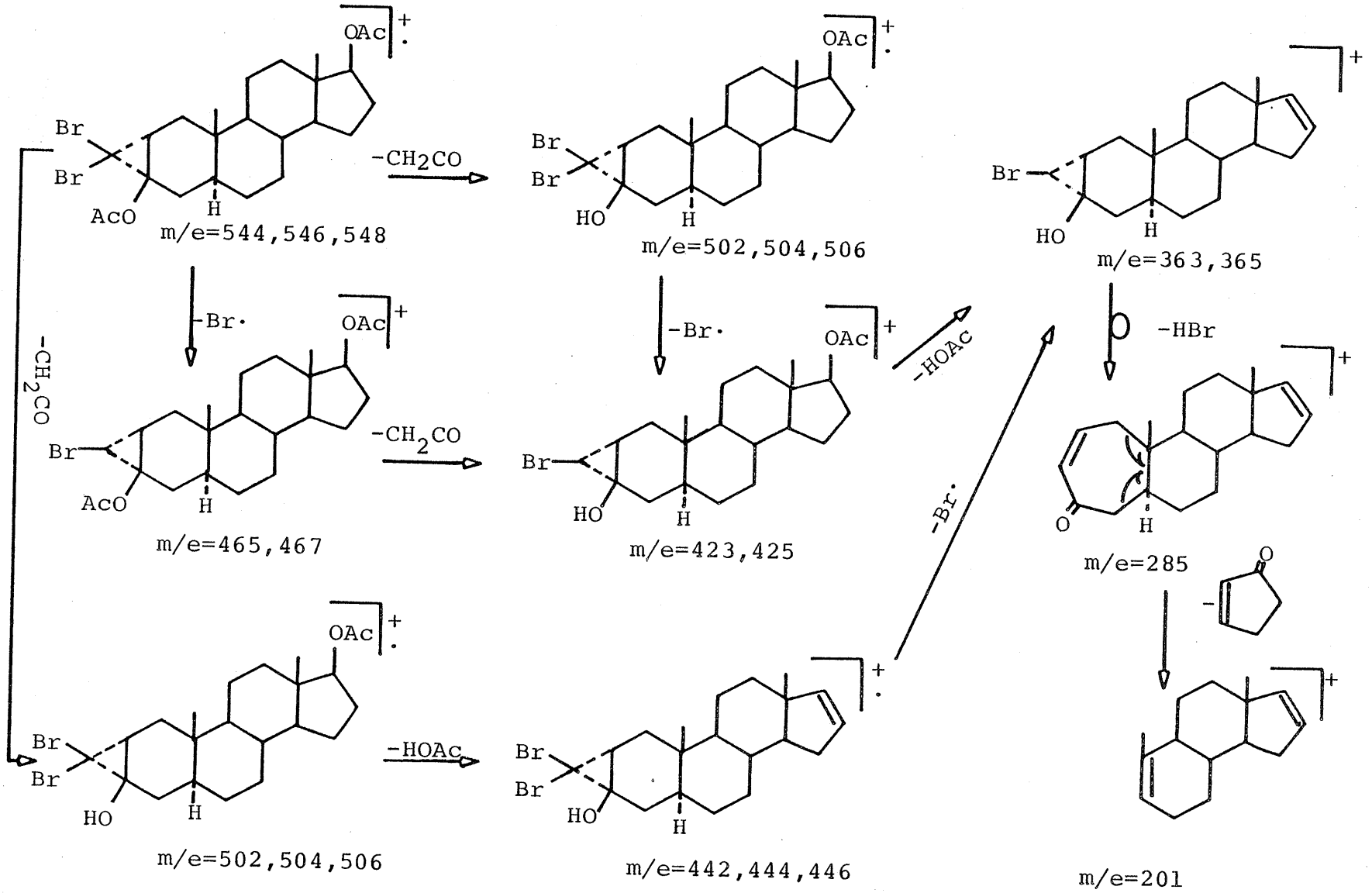
Table XXIX. Mass spectral fragments of 2α,3α-cyclopropane-5α-androstane derivatives (relative intensity m/e).

Compound	M^+	M^+-Br	M^+-CH_3	M^+-42	M^+-HOAc	M^+-70	M^+-(Br +42)	M^+-(42 +CH_3)	M^+-Br -HOAc)	M^+-HOAc +CH_3)	M^+-(70 +HOAc)	M^+-(42 +84)	M^+-(Br+ 42+ HOAc)	M^+-(Br+ 42+HOAc +$H_2$0+66)	M^+-(Br +2HOAc +66)	M^+-(70 +HOAc +CH_3)	M^+-(Br+ 42+HOAc +HBr+82)	M^+-(Br+ 42+HOAc +HBr)
94	-	3 465 2.5 467		5.5 502 12 504 5.5 506			11.5 423 11 425						4.5 363 3.5 365				46 201	5 283
97	-	15 387					100 345		0.5 327				9.6 285	74.5 201	9.1 267			
114	73.9 388		26.9 373	64.4 346				100 331				69.1 262						
123	3.3 332				3.0 272	5.9 262				3.8 257	11.7 202					9.61 187		

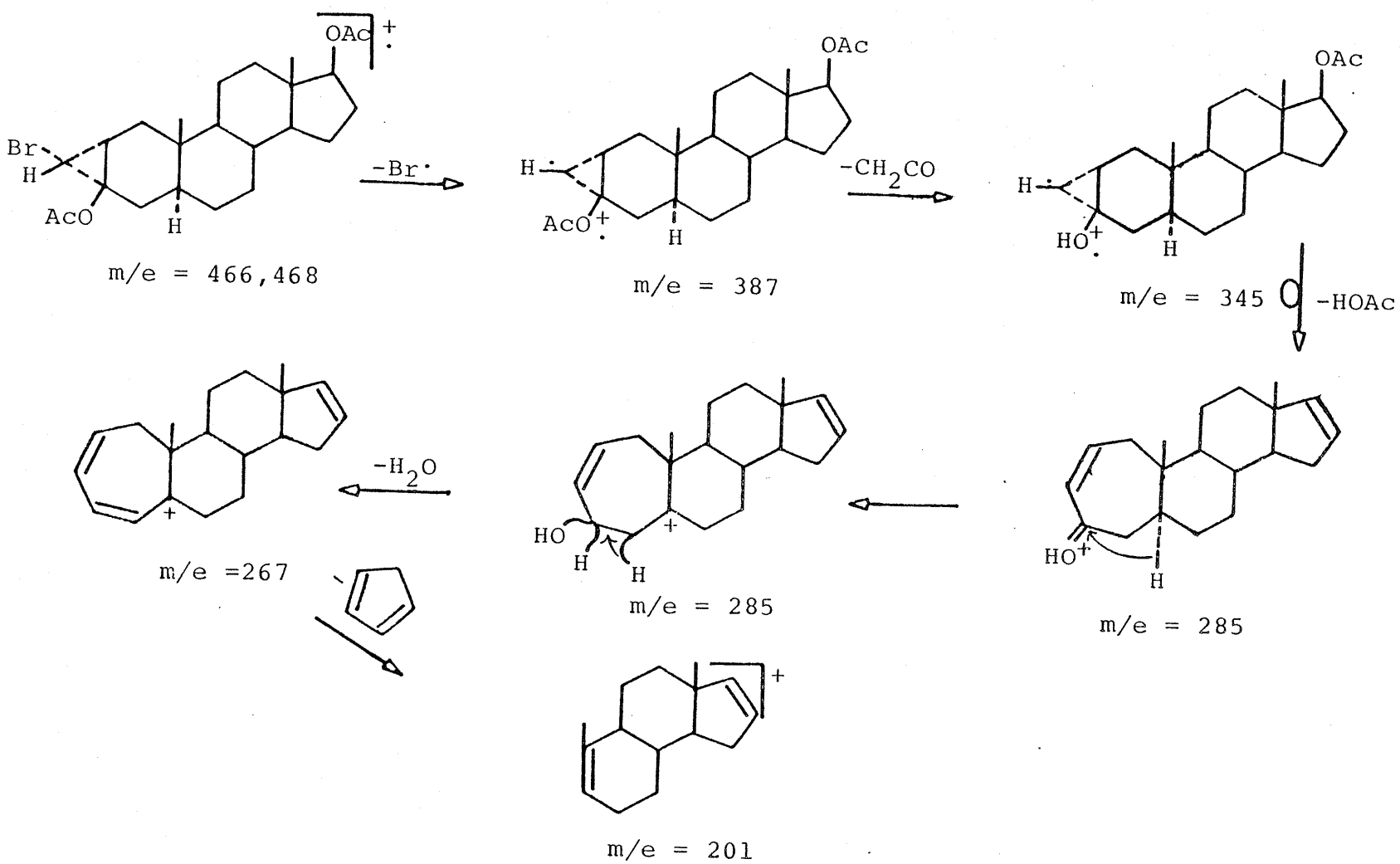
Scheme XXVII



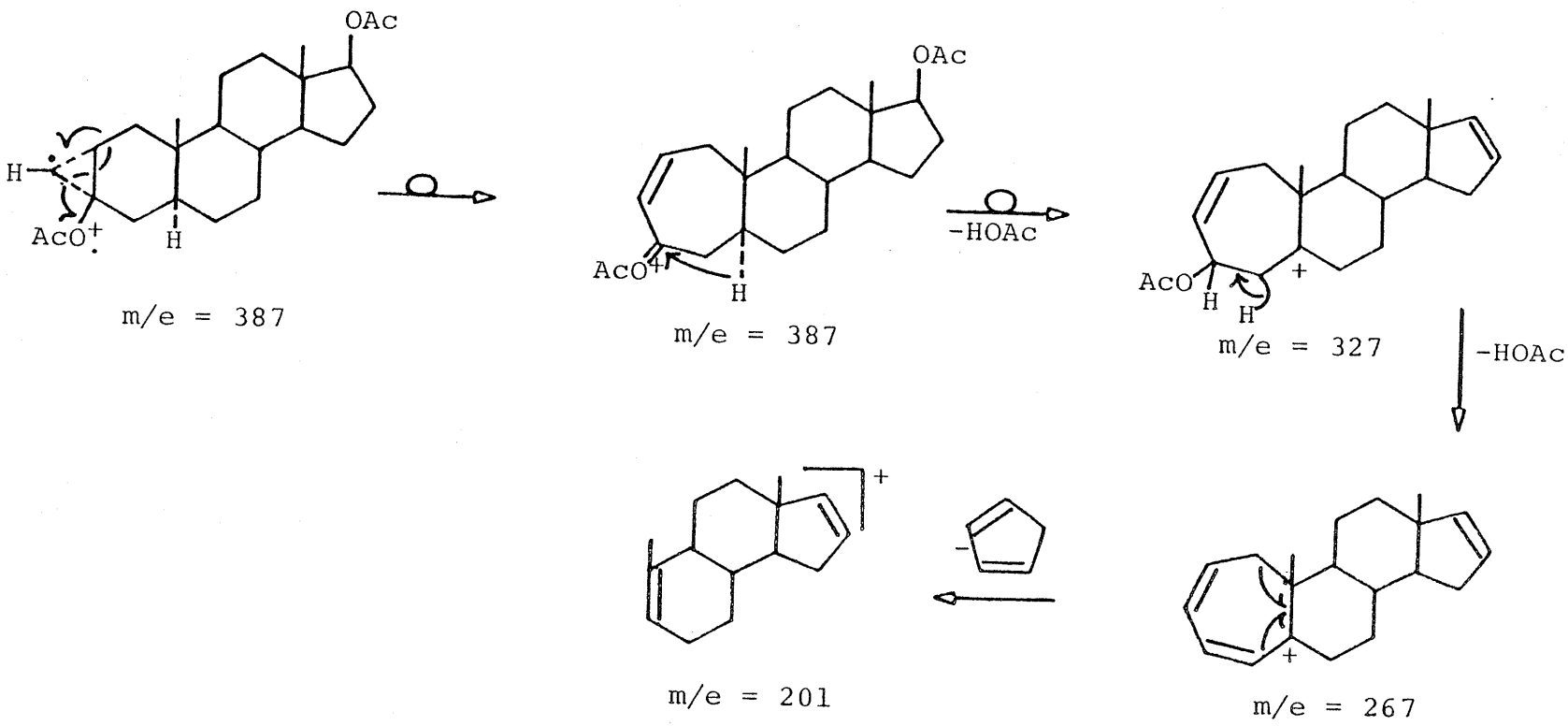
Scheme XXVIII



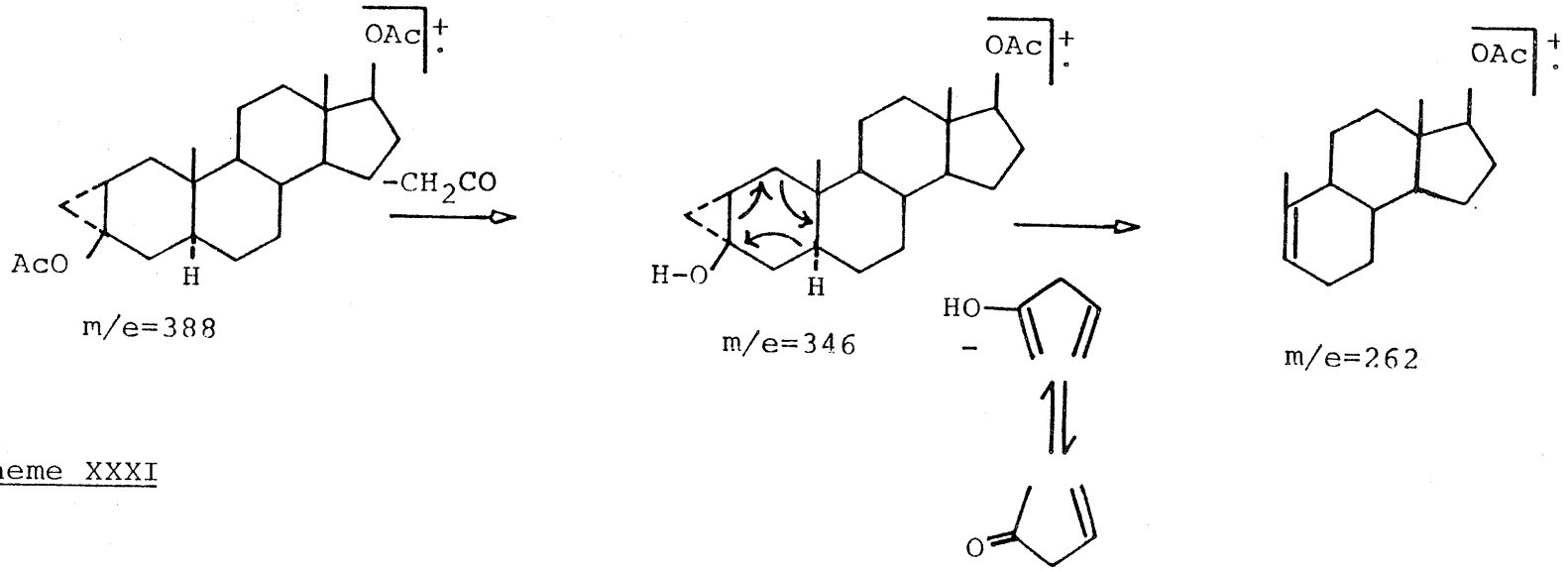
Scheme XXIX


OAc
Br
H
AcO
H
-Br·
m/e = 466,468
OAc
H
AcO
H
-CH2CO
m/e = 387
OAc
H
HO
H
m/e = 345
-HOAc
HO
H
H
m/e = 285
HO
H
H
m/e = 285
-H2O
m/e =267
m/e = 201

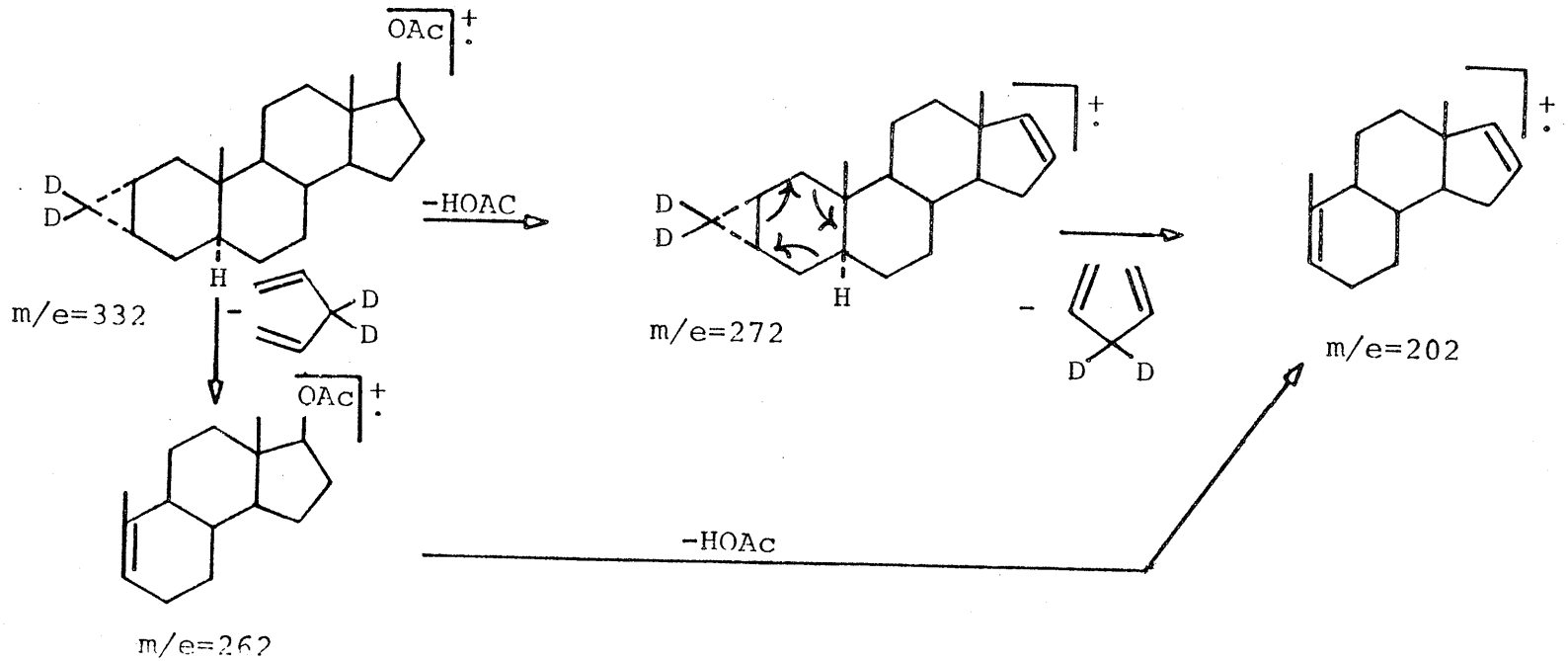
Scheme XXIX (Continued)



Scheme XXX



Scheme XXXI



arise through elimination of the A-ring by the retro-Diels-Alder mechanism (Scheme XXX).

The characteristic elimination of one of the acetate substituents as ketene in the mass spectra of the A-ring substituted cyclopropanosteroids 94, 97 and 114, can be attributed to the elimination of the 3β-acetoxy substituent. Mass spectral ketene elimination is a prominent feature in the mass spectra of phenylacetates[115]. In these compounds, as in the 3β-acetoxy substituted cyclopropanosteroids, the acetate substituent cannot be eliminated as acetic acid and is, therefore, eliminated as ketene. The 17β-acetoxy substituent, nevertheless, can be readily eliminated as acetic acid and this is experimentally observed.

I. Androgenic/Anabolic Activity and the Significance of Metabolic Transformation.

It is generally accepted that the anabolic and androgenic activity of 5α-androstane derivatives which do not possess an A-ring oxygen function exert their biological effect through metabolic conversion to 3-oxygenated derivatives more closely corresponding to the endogenous steroids[31,87]. Alternatively, the presence of a C-3 oxygen function is considered important for anabolic-androgenic activity. That the relationship of chemical structure to biological activity for these derivatives is more complex than indicated by this biotransformation follows from the activity of 1,4-seco-5α-androstan-17β-ol (82) which has anabolic and androgenic activity yet does not possess a C-3 atom[84]. A number of steroid compounds which have no A-ring oxygen function have been shown to undergo C-3 oxidation under *in vitro* and *in vivo* conditions[31,193,194,195,196,197]. 17β-hydroxy-17α-methyl-5α-androstane has been shown to form C-3 oxygen derivatives under *in vitro* conditions from which it has been concluded that metabolic activation of the hydrocarbon A-ring is responsible for its biological properties[197]. Wolff *et al.* has shown that this unoxygenated C-3 ring compound (82) is bound to a different portion of the rat ventral prostate from the endogenously active 5α-dihydrotestosterone[193]. It is not known whether this binding site is an alternative anabolic-androgenic receptor or possibly that 82

possesses selectivity between these properties. To obtain evidence to evaluate the contention that metabolic oxidation at C-3 is necessary for biological activity, the relative anabolic-androgenic activity of 3,3-deuterio-17β-hydroxy-5α-androstane-d_2 (125) and 17β-hydroxy-5α-androstane (126) were evaluated.

Biological tests were carried out by subcutaneous injection of compounds as suspensions in carboxymethyl cellulose solution to castrate rats for seven days with autopsy on the day after the last administration[198] (Table XXX). Local tissue reaction at the injection site was observed.

If metabolic activation is required for activity then substitution of the C-3 hydrogens with deuterium should, through the isotope effect[199,200,201], decrease the rate of oxidation at C-3. This decrease should then result in a diminished biological activity of the deuterated derivative. Surprisingly, the results of anabolic-androgenic tests on these substances indicated that the deuterated derivative is moderately more active than the unsubstituted analogue (Table XXX). Furthermore, the results also suggest that the deuterated compound demonstrates a more favourable separation between the anabolic and androgenic activity relative to the unsubstituted compound. Both substances, nevertheless, show relatively high biological activity. If the rate of metabolic oxidation at C-3 is decreased through deuterium substitution favouring alternative metabolic pathways, the results suggest that a more active metabolite may be formed. In vitro studies

of the metabolism of 17β-hydroxy-17α-methyl-5α-androstane show the formation of C-3 oxidized derivatives. Decreasing the rate of metabolism at both C-2 and C-3 appears to favour metabolism at C-4. C-4 oxygenated derivatives may show greater anabolic-androgenic activity[49]. Alternatively the unmetabolized compound may be more effective than the C-3 oxygenated derivatives. These results then suggest that the hydrocarbon receptor site is also effective in eliciting the anabolic-androgenic response, possibly with a relative increase of anabolic over androgenic affect.

The cyclopropanosteroid 2α,3α-cyclopropano-17β-hydroxy-5α-androstane (118) has been shown to have high myotropic and androgenic activity with a relative increase of myotropic over androgenic activity compared with testosterone[31]. The biological activity of this substance has been used to argue that an A-ring oxygen function is not required for myotropic/androgenic activity, and that the cyclopropane ring is sufficient to produce the desired changes. It has also been stated that this molecule should be resistant to metabolic oxidation but no supporting evidence was provided[31,202,203]. Recently, Templeton and Kim[180] have shown that in the rabbit, the metabolism of 2α,3α-cyclopropano-17β-hydroxy-5α-androstane (118) proceeds to give an A-ring C-2 oxygen containing isolate indicating that attack occurs on the cyclopropane ring[180].

The assumption that the cyclopropanosteroid 118 demonstrates decreased susceptibility to metabolic oxidation compared with the corresponding olefin and possibly the hydrocarbon is probably valid as this metabolite represented only a small percentage of the metabolites identified (6.5% of the urinary excretion products). That oxidative attack occurred at the C-2 rather than the C-3 position of the steroid A-ring is somewhat surprising since experiments with rabbit liver homogenate on 17β-hydroxy-17α-methyl-5α-androstane show that attack at C-3 of the saturated A-ring accounts for the isolated metabolic products[197]. Oral dosing of rabbits with 3-deoxyhydroepiandrosterone (androst-5-en-17-one) gave C-3 hydroxylated products as the major metabolites although some C-2 oxidation also occurred[194,195]. Similar results were obtained with 3-deoxyesterone[196]. C-3 oxygen substituted derivatives of 17β-hydroxy-2α,3α-cyclopropano-5α-androstane have been shown to have no appreciable myotropic/androgenic effectiveness in screening tests on castrated rats[198,204,205].

To test further the hypothesis that 17β-hydroxy-2α,3α-cyclopropano-5α-androstane possessed intrinsic activity, the 2β,4,4-d_3 deuterated derivative 121 was prepared with the view to decreasing the rate of metabolic oxidation at C-2 and C-4 through the deuterium isotope effect[199,200,201]; and thereby giving an increased activity to the molecule if the activity were due to the presence of the unaltered compound.

Comparison of the myotropic/androgenic activity of the hydrogen and deuterium compounds shows that the activity is halved on deuterium substitution (Table XXXI). Since the physical properties of the deuterated compound should not be significantly altered, it follows that the biological effect results from a C-2 and/or C-4 metabolite. 17β-hydroxy-17α-methyl-5α-androstan-2-one is essentially devoid of activity[206] and methyl substitution at C-3 is also expected to reduce activity[207], therefore, the C-4 metabolites appear to be the active form. This is substantiated by the relatively high myotropic/androgenic activity reported for 17β-hydroxy-2α,3α-cyclopropano-5α-androstan-4-one[208]. These results strongly suggest that the myotropic/androgenic effect of 17β-hydroxy-2α,3α-cyclopropano-5α-androstane is a result of metabolic activation through oxidation at C-4 and not due to the unoxygenated A-ring. Recent evidence has shown that some anabolic/androgenic A-ring unoxygenated androstane derivatives e.g. 17β-hydroxy-5α-androstane, bind to a different androgen receptor in rabbit ventral prostate tissue from 3-oxygenated compounds like the endogenous dihydrotestosterone[49,193]. Whether this binding site elicits the observed biological response is not known. The above evidence suggests that it may not.

TABLE XXX. Anabolic/androgenic assays of 5α-androstan-17β-ol derivatives.

Compound	Ventral Prostate	Seminal Vesicle	Levator Ani
Control[a,b]	21.1 ± 1.20	12.4 ± 0.35	32.5 ± 1.38
125	91.6 ± 4.20	39.7 ± 3.82	71.1 ± 3.63
126	68.6 ± 5.53	31.9 ± 2.07	64.6 ± 2.69

[a]Carboxymethylcellulose solution.

[b]The numbers indicated are organ weights in mg ± standard error.

TABLE XXXI. Anabolic/androgenic assays of 2α,3α-cyclopropano-5α-androgstan-17β-ol derivatives.

Compound	Ventral Prostate	Seminal Vesicle	Levator Ani
Control[a,b]	15.4 ± 0.87	12.2 ± 0.44	33.5 ± 2.61
Testosterone	51.6 ± 9.41	19.9 ± 8.94	80.8 ± 3.28
121	39.8 ± 2.45	31.6 ± 1.30	58.1 ± 3.26
118	72.6 ± 6.69	42.1 ± 3.42	77.6 ± 3.56

[a]Carboxymethylcellulose solution.

[b]The numbers indicated are organ weights in mg ± standard error.

III. EXPERIMENTAL

Unless otherwise stated, the following instruments and procedures were used.

Melting points (mp) 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 1.0% w/v) in a 1 dm tube at 23°C. Infra-red (ir) spectra were determined on a Perkin-Elmer (Model 267) Grating Infra-red Spectrophotometer. Proton magnetic resonance spectra were determined on a Varian Anaspect (EM 360) NMR Spectrometer: 220 MHz spectra were recorded on a Varian HR 220 instrument by the Canadian 220 MHz NMR Centre, Ontario Research Foundation, Sheridan Park, Ontario; tetramethyl silane (TMS) was used as an internal standard unless indicated otherwise. Mass spectra (ms) were recorded on a Finnigan Quadrupole (Model 1015) instrument at 70 eV using a direct probe method by Mr. Wayne Buchannon, Chemistry Department, University of Manitoba. The mass spectrometer was operated at an accelerating voltage of 70 eV. Elemental analyses were performed by Mr. G. Crouch, School of Pharmacy, University of London, England.

Petroleum ether refers to the fraction boiling within the range of 60-80°C. Thin layer chromatography (t.l.c.) was carried out on silica gel coated (0.50 mm) glass plates and developed with the following solvent systems: (I) 10% ethyl acetate/petroleum ether; (II) 10% ethyl acetate/petroleum ether and silver nitrate (10% w/v solution) impregnated silica gel

coated glass plates; (III) 25% ethyl acetate/petroleum ether; (IV) 25% ethyl acetate/petroleum ether and silver nitrate impregnated silica gel coated glass plates; (V) 50% ethyl acetate/petroleum ether; (VI) ethyl acetate visualized by spraying with 4% v/v concentrated sulfuric acid in ethanol followed by heating at approximately 115°C for fifteen to thirty minutes.

Acetylation: To a solution (10% w/v) 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 diluted sulfuric acid and extracted with ether.

Zinc/copper couple: A homogeneous, consistently active zinc/copper couple was prepared by either Method I or Method II as outlined below.

Method I: Analytical grade, finely powdered zinc (6.57 g, 100 mmole) and cuprous chloride (9.90 g. 100 mmole) were successively added to vigorously stirred anhydrous ether (25 ml), maintained under a nitrogen atmosphere. This mixture was refluxed for thirty to forty minutes. The couple was ready for use when the mixture had taken on a reddish-brown hue.

Method II: To vigorously stirred, boiling glacial acetic acid (200 ml) was cautiously added, in two portions, a homogeneous triturate of analytical grade zinc (90.6 g, 1.4 mmole) and cupric acetate monohydrate (5.6 g, 31 mmole). The resultant mixture was used directly.

Simmons-Smith Reaction with the Unsaturated Steroid: To a vigorously stirred, freshly prepared, refluxing zinc/copper couple in ether (Method I) was added in two portions, dry methylene iodide (10 molar excess with respect to substrate) dissolved in anhydrous ether (approximately 75% w/v). The exothermic reaction was allowed to subside and the reaction mixture was refluxed for an additional ten to fifteen minutes. Olefinic substrate was added as a solution (approximately 15% w/v) in anhydrous ether.

2α,3-Dibromocyclopropano-5α-androstane-3β,17β-diol diacetate (94)

To a magnetically stirred refluxing solution of cetyltrimethylammonium bromide (2.0 g) in aqueous sodium hydroxide (50% w/v) (100 ml), was added, drop-wise, a solution of 5α-androst-2-ene-3,17β-diol diacetate (93) (4.6 g) in tribromethane (25 ml). After 15 minutes, and lasting approximately 20 minutes, the exothermic reaction began to boil vigorously. After 60 minutes, a dark gummy semi-solid material, showing two major spots on t.l.c. (System III), had precipitated from the aqueous solution. The black precipitate was dissolved in chloroform and poured into brine to avoid emulsion formation. The reaction mixture was neutralized with 6N-sulfuric acid and the chloroform layer was successively washed with brine to avoid emulsion formation and dried over sodium sulfate. Filtration of the organic phase followed by evaporation at reduced pressure gave a dark gummy residue which was dissolved in benzene and filtered over aluminum oxide (270 g).

Elution with benzene (700 ml) gave a crystalline material (4.0 g) which after recrystallization from warm acetone gave 2α,3-dibromocyclopropano-5α-androstane-3β,17β-diol diacetate (94) (1.32 g), mp 199-200°C; $[\alpha]_D$ 23°+20°(0.9945% w/v); ir ν_{max} (CCl_4): 1765 (3β-acetoxy C=0), 1735 (17β-acetoxy C=0) cm^{-1}; pmr ($CDCl_3$): δ4.585 (1, t, J = 8.5 Hz, 17α-proton), 2.116 (2, s, 3β-acetoxy methyl protons), 2.050 (3, s, 17β-acetoxy methyl protons), 0.816 (3, s, 10β-methyl), 0.786 (3, s, 13β-methyl), 0.663 (1, sextet, J = 4.0 Hz, J = 12.0 Hz) ppm; ms m/e 502, 504, 506 (M^+ - ketene), 423, 425 (M^+ - ketene - Br), 363, 365 M^+ - (ketene + Br) - HOAc , 363, 365 M^+ - (ketene + Br + HOAc) - Br , 283 M^+ - (ketene + Br + HOAc + HBr) , 201 M^+ - (ketene + Br + HOAc + HBr) - 82 . Anal. calcd. for $C_{24}H_{34}Br_2O_4$: C 52.76, H 6.27, Br 29.25; found: C 53.04, H 6.363, Br 29.33.

2α,3-(endo)-Bromocyclopropano-5α-androstane-3β,17β-diol diacetate (97)

(a) To vigorously stirred anhydrous ether (10 ml), under a nitrogen atmosphere, was added zinc (3.27 g = 50 mmole) and curprous chloride (4.91 g = 50 mmole). After refluxing the heterogeneous mixture for 30 minutes, 2α,3-dibromocyclopropano-5α-androstane-3β,17β-diol diacetate (94) (546 mg) was added. After four and one-half hours, the reaction was poured into an ice-cold saturated sodium bicarbonate and extracted with ether. The ether phase was washed with water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at

reduced pressure gave a crystalline material (344 mg) which after recrystallization from ether/methanol gave 2α,3-(endo)-bromocyclopropano-5α-androstane-3β,17β-diol diacetate (97) (143 mg); mp 165-166°C; ir ν_{max} (CCl_4); 1752 (17β-acetoxy C=0), 1735 (3β-acetoxy C=0) cm^{-1}; pmr ($CDCl_3$): δ4.580 (1, t, $J_{16\alpha,17\alpha} = J_{16\beta,17\alpha} = 7.5$ Hz, 17α-proton), 3.364 (1, d, $J_{cis} = 8.8$ Hz, exo-cyclopropane proton), 2.027 (3, s, 3β-acetoxy methyl protons), 1.972 (3, s, 17β-acetoxy methyl protons), 0.882 (3, s, 10β-methyl protons), 0.772 (3, s, 13β-methyl protons), 0.659 (1, sextet) ppm; ms m/e 387 (M^+ - Br), 345 (M^+ - Br - ketene), 285 M^+ - (Br + ketene) - HOAc , 201 M^+ - (Br + ketene + HOAc + H_2O) - 66 . Anal. calcd. for $C_{24}H_{35}O_4Br$: C 61.66, H 7.55; found: C 61.87, H 7.586.

(b) To a solution of 2α,3-dibromocyclopropano-5α-androstane-3β,17β-diol diacetate (94) (292 mg) in dry dioxane (30 ml) and triethylamine (0.2 ml) was added two teaspoonful of Raney nickel which had been stored under ethanol. The heterogeneous mixture was vigorously stirred under a hydrogen atmosphere for 32 hours after which time the catalyst was filtered off and cautiously washed with diethyl ether. The ether filtrate was washed with dilute hydrochloric acid, distilled water and brine. After drying over magnesium sulfate, the organic phase was filtered and evaporated at reduced pressure to give a crystalline material (106 mg), mp 160-161°C. Recrystallization from hot methanol gave 2α,3-(endo)-bromocyclopropano-5α-androstane -3β,17β-diol diacetate (97), mp 162-162.5°C; ir ν_{max} (KBr): 3070 (cyclopropyl C-H), 1740

(3β-acetoxy C=0), 1734 (17β-acetoxy C=0) cm^{-1}; pmr ($CDCl_3$): δ4.495 (1, t, J = 8 Hz, 17α-proton), 3.289 (1, d, J_{cis} = 10 Hz, exo-cyclopropane proton), 1.961, 1.909 (3, s, 3β- and 17β-acetoxy methyl protons), 0.818, 0.716 (3, s, 10β- and 13β-methyl protons), 0.591 (1, sextet) ppm; ms m/e 387 (M^+ - Br), 345 (M^+ = Br - ketene), 285 M^+ - (Br + ketene) - HOAc , 267 M^+ - (Br + ketene + HOAc) - H_2O , 201 M^+ - (Br + ketene + HOAc + H_2O) - 66 .

A-Homo-3-bromo-5α-androst-2-ene-4ξ-17β-diol diacetate (103)

To magnetically stirred, refluxing anhydrous ether (15 ml), maintained under a nitrogen atmosphere, was added 2α,3-dibromocyclopropano-5α-androstane-3β,17β-diol diacetate (94) (55 mg) and lithium aluminum hydride (525 mg). After 40 hours reflux, excess metal hydride was neutralized with ethyl acetate. The reaction mixture was poured into water and extracted with ether. The ether phase was washed with dilute sulfuric acid, distilled water and brine and dried over anhydrous sodium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a crystalline material (442 mg) which was acetylated and chromatographed over aluminum oxide. A crystalline material (131 mg) which was eluted with petroleum ether ($60^o/90^o$) was identified as A-homo-3-bromo-5α-androst-2-ene-4ξ,17β-diol diacetate (103), mp 155-156.5°C; ir ν_{max} (CCl_4) 1740, 1733 (ester C=0), 1635 (olefinic C=C), 1372, 1365 (ester C=0) cm^{-1}; pmr ($CDCl_3$) δ 6.132 (1, t, $J_{2,1\alpha}$ = 10 Hz, $J_{2,1\beta}$ = 6 Hz, $J_{allylic}$ = 2.0 Hz, 2-proton), 5.489 (1, d, J_{cis} = 11 Hz, J_{trans} = 3.5 Hz,

$J_{allylic}$ = 1.9 Hz, 4ξ-proton), 4.586 (1, t, $J_{16\alpha,17\alpha}$ = 8.5 Hz, $J_{16\beta,17\alpha}$ = 7.5 Hz 17α-proton), 2.125 (3, s, 4ξ-acetoxy methyl protons), 2.030 (3, s, 17β-acetoxy methyl protons), 0.773, 0.752 (3, s, 10β- and 13β-methyl protons) ppm; ms m/e 404, 406 (M^+ - ketene), 387 (M^+ - Br), 327 (M^+ - Br - HOAc), 267 (M^+ - Br - 2 HOAc), 201 (M^+ - Br - 2HOAc - 66). Anal. calcd. for $C_{24}H_{35}O_4Br$: C 61.67, H 7.55, Br 17.10; found: C 62.47, H 7.54, Br 17.46.

2α,3-Cyclopropano-5α-androstane-3β,17β-diol diacetate (114)

To a freshly prepared, vigorously stirred, refluxing zinc/copper couple was added, in two portions, methylene iodide (2.4 ml) dissolved in anhydrous ether (2.0 ml). The exothermic reaction was allowed to subside and 5α-androst-2-ene-3,17β-diol diacetate (92) (1.05 g) dissolved in anhydrous either (15.0 ml) was added. The mixture was refluxed for 24 hours. At the end of this time period t.l.c. (System II) indicated two major spots. The faster eluting fraction corresponded to the starting material. The mixture was again treated with a ten molar excess of the Simmons-Smith reagent. After 19 hours reflux t.l.c. (System II) indicated no major changes. The reaction was allowed to cool, poured into water and extracted with ether. The ether phase was washed with saturated sodium bicarbonate solution, water, brine and dried over sodium sulfate. Filtration of the organic phase followed by evaporation at reduced pressure gave a gummy residue, which, after chromatography over aluminum oxide (35 g) (elution with 25% benzene/petroleum ether $60^{\circ}/90^{\circ}$) gave a crystal-

line material (87 mg). Recrystallization from hot ether gave 2α,3-cyclopropano-5α-androstane-3β,17β-diol diacetate (114) (36 mg), mp 163-164°C; ir (KBr): 3075 (cyclopropane C-H), 1738, 1732 (ester C=0), 1440 (ester C=0) cm^{-1}; pmr ($CDCl_c$): δ4.566 (1, t, $J_{16\alpha,17\alpha} = J_{16\beta,17\alpha}$ = 8.0 Hz, 17α-proton), 2.023 (3, s 3β-acetoxy methyl protons), 1.962 (3, s, 17β-acetoxy methyl protons), 0.914 (3, s, 10β-methyl protons), 0.764 (3, s, 13β-methyl protons), 0.514 (1, sextet, J = 12 Hz, J = 12 Hz, J = 5 Hz), 0.462 (1, t, $J_{\underline{gem}} = J_{\underline{cis}}$ = 6.0 Hz, <u>endo</u>-cyclopropane proton) ppm; ms m/e 388 (M^+), 346 (M^+ - ketene), 262 (M^+ - ketene - 84).

<u>3-Trimethylsilyloxy-5α-androst-2-en-17β-yl acetate (115)</u>

To triethylamine (15.0 ml), previously distilled from calcium hydride, under a nitrogen atmosphere was added freshly distilled dimethylformamide and 17β-acetoxy-5α-androstane-3-one (92) (1.0 g). The solution was heated to reflux in an oil bath and trimethylsilylchloride (7.0 ml) was added. After 3 hours reflux, the reaction mixture was poured into water and extracted with hexane. The organic phase was washed with distilled water and brine and dried over anhydrous sodium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a crystalline material (1.04 g). Recrystallization from warm methylene chloride/methanol gave 3-trimethylsilyoxy-5α-androst-2-en-17β-yl acetate (115) (325 mg), mp 98-103°C; ir ν_{max} (CCl_4): 1733 (ester C=0) 1670 (olefinic C=C); pmr (CCl_4): δ4.44 (1, q, $J_{2,1\beta} = J_{2,1\alpha}$ = 2.0 Hz, 2-proton), 4.24

(1, t, $J_{16\alpha,17\alpha} = J_{16\beta,17\alpha} = 8.0$ Hz, 17α- proton, 1.81 (3, s, 17β - acetoxy methyl protons), 0.63 (6, s, 10β- and 13β-methyl protons), 0 (9, s, trimethylsilyloxy methyl protons) ppm; ms m/e 404 (M^+), 262 (M^+ - 142), 202 (M^+ - 142 - HOAc). Anal. calcd. for $C_{24}H_{40}O_3Si$: C 71.24, H 9.96; found: C 71.23, H 10.01.

Treatment of 3-trimethylsilyloxy-5α-androst-2-en-17β-yl-acetate (115) with the Simmons-Smith reagent.

To a vigorously stirred zinc/copper couple, refluxing in anhydrous ether (25.0 ml) under a nitrogen atmosphere, was added in two portions, dry methylene iodide (4.0 ml), dissolved in anhydrous ether (6.0 ml). The exothermic reaction was allowed to subside and 3-trimethylsilyloxy-5α-androst-2-en-17β-yl acetate (115) (1.5 g), dissolved in anhydrous ether (15.0 ml), was added. After 29 hours reflux, t.l.c. (System IV) showed three major spots, the faster eluting fraction corresponding to starting material. The reaction was poured into water and extracted with ether. The ether phase was neutralized, washed with brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a gummy residue: ir ν_{max} (CCl_4): 3063 (cyclopropyl C-H), 1737 (17β-acetoxy C=0); pmr ($CDCl_3$): δ4.55 (1, q, J = 7.5 Hz, 17 -proton), 1.989 (3, s, 17β-acetoxy methyl protons), 0.800, 0.766 (10β- and 13β-methyl protons), 0.402 (2, m), 0.05 (2, m) ppm. Chromatography over aluminum oxide (70 g) gave no clearly defined products or crystalline fractions.

2,4,4-Deuterio-17β-acetoxy-5α-androst-2-ene-d_3 (119)

To a magnetically stirred solution of deuterium oxide (26 ml) in dry dioxane (100 ml) was added sodium metal (6.2 g). The exothermic reaction was allowed to subside and a solution of 17β-acetoxy-5α-androstan-3-one (92) (5.8 g) in dry dioxane was added. The solution was refluxed for 2.5 hr., cooled and poured in dilute hydrochloric acid. The reaction was extracted with ether. The ether was washed with distilled water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a crystalline product (4.4 g); (d_4 = 22%, d_3 = 43%, d_2 = 30%, d_1 = 3%, d_o = 2%).

The crystalline product (4.4 g) was immediately dissolved in methylene chloride and treated with one equivalent of bromine in methylene chloride. The reaction was complete within 10 minutes as indicated by the disappearance of the reddish-brown colour. The reaction solution was poured into saturated aqueous sodium bicarbonate and extracted with methylene chloride. The organic phase was washed with water and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase a reduced pressure gave a crystalline product which was immediately treated with sodium borohydride (3.74 g) in dry dioxane (185 ml) containing deuterium oxide (8 ml). After 10 hours, the reaction was poured into distilled water and extracted with ether. The ether phase was washed with water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evapora-

tion of the organic phase gave a white crystalline material (4.4 g) identified as an epimeric mixture of 2ξ-bromo-2ξ,4,4-deuterio-17β-acetoxy-5α-androstane 3α- and 3β-alcohols: pmr ($CDCl_3$): δ4.56 (1, 5, J = 8 Hz, 17α-proton), 3.99 (broad, methine proton), 2.03 (1, s, 17β-acetoxy methyl protons), 0.88 (3, s, 10β-methyl protons), 0.79 (3, s, 13β-methyl protons) ppm; ms m/e 413, 414, 415 (M^+), 353, 354, 355, 356, (M^+-HOAc). The epimeric mixture of bromoalcohols was not purified further and was immediately dissolved in glacial acetic acid (25 ml) and treated with the zinc/copper couple (Zn = 90.6 g; $Cu(OAc)_2 \cdot H_2O$ = 5.6 g) (Method II) for 24 hr. The reaction mixture was poured into water, extracted with ether and neutralized with saturated aqueous sodium bicarbonate solution. The ether phase was washed with brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a crude reaction product which was dissolved in benzene and passed through an ethyl acetate washed aluminum oxide column (170 g). Identical fractions were combined (t.l.c, System III). Evaporation of the benzene at reduced pressure gave a crystalline product (2.5 g) which, after recrystallization from ether/methanol, gave 2,4,4-deuterio-17β-acetoxy-5α-androst-2-ene-d_3 (119) (1.3 g), mp 95-98°C (literature 97-98°C[180]); ir ν_{max} (CCl_4): 3024 (olefinic C-H), 1735 (ester C=0), 1642 (C=C) cm^{-1}; pmr ($CDCl_3$): δ5.61 (1, s, broad, C - 3 proton), 4.60 (1, q, J = 7 Hz, 17α-proton), 2.04 (3, s, 17β-acetoxy methyl protons), 0.80 (3, s, 10β-methyl protons), 0.77 (3, s, 13β- methyl protons), ppm; ms (d_3 = 35%, d_2 = 40%,

d_1 = 21%, d_o = 4%).

2α,3α-Cyclopropano-2β,4,4-deuterio-5α-androstan-17β-yl-acetate-d_3 (120)

To a refluxing zinc/copper couple (Zn = 2.6 g, CuCl = 3.92 g) (Method I) was added, in two portions, dry methylene diiodide (1.6 ml) dissolved in anhydrous ether (2.0 ml). The exothermic reaction was allowed to subside and 2,4,4-deuterio-17β-acetoxy-5α-androst-2-ene-d_3 (119) (650 mg), dissolved in anhydrous ether (3.0 ml) was added. The heterogeneous mixture was allowed to reflux for 8 hours and then poured into water and extracted with ether. The ether phase was washed with water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a crystalline material (790 mg) which after recrystallization from hot ether/methanol gave 2α,3α-cyclopropano-2β,4,4-deuterio-17β-acetoxy-5α-androstane-d_3 (120) (130 mg), pm 102-103°C (literature 105-106°C)[31]; pmr ($CDCl_3$): δ4.545 (1, t, J = 7.5 Hz, 17α-proton), 2.039 (3, s, 17β-acetoxy methyl protons), 0.793 (3, s, 10β-methyl protons), 0.770 (3, s, 13β-methyl protons), 0.525 (2, heptet), -0.175 (1, t, $J_{trans} = J_{gem}$ = 5 Hz, endo-cyclopropane proton).

2α,3α-Cyclopropano-2β,4,4-deuterio-17β-hydroxy-5α-androstane-d_3 (121)

A methanolic potassium hydroxide solution (2% w/v) (4.0 ml) of 2α,3α-cyclopropano-2β,4,4-deuterio-17β-acetoxy-5α-androstane-d_3 (120) (200 mg) was refluxed for 1.5 hr. After

cooling, the reaction solution was poured into dilute sulfuric acid and extracted with ether. The ether extract was washed with water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic solvent at reduced pressure gave a product which after crystallation from hot ether/methanol gave 2α,3α-cyclopropano-2β,4,4-deuterio-17β-hydroxy-5α-androstane-d_3 (121) (150 mg), mp 128-129°C (literature 129-130°C[180]); ms (d_3 = 31%, d_2 = 43%, d_1 = 21%, d_o = 4%).

17β-Acetoxy-2α,3α-deuteriocyclopropano-5α-androstane-d_2 (123)

To a magnetically stirred, refluxing zinc/copper couple (Zn = 1.2 g, CuCl = 18. g) (Method I) was added in one portion diiodomethane-d_2 (0.6 mg) dissolved in anhydrous ether (1.5 ml). The exothermic reaction was allowed to subside and 17β-acetoxy-5α-androst-2-ene (122) dissolved in anhydrous ether (1.0 ml) was added. After 5 and 7.5 hrs., t.l.c. (System II) showed two fractions, the slower eluting fraction corresponding to starting material. After 21 hrs., the reaction mixture was cooled, diluted with ether and filtered over celite at reduced pressure. The filtrate was poured into ice-cold saturated sodium bicarbonate and extracted with ether. The ether phase was washed with water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a gummy residue (270 mg) which was dissolved in chloroform and treated with ozone. The crude product of ozonolysis, which showed two fractions on t.l.c. (System I), was dissol-

ved in benzene and passed through an aluminum oxide column. The first two fractions, which contained all the material (96 mg), were crystalline. Recrystallization of these two combined fractions from hot methanol gave 17β-acetoxy-2α,3α-deuteriocyclopropano-5α-androstane-d_2 (123) (41 mg), mp 99-101.5°C (literature 97-98°C[31]); ir ν_{max} (CCl_4): 3065 (cyclopropyl C-H), 1732 (17β-acetoxy C=0) cm^{-1}; pmr ($CDCl_3$): δ4.545 (1, t, J = 8.0 Hz, 17α-proton), 2.003 (17β-acetoxy methyl protons), 0.753 (10β-methyl protons), 0.730 (13β-methyl protons), 0.730 (13β-methyl protons), 0.466 (1, sextet) ppm; ms m/e 332 (M^+), 272 (M^+ - HOAc), 262 (M^+ - HOAc - 74), 257 M^+ - (HOAc + 74) - CH_3, 202 M^+ - (HOAc - 74 - CH_3) - 55 (98% d_2).

3-(p-Toluenesulfonylhydrazono)-17β-hydroxy-5α-androstane (124)

A magnetically stirred solution of 17β-hydroxy-5α-androstan-3-one (81) (1.5 g) and p-toluenesulfonylhydrazine (990 mg) in methanol (30 ml) was heated to 60°C. After 15 minutes the reaction was removed from the heat and the methanol was evaporated at reduced pressure leaving a yellow crystalline material. Recrystallization of this material from hot benzene/methanol gave white crystals of 3-(p-toluenesulfonylhydrazono)-17β-hydroxy-5α-androstane (124) (2.6 g); ir ν_{max} ($CHCl_3$): 3610 (free O-H), 3290, 3210 (hydrazonyl N-H), 1603 (C=N), 1384, 1157 (O=S=O) cm^{-1}.

3,3-Deuterio-17β-hydroxy-5α-androstane-d_2 (125)

A magnetically stirred mixture of the tosylhydrazone (124) (880 mg) and lithium aluminum deuteride (1.0 g) in dry dioxane (30 ml) was refluxed for 2 hours. After this time, the reaction was removed from the heat and deuterium oxide (3.5 ml) was cautiously added over a period of 10 minutes. The reaction mixture was then refluxed for an additional 5 minutes, allowed to cool, diluted with ether and filtered at reduced pressure. The filtrate was washed with water, brine and dried over anhydrous magnesium sulphate. Filtration followed by evaporation of the organic phase at reduced pressure gave a white crystalline material (332 g) that showed four fractions on t.l.c. (R_f = 0.40, 0.11, 0.05, 0.00) (System III). The crude product was dissolved in warm benzene and passed through an aluminum oxide column (35 g). After 650 ml of benzene had been collected, the alcohol had been completely eluted (t.l.c.). Identical fractions were combined to give 3,3-deuterio-17β-hydroxy-5α-androstane-d_2 (125) (161 mg), mp 165-166°C (literature 163-167°C[209]); ms m/e 278 (M^+) (98% isotopic purity).

17β-Hydroxy-5α-androstane (126)

A solution of high grade ethylene glycol (75 ml), potassium hydroxide (8 g), 17β-hydroxy-5α-androstan-3-one (81) (10 g) and hydrazine monhydrate (5 ml) was refluxed for 60 minutes. The reaction temperature was then raised to 200°C by distillation and reflux was continued for an additional 2.5

hrs. The hydrazone was decomposed by the addition of distilled water and the white solid precipitate which formed was dissolved in ether. The ether phase was washed with dilute sulfuric acid, brine and dried over magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a crystalline material (7.7 g) identified as 17β-hydroxy-5α-androstane (126), mp 166-167°C (literature 163-167°C[209]); ir ν_{max} ($CHCl_3$): 3617 (free O-H).

Attempted synthesis of 2β,4-cyclo-5α-androst-5-ene-3ξ, 17β-diol diacetate (132)

To a magnetically stirred solution of 17β-acetoxy-5α-androst-4-en-3-one (127) (1.60 g) in methylene chloride was added two equivalents of liquid bromine dissolved in methylene chloride (50 ml). After the reddish-brown solution had cleared (approximately 10 minutes), the reaction solution was poured into ice-water and extracted with methylene chloride. The methylene chloride was washed with sodium bicarbonate solution (10 meq), water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a crude mixture of 128 (2.6 g) ir (CCl_4): 1735 (17β-acetoxy C=O), 1695 (C-3 ketone C=O), 1620 (conjugated C=C) cm^{-1}. The epimeric mixture of dibromosteroids was immediately solubilized in absolute ethanol and added to a slurry of sodium borohydride in absolute ethanol (5.0 ml). The reaction effervesced and after 10 minutes, the reaction mixture was poured into ice-cold diluted sulfuric acid. The reaction mixture was extracted with methy-

lene chloride. The organic phase was washed with water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a gummy mixture (2.37 g): ir (CCl_4): 3580 (free O-H), 3020 (olefinic C-H), 1738 (17β-acetoxy C=O), 1680 (C=C) cm^{-1}. The epimeric mixture of dibromo alcohols (2.37 g) was dissolved in anhydrous ether (5.0 ml) and transferred to a reaction flask, at 25°C, in which had been previously prepared a zinc/copper couple (Zn = 14.7 g; CuCl = 22 g) (Method I) in anhydrous ether (45 ml) under a nitrogen atmosphere. After 2.5 hr., the reaction mixture showed three fractions on t.l.c. (System III) (R_f = 0.52, 0.24 and 0.13). The reaction mixture was diluted with ether and filtered at the pump. The ether filtrate was washed with ice-water and brine and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the organic phase at reduced pressure gave a gummy residue (1.37 g): ir (CCl_4): 3020 (olefinic C-H), 1736 (17 -acetoxy C=O), 1680 (six membered ring ketone C=O), 1620 (conjugated C=C). No changes in the ir spectrum were observed after acetylation of the crude reaction mixture. Chromotography of the crude acetylated reaction mixture over ethyl acetate washed aluminum oxide (82 g) gave no clearly defined products or crystalline fractions.

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V. APPENDICES

APPENDIX I

Table of substituent constant (π) values[5b].

Substituent	$\pi^{a,b}$
-F	-0.17
-Cl	+0.39
-Br	+0.60
-I	+1.00
-OH	-1.16
-COOH	-0.65
$-COOCH_3$	-0.27
-C=O	-1.21
-O-	-0.98
-CN	-0.84
$-CH_2-$	+0.50
-HC:CH-	+0.73
-HC:CH-CH:CH-	+1.36
-C:C-	+0.44

[a]The substituent partitient coefficient of the hydrogen atom is zero.

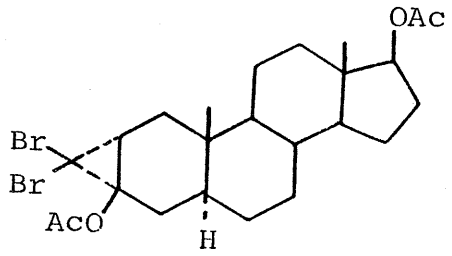
[b]The unsaturated substituent constant values are relative to the carbon-carbon single bond.

APPENDIX II

List of abbreviations.

br	broad
h	heptet
m	multiplet
q	quartet
s	singlet
sx	sextet
t	triplet

APPENDIX III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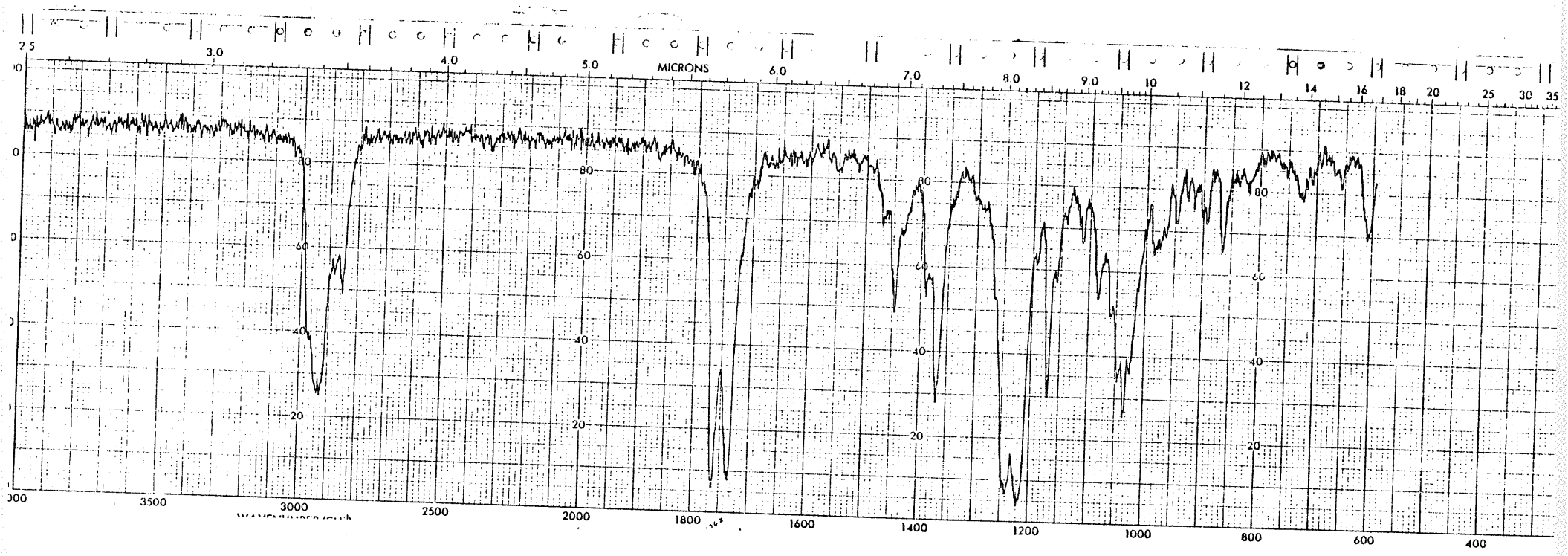
94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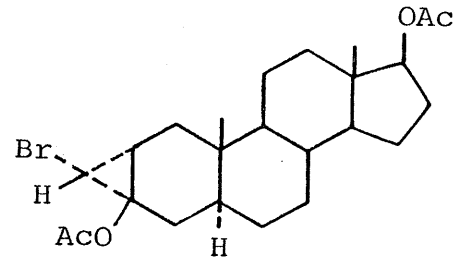
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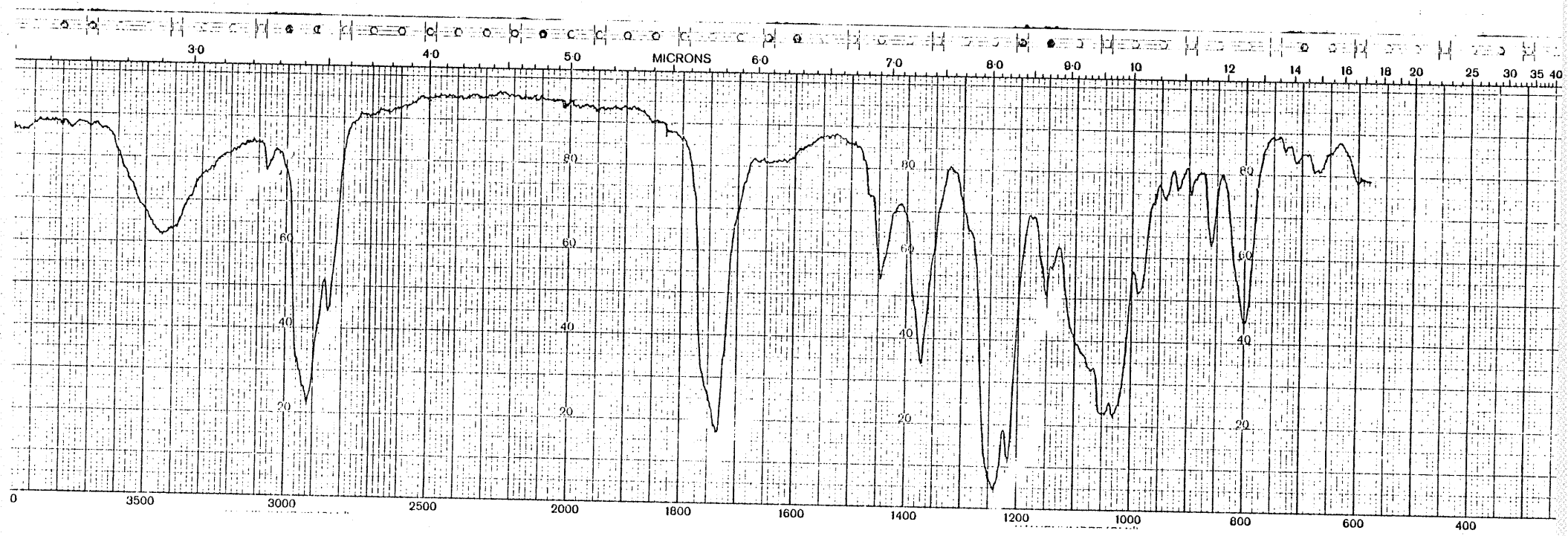
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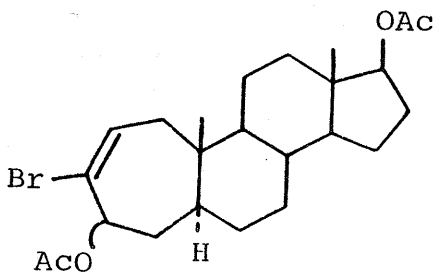
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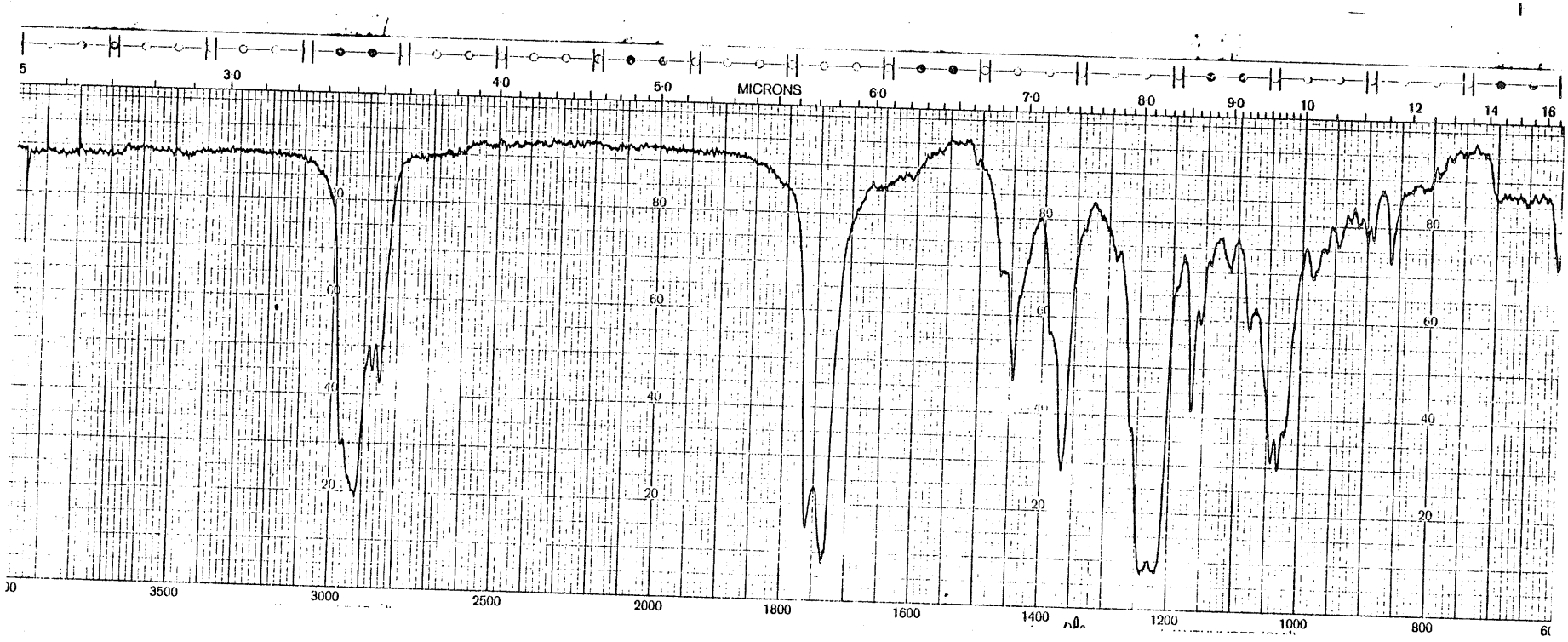
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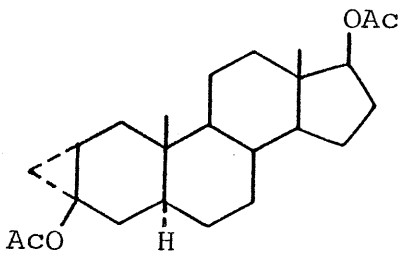
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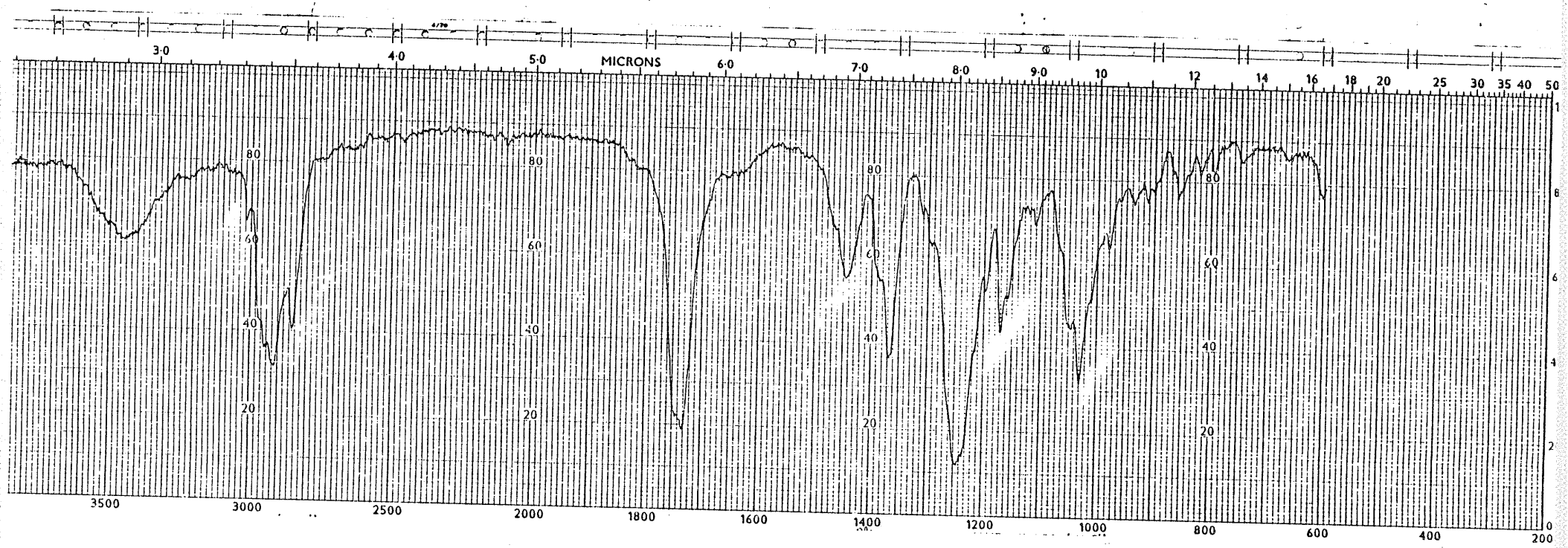
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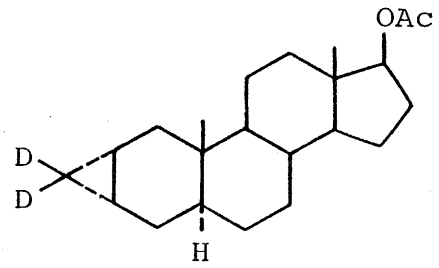

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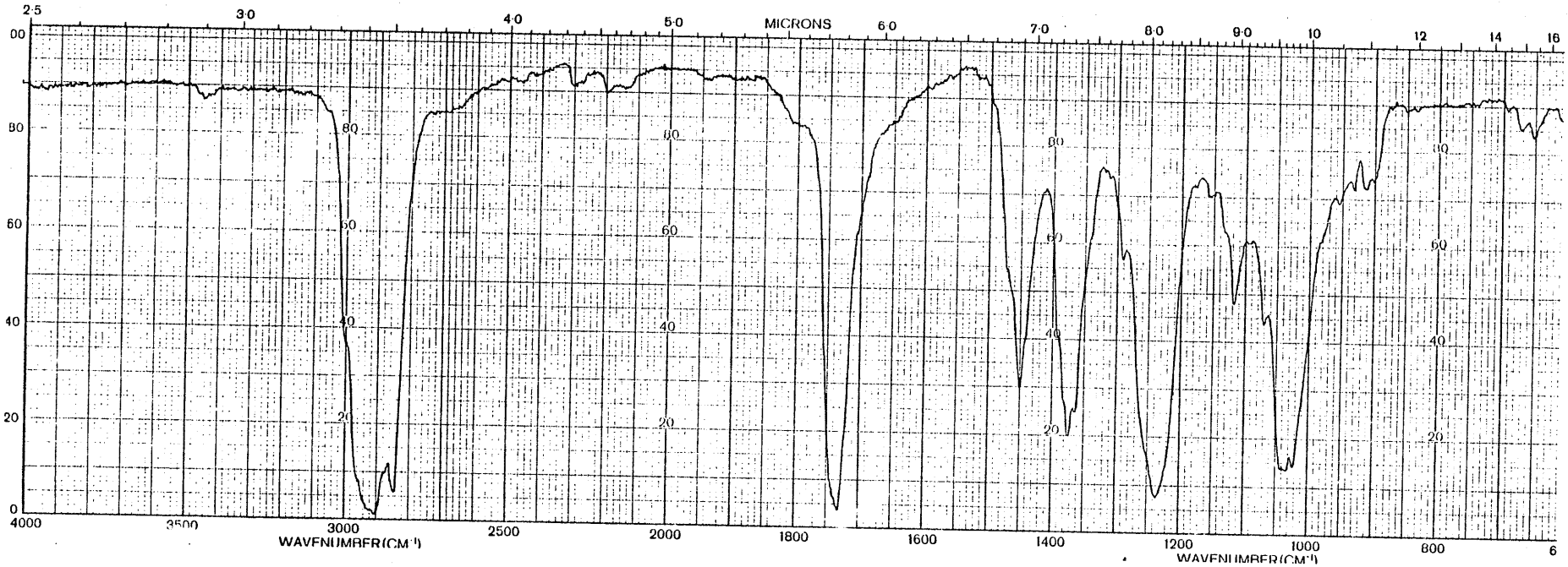

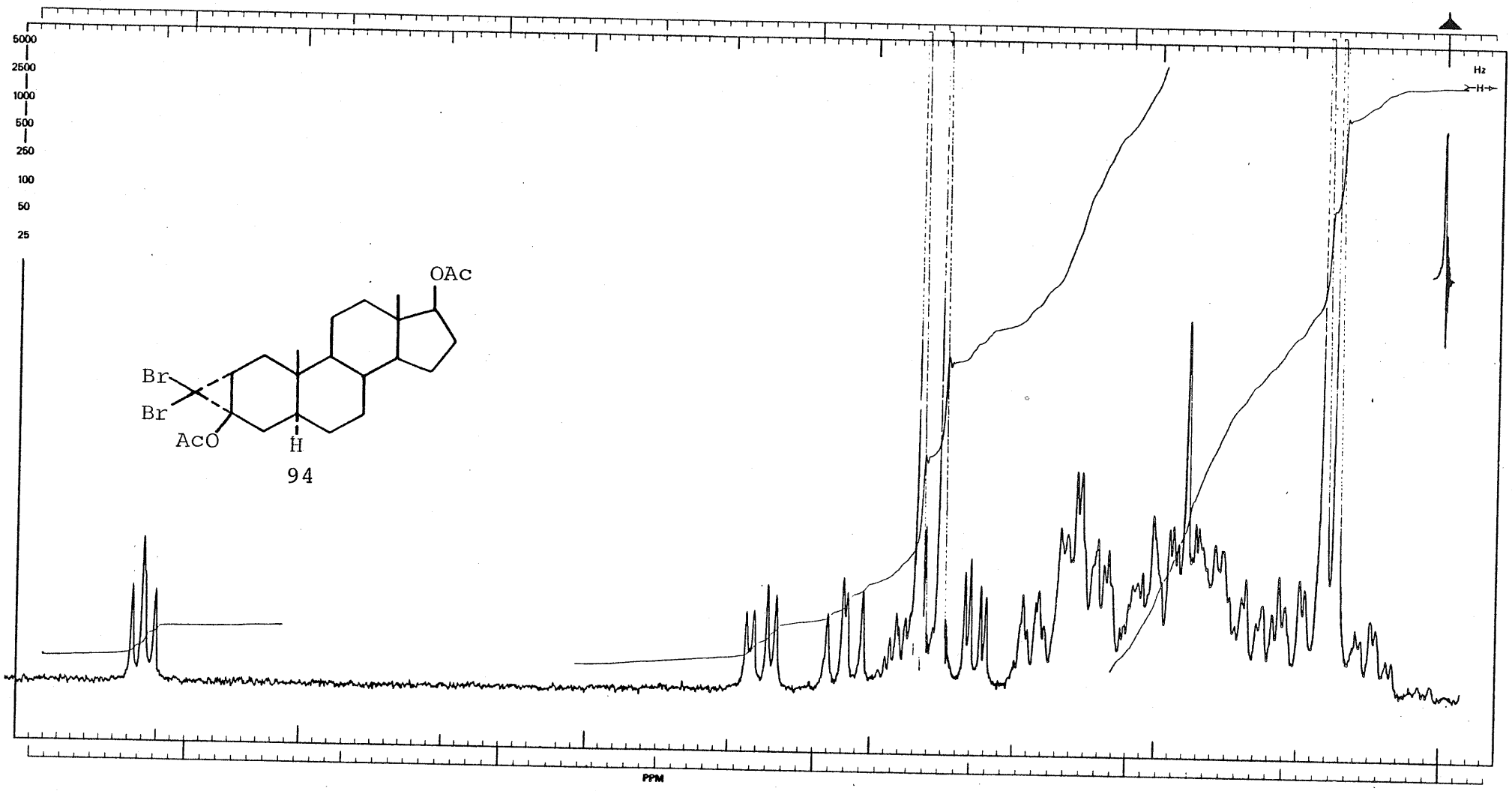
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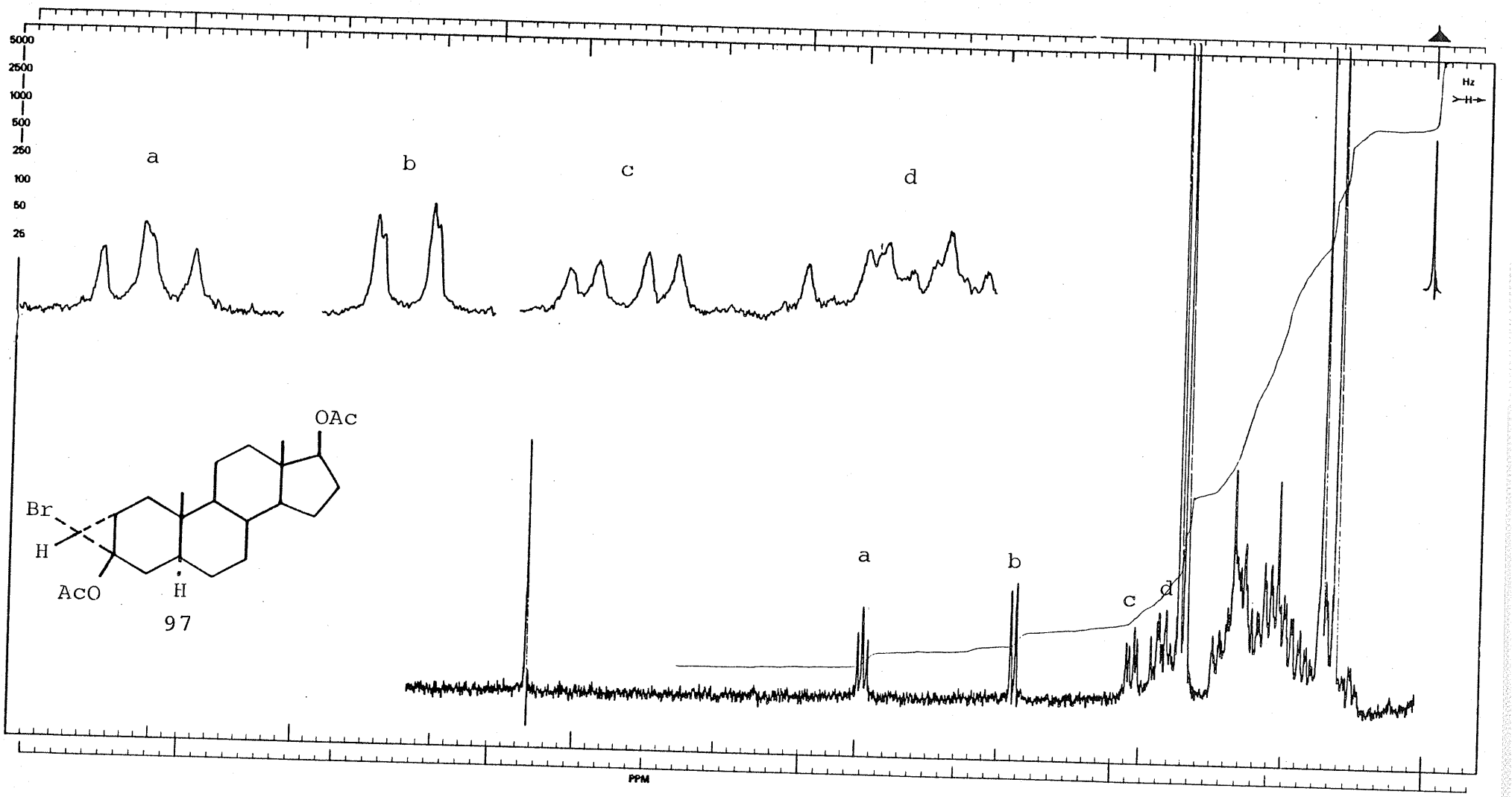
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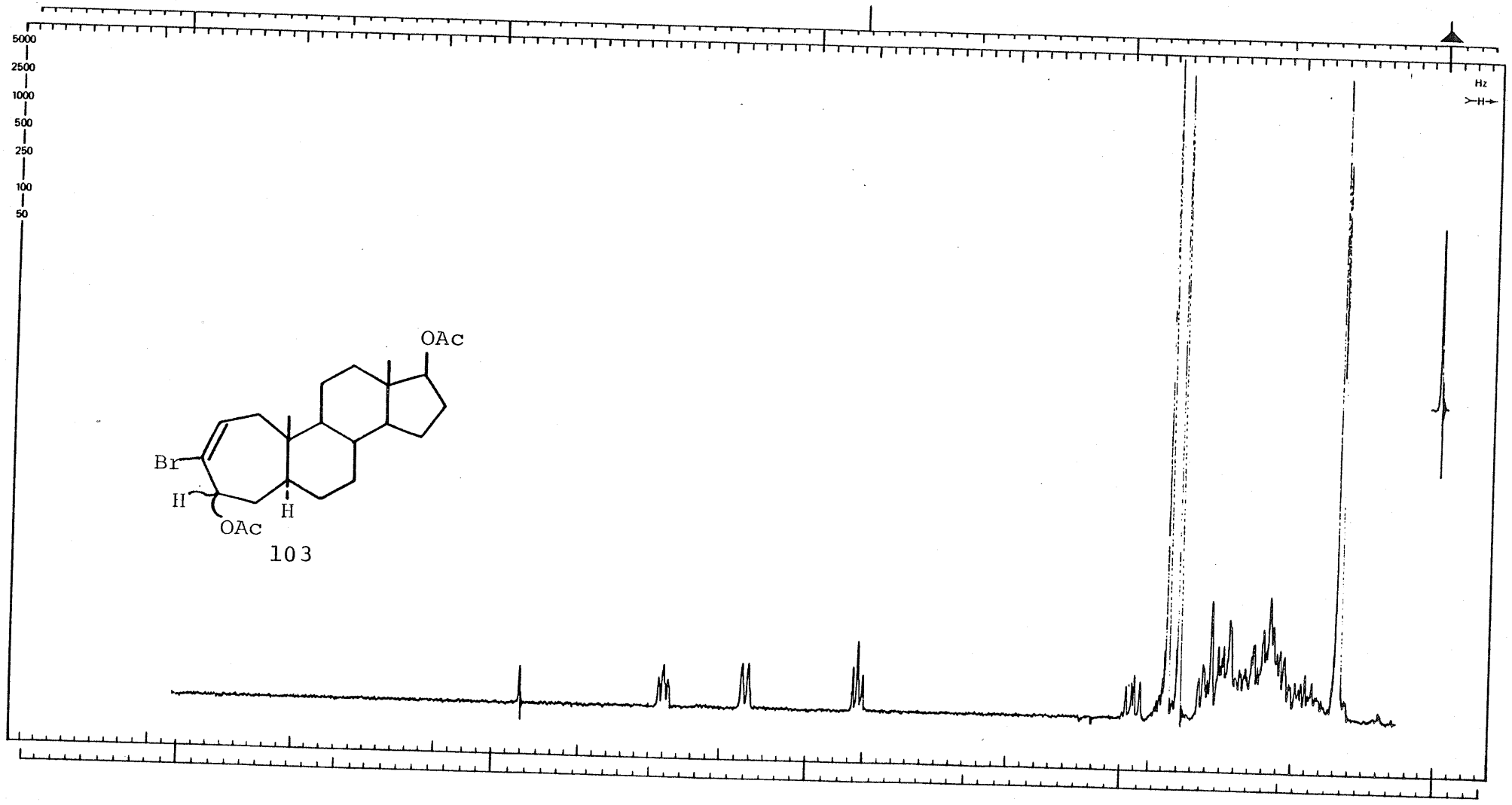

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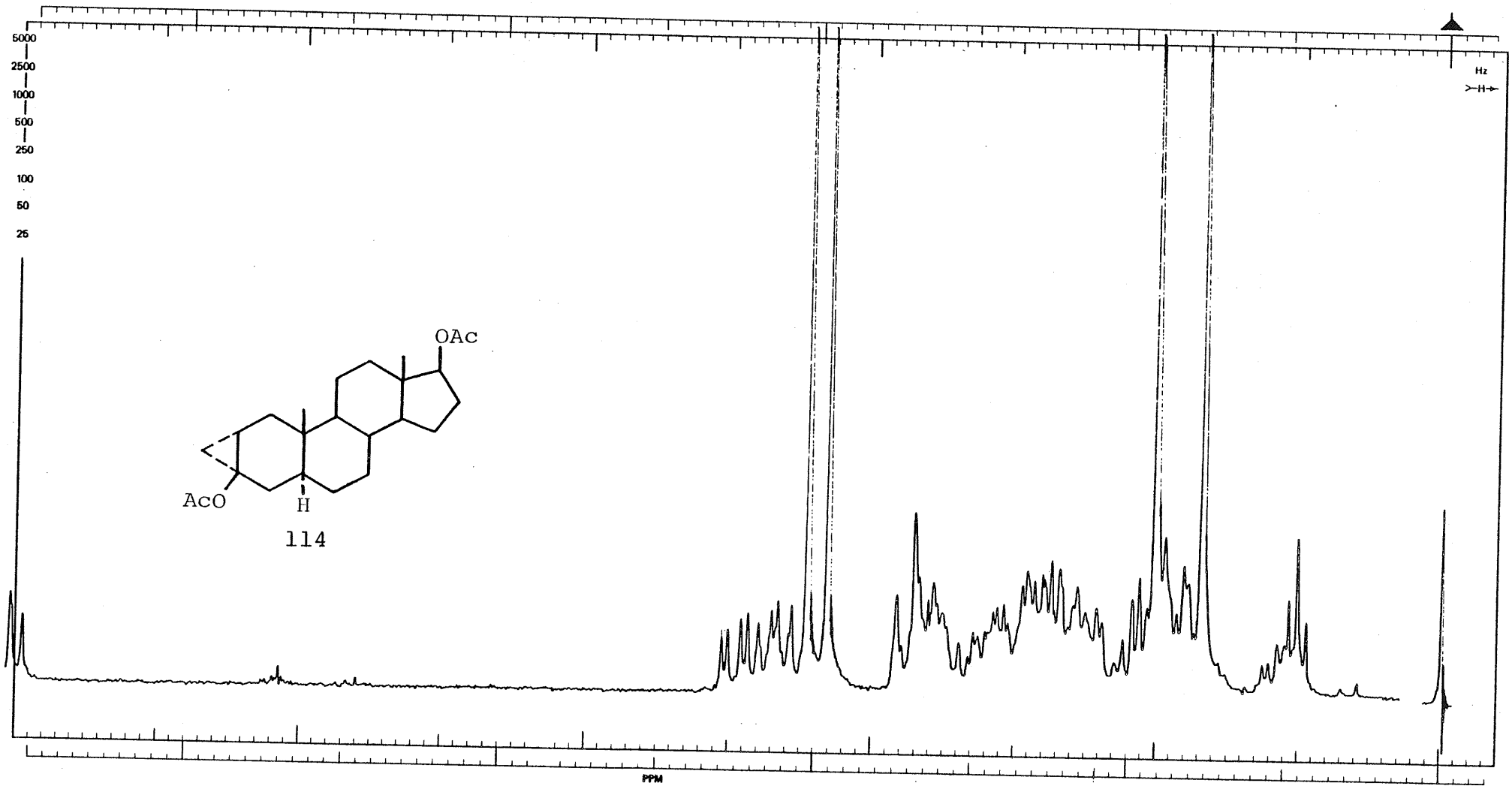

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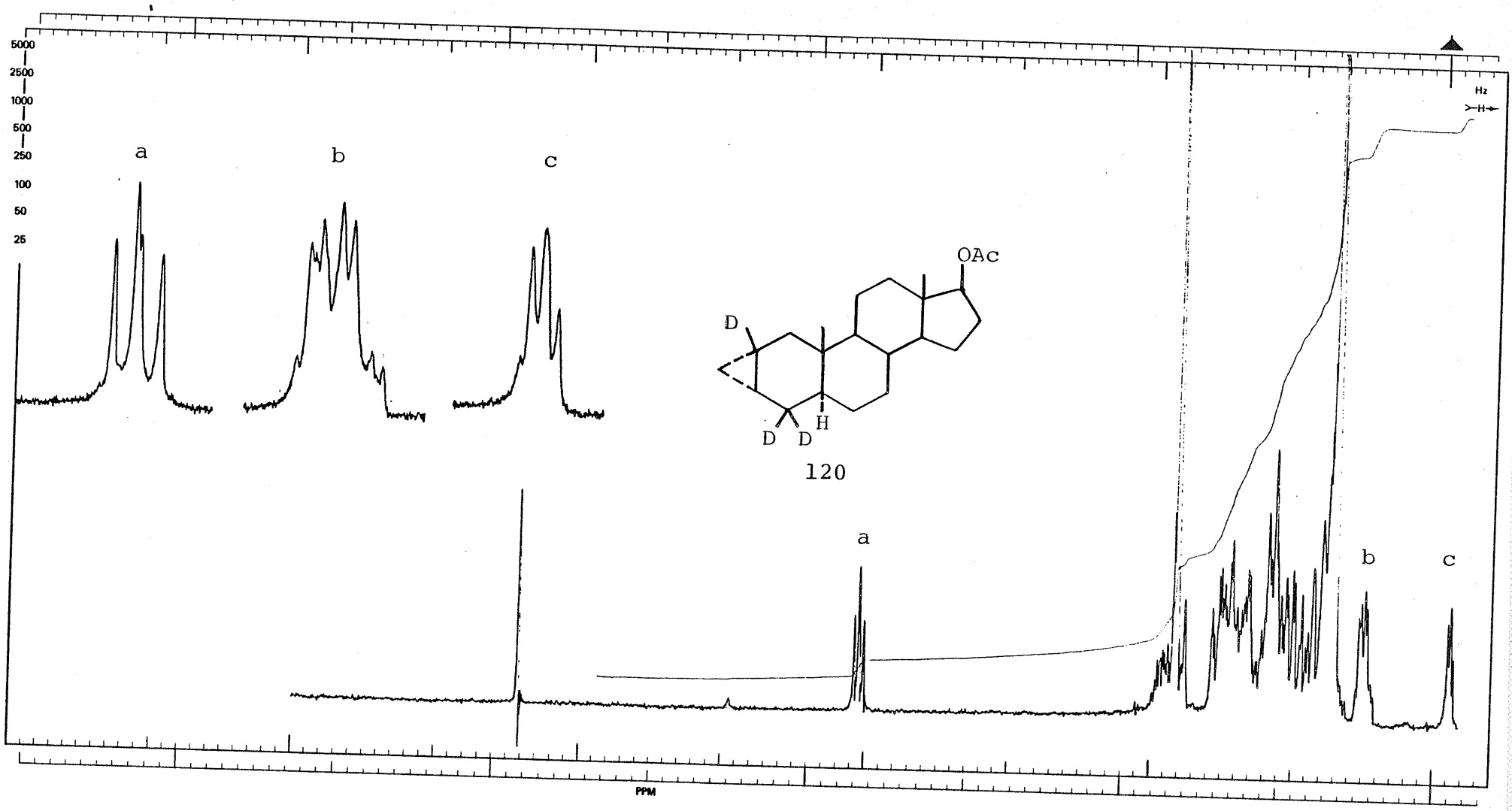
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D
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D
H
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c
Hz
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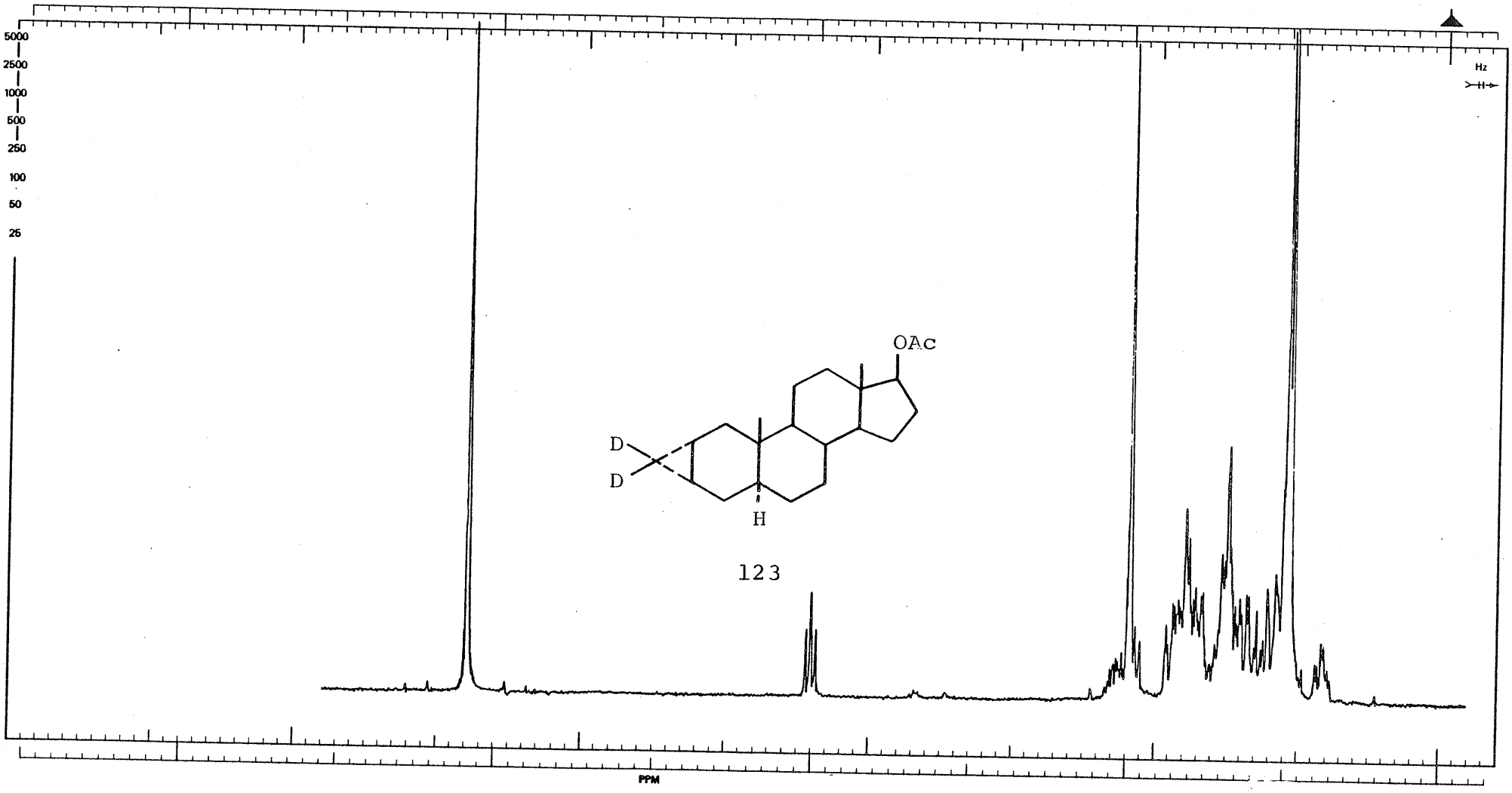

123