The University of British Columbia

FACULTY OF GRADUATE STUDIES

PROGRAMME OF THE

FINAL ORAL EXAMINATION

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

OF

JUDITH ELEANOR HALL

B.Sc.,M.Sc., University of Alberta, Calgary

MONDAY, SEPTEMBER 11, at 3:30 p.m.

IN ROOM 261, CHEMISTRY BUILDING

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SYNTHETIC AND STRUCTURAL STUDIES IN NATURAL PRODUCTS

ABSTRACT

Part 1 describes the synthesis of ring A-oxygenated 6-aza steroids in the cholestane series. Methyl-5-oxo-5,7-seco-6-nor-3-cholesten-7-oate (89) was reacted with benzylamine to give N-benzyl-6-aza-2,4-cholestadien-7-one (88). Selective hydroboration of the 2,3-double bond of this compound yielded three alcohols which were identified as 3 -α, 3β- and 2α-hydroxy-N-benzyl-6-aza-4-cholesten-7-one (91,92 and 90 respectively). Oxidation of the first two with chromium trioxide in either acetone or pyridine gave N-benzyl-6-aza-4-cholesten-3,7-dione (93).

In Part 2 the ORD curves of 6- and 11-aza steroids possessing lactam, enol lactam and amide functions are discussed. All the compounds studied exhibited positive Cotton effects. An attempt is made to interpret the sign of the Cotton effects observed for the 6-aza steroid lactams in terms of the configuration at C_5.

An investigation of the spores of Equisetum telmateia is described in Part 3. Equisporoside was isolated from the methanol extracts and shown

to be identical with the known flavonoid, gossypitrin (22). It was concluded that the structure of equisetolic acid which was isolated from the ether extracts of the spores was $HOOC(CH_2)_{28}COOH$. This work corrects the previous formulations suggested for these compounds by other workers. The alkaloid content of the spores was found to be negligible.

GRADUATE STUDIES

Field of Study: Chemistry

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Seminar in Chemistry	A. Rosentha
Physical Organic Chemistry	R. Stewart
Structure of Newer Natural Products	T. Money

PUBLICATIONS

M.H. Benn and J.E. May, The Biosynthesis of Diterpenoid Alkaloids, Experientia, 20, 252 (1964)

M.H. Benn and J.E. May, Raney Nickel Desulphurisation of Catechol Thionocarbonate: A New Synthesis of Methylenedioxybenzene, Chem. and Ind., 499 (1964)

J.P. Kutney, G. Eigendorf, and J.E. May, Optical Rotatory Dispersion Studies on Aza Steroids, Chem. Commun., 59 (1966)

J.P. Kutney, G. Eigendorf and J.E. Hall, Synthesis of Ring A-Oxygenated 6-Aza Steroids, Tetrahedron, 23, in press (1967)

SYNTHETIC AND STRUCTURAL STUDIES IN NATURAL PRODUCTS

Part 1. Synthesis of Ring A-Oxygenated 6-Aza Steroids

Part 2. ORD Studies of Lactam and Amide Chromophores

Part 3. Investigation of the Spores of Equisetum telmateia

by

JUDITH ELEANOR HALL

B.Sc., The University of Alberta, Calgary, 1962.

M.Sc., The University of Alberta, Calgary, 1964

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Chemistry

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

May, 1967.

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Department of Chemistry

The University of British Columbia
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I wish to express my thanks to Professor J.P. Kutney for the opportunity of working with him and for his guidance during the course of this research.

We are indebted to Professor T.A. Geissman for samples of gossypitrin, gossypetin and gossypetin hexamethyl ether.

PART I

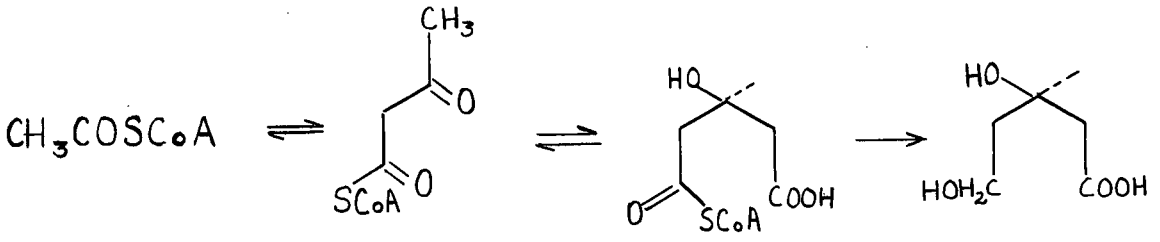
SYNTHESIS OF RING A-OXYGENATED 6-AZA STEROIDS

INTRODUCTION

In recent years there have been numerous investigations concerned with the effect of substituents attached to the normal steroid skeleton. These investigations led to the realization that very dramatic alterations in biological properties are encountered when substituents such as methyl, hydroxyl, and halogen, particularly fluorine, are placed at specific positions in the molecule.[1-3] A more significant alteration in the structure and chemical nature of steroids, replacement of one or more carbon atoms with a nitrogen atom, is known in some cases to provide biologically active substances. The object of this research was to synthesize a ring B aza steroid which possessed the C-3 oxygen function lost during the early stages of previous syntheses. This was of interest as the Δ^4-3-keto moiety is present in most of the active steroidal hormones. Recent biological tests with a number of A and B ring modified 20,25-diazacholesterol analogues showed that those possessing an oxygen function at C-3 were more active than those without. It therefore appears that hypocholesterolemic activity is associated with the localization of electrons near the C-3 atom.[4]

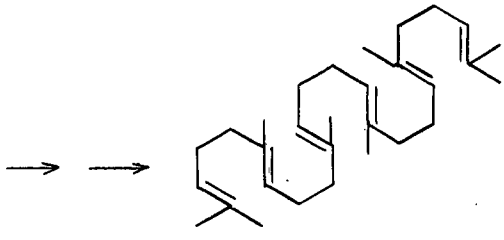
The activity of aza steroids is due to their ability to block the biosynthesis of cholesterol (Figure 1) apparently either by inhibiting the conversion of 3-hydroxy-3-methylglutaryl coenzyme A into mevalonic acid or by inhibiting the reduction of desmosterol to cholesterol. Aza steroids may therefore be of clinical value for the treatment of atherosclerosis, a disease associated with abnormally high serum cholesterol levels.

A number of diaza steroids having the nitrogen atoms in the side chain were found to be extremely potent inhibitors of cholesterol biosynthesis in animals. Further studies[5] indicated that the monoaza steroids, 24- and 25-aza cholesterol, were the most active of those tested.

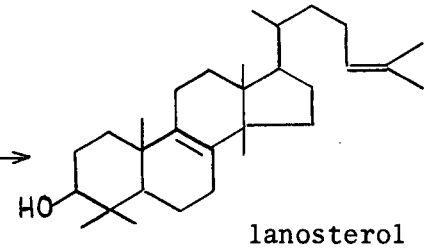


3-hydroxy-3-methyl glutaryl CoA

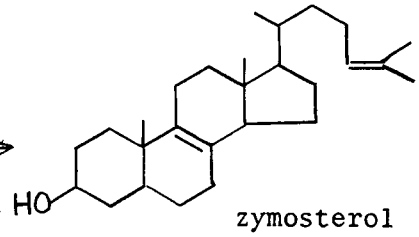
mevalonic acid



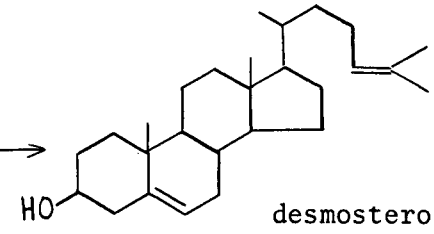
squalene



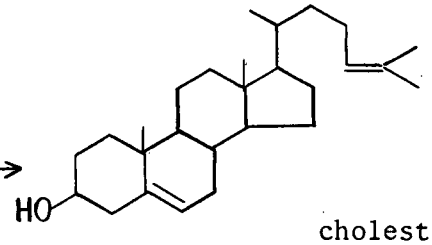
lanosterol



zymosterol



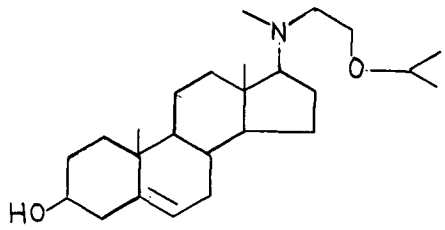
desmosterol



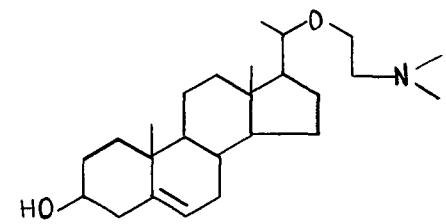
cholesterol

Figure 1. Important Intermediates in the Biosynthesis of Cholesterol

This is consistent with results of more recent tests with 20-aza-24-oxa- (1), 22-oxa-25-aza- (2), and 20-oxa-21-nor-25-azacholesterol (3), which indicated that 24- and 25-azacholesterols and related compounds are potent



1



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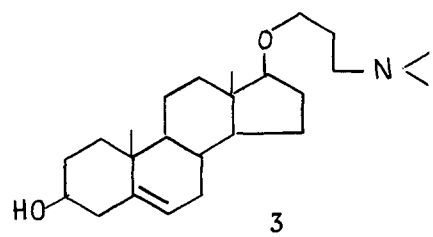
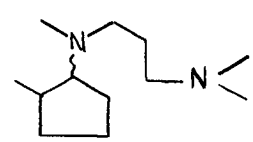


3

hypocholesterolemic agents regardless of whether C-20 and C-22 are present as carbon, oxygen, or nitrogen atoms. An absolute reduction of cholesterol and an almost constant total sterol level was observed.[6] Results of structure-activity relationship studies suggested that a receptor site with dimensions specific for cholesterol is involved[7] and a non-steroidal analogue (4) was found to be inactive when tested on rats.[4]



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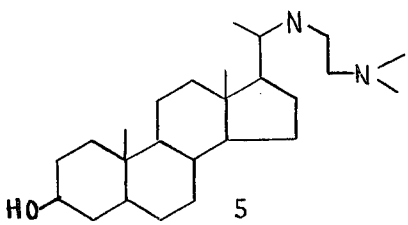
Subsequent clinical studies demonstrated that 22,25-diazacholestanol (5)[8] and 20,25-diaz a cholesterol (6)[9,10] caused a significant reduction in



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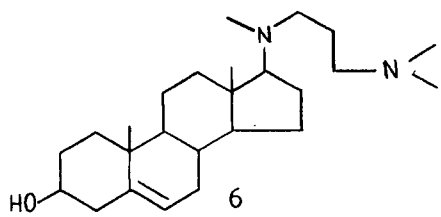

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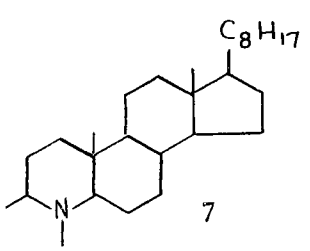
serum cholesterol levels in subjects with hypocholesterolemia and coronary atherosclerosis .

N-methyl-4-aza-3β-methyl-5α-cholestane (7) and N,N-dimethyl-4-aza-3β-benzyl-5α-cholestane iodide (8) also inhibited the reduction of desmosterol to cholesterol. Certain aza steroids, however, cause a marked



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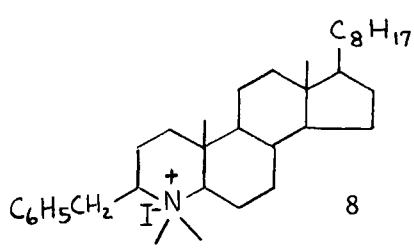

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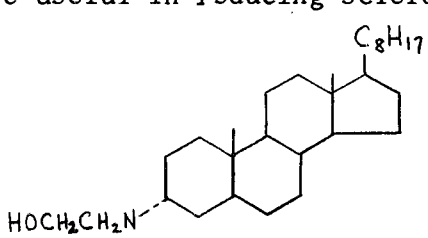
increase in cholesterol biosynthesis. The most active of these were 3α-N-ethanolaminocholestane (9) and N-phenyl-4-aza-5-cholestan-3-one (10) which are useful in reducing sclerotic lesions in laboratory animals without



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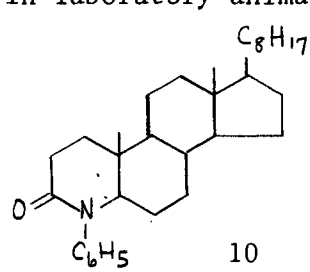

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resorting to high levels of cholesterol in the diet. A detailed discussion of the biological activity of aza steroids is available.[11,12]

In addition to the properties already mentioned aza steroids have been reported to possess anabolic, anti-bacterial, anti-fungal, hypotensive, coronary artery dilating, CNS stimulant, CNS depressant, neuromuscular blocking, anti-inflammatory, and androgenic activities.[13]

The introduction of a nitrogen atom into the steroid nucleus has attracted the attention of chemists for some time. As a result it has been introduced into virtually every position of the steroid nucleus. An excellent review of the literature up to 1962 describes the various methods which have been used.[14] The more recent work will be briefly discussed in the following pages.

The Beckmann rearrangement has been utilized frequently in these syntheses and a recent review article on the uses of this reaction and the Schmidt reaction has been published.[15] Beckmann rearrangement of the oximes of a 1-keto steroid (11) and the 1-keto-A-nor derivative (14) yielded 1-aza-A-homocholesterol (12) and 1-azacholesterol (15) respectively. The 1,10-

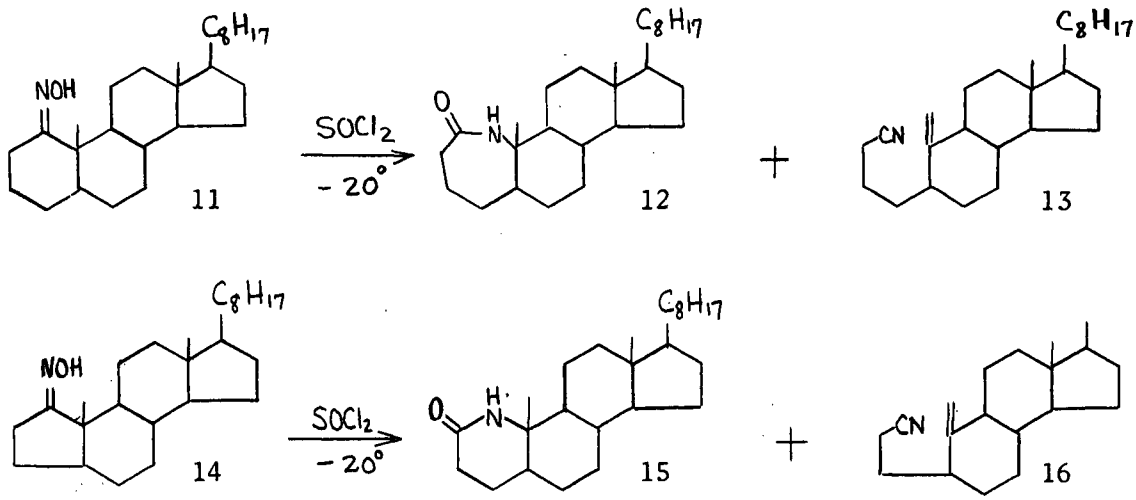


Figure 2. Synthesis of 1-Aza and 1-Aza-A-Homo Steroids.

seco-1-cyano compounds (13) and (16) were also obtained via abnormal rearrangement (Figure 2).[16] The oxime of 5α-cholestan-2-one (17) provided a mixture of 2- and 3-aza-A-homo lactams, (18) and (19), which were reduced to the corresponding 2- and 3-aza-A-homo-5α-cholestanes, (20) and (21). (Figure 3).[16] On the other hand, Beckmann rearrangement of the oxime of A-nor-5α-cholestan-2-one (22) led to an inseparable mixture of 2-aza-5α-cholestan-3-one (23) and 3-aza-5α-cholestan-2-one (24). They were separated

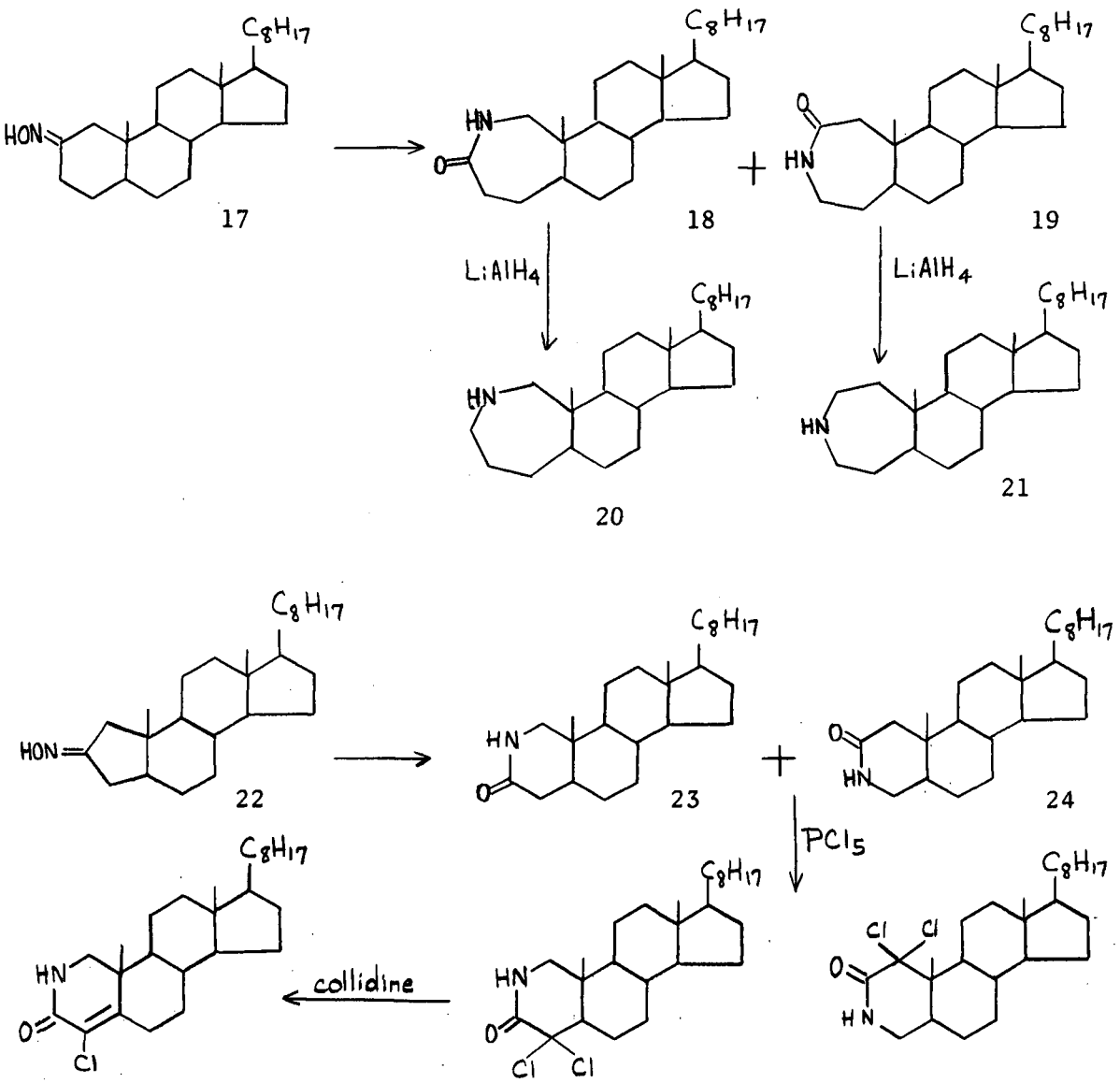


Figure 3. Synthesis of 2- and 3-Aza Steroids.

as the dichloro derivatives which were characterized by their reactivity with collidine (Figure 3).[17] Similarly, 5α-cholestan-3-one oxime (25) yielded an inseparable mixture of the 3- and 4-aza-A-homo derivatives (26) and (27). (Figure 4).[18] The Δ^4-3-keto steroid oxime (28), however, gave only the unsaturated lactam (29) which, after hydrogenation and lithium aluminum hydride reduction, yielded the 3-aza-A-homo steroid analogue (30), Figure 4).[19,20]

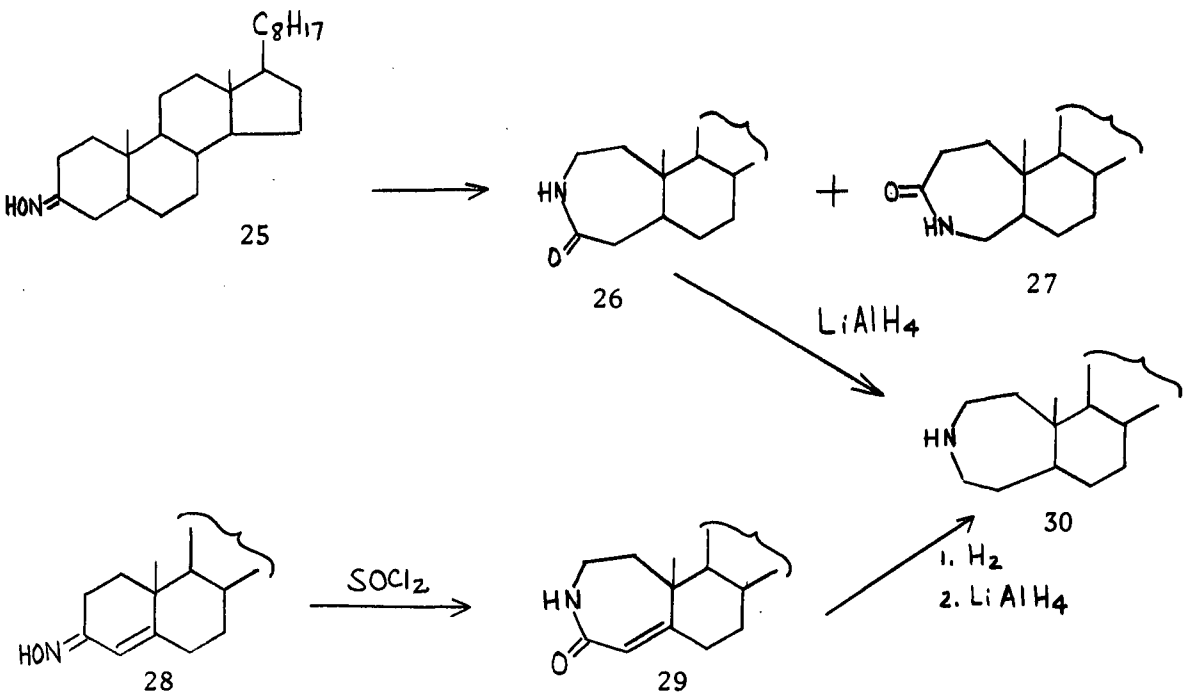


Figure 4. Synthesis of 3- and 4-Aza-A-Homo Steroids.

Recently A-homo-4-aza-5α-pregnane-3,20-dione (31) was synthesized in 93% yield from the oxime of 5α-pregnane-3,20-dione (32) using benzene sulfonyl chloride as the reagent. Standard Beckmann conditions, thionyl chloride in dioxane, gave a 39% yield of the same product. (Figure 5).[21]

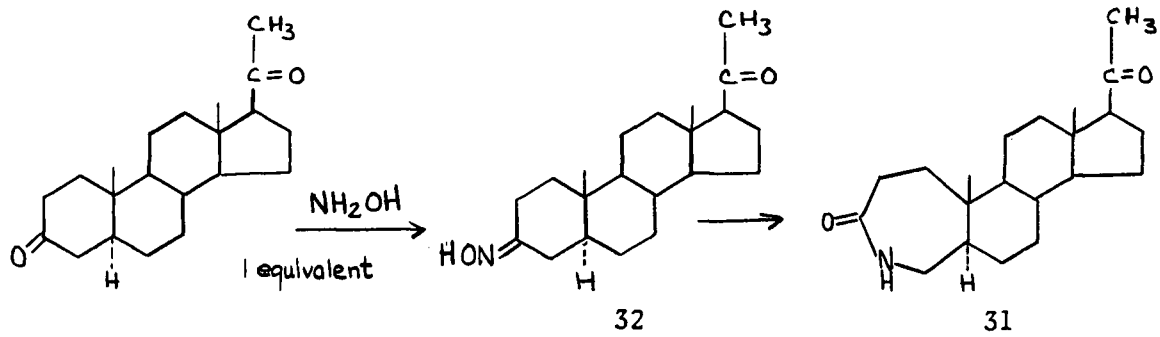
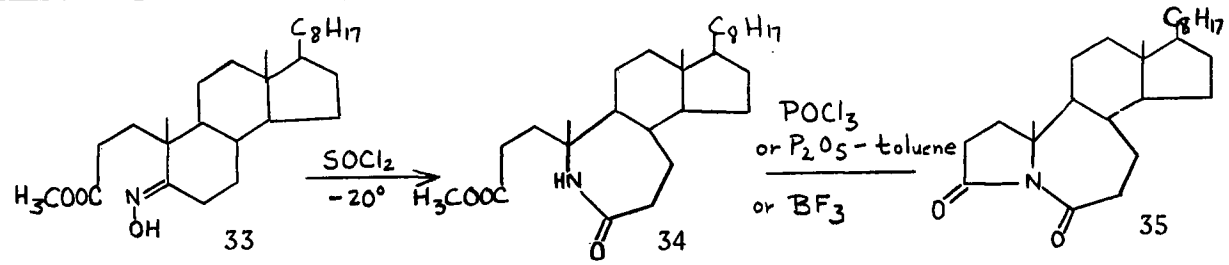


Figure 5. Synthesis of A-Homo-A-Azapregnanedione.

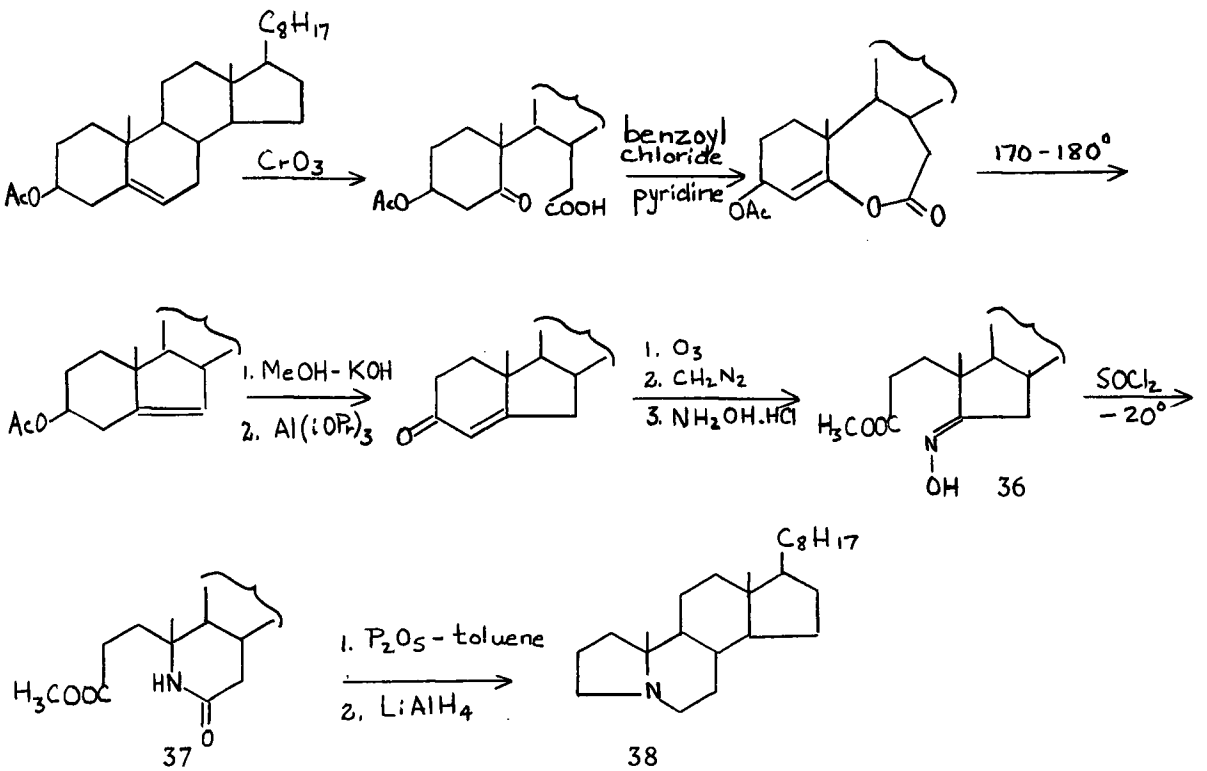
The synthesis of 5-aza-A-nor-B-homocholestane (35)[22,23] and 5-aza-A-norcholestane (38)[24,25] via a Beckmann rearrangement of the keto-ester oximes (33) using phosphorous oxychloride, boron trifluoride, or phosphorous pentoxide in toluene, and its application to the conversion of testosterone acetate (39) into A-nor-B-homo-5-azaandrostan-17β-ol (40)[26] have recently appeared in the literature. (Figure 6). The Beckmann rearrangement has also been used to synthesize a 6-aza-B-homo steroid (41),[27] and 7-aza-B-homo steroid (42),[28] and a 7-aza steroid (43).[29] (Figure 7).

Other methods of synthesizing aza steroids have also been employed in the past few years. The synthesis of a 3-azaequilenin derivative (46) via an intramolecular conversion of 2-aminoequilenin-3,4-quinone (44) with peracetic acid and subsequent decarboxylation of the acid (45) with copper powder has been reported. (Figure 8).[30] 6-Aza steroids have been successfully prepared using a modified Curtius reaction. (Figure 9)[31,32] Elimination of the C-3 oxygen function was overcome by reducing the intermediate isocyanate (47) with lithium aluminum hydride to yield (49), or catalytically to yield (50).[33] To explain these results an equilibrium between the isocyanate (47) and the lactone (48) was suggested. (Figure 10).

A-nor-B-homocholestane



A-norcholestane



A-nor-B-homoandrostane-17β-ol

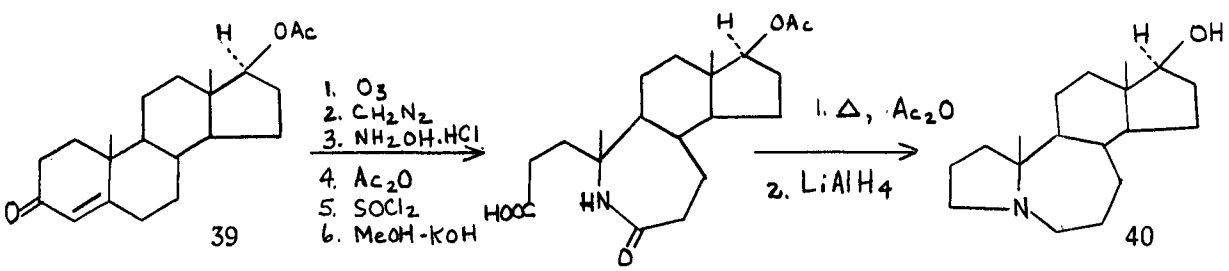


Figure 6. Synthesis of 5-Aza Steroids.

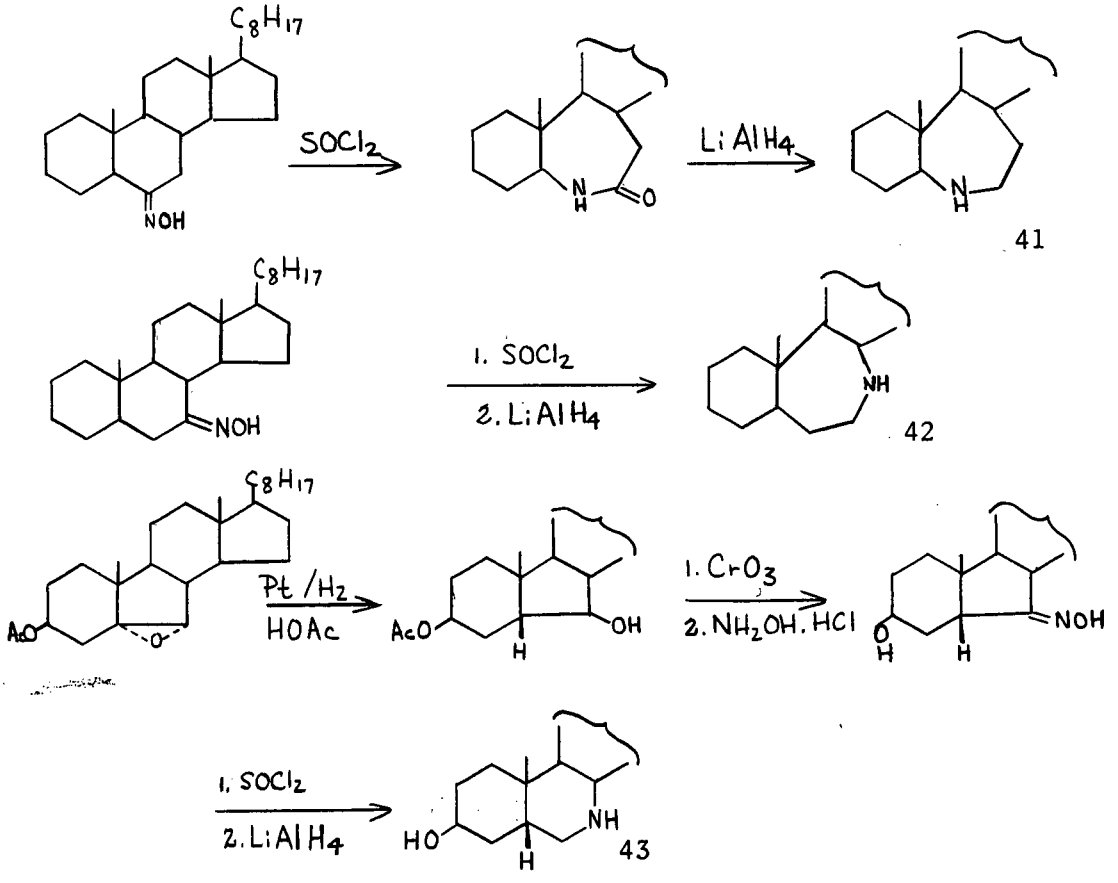


Figure 7. Synthesis of 6- and 7-Aza Steroids.

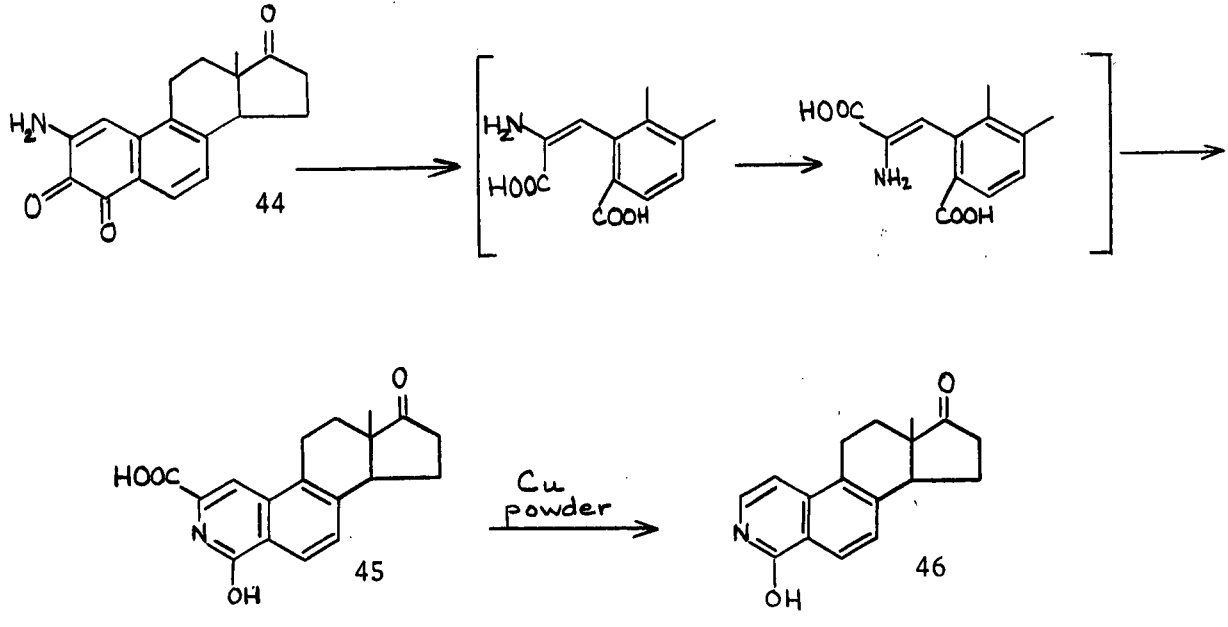


Figure 8. Synthesis of 3-Aza-4-Hydroxy e quilenin.

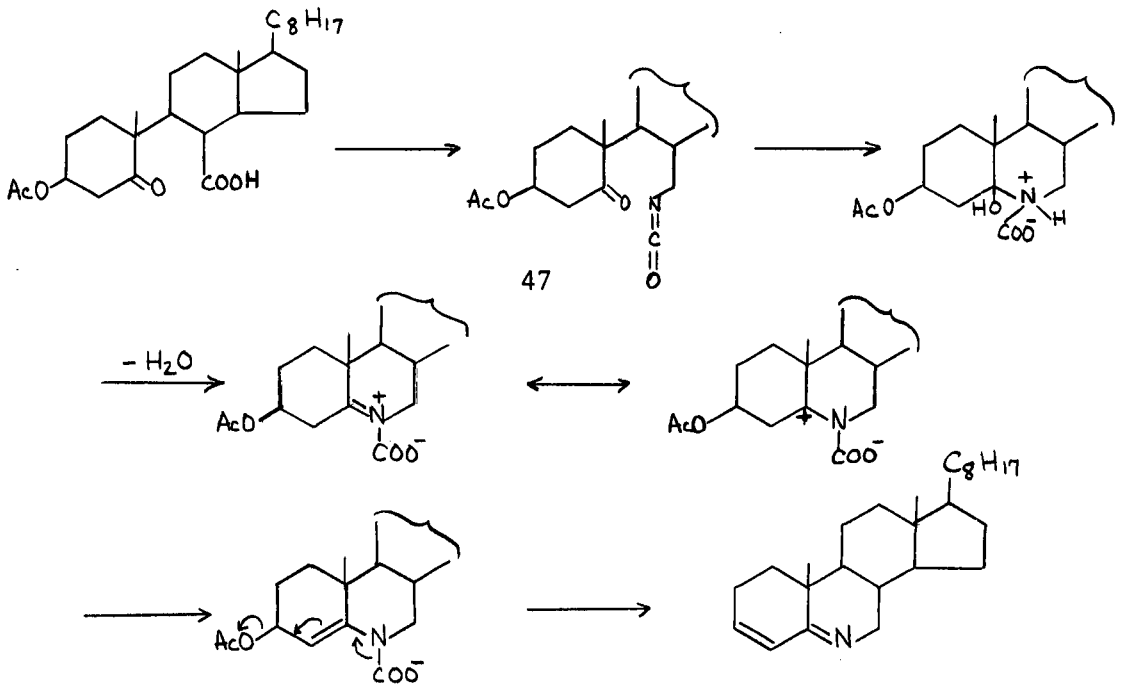


Figure 9. Synthesis of 6-Aza Steroids via a Modified Curtius Reaction.

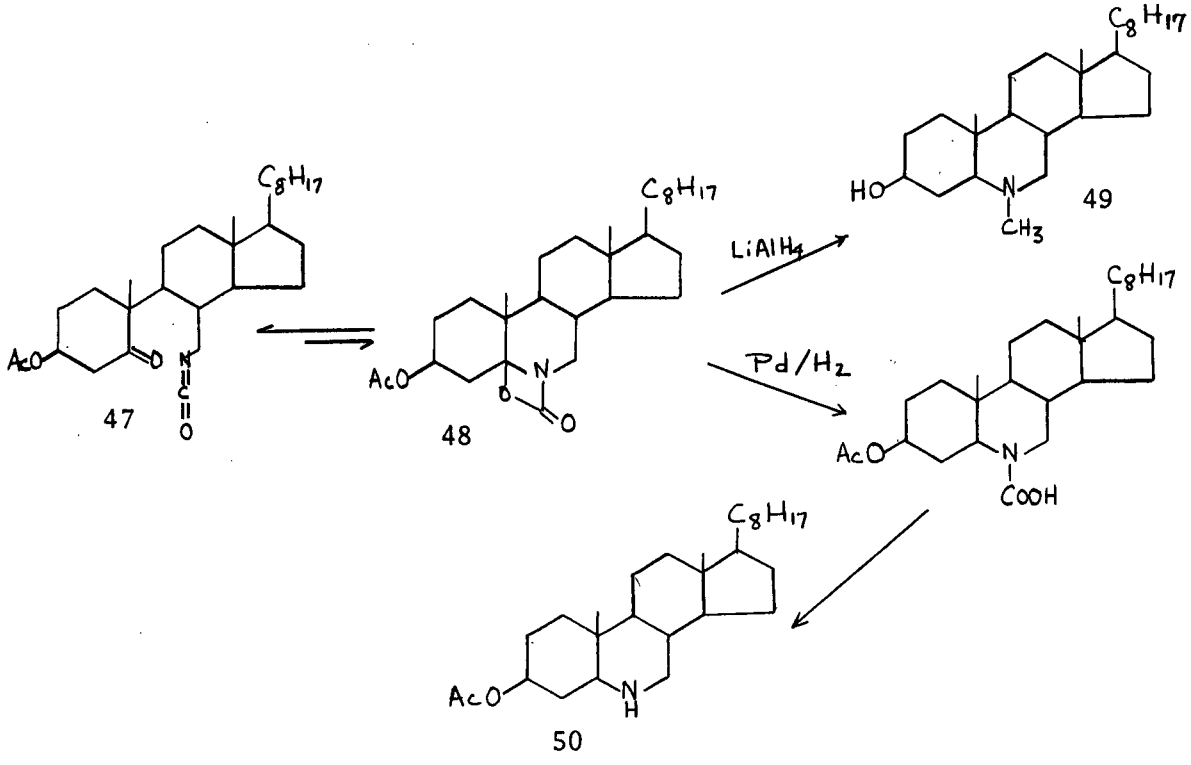


Figure 10. 6-Aza Steroids.

Introduction of a nitrogen atom into the steroid nucleus has also been accomplished by the cyclization of intermediate keto acids with amines. This has recently been applied to the synthesis of 4-hydroxy (51),[34] 4-amino- (52),[35] 4-alkylamino- (53),[36] and 4,6β-dimethyl- (54)[37] derivatives of 4-aza steroids, as well as to other heterocyclic steroids (55).[38] (Figure 11).

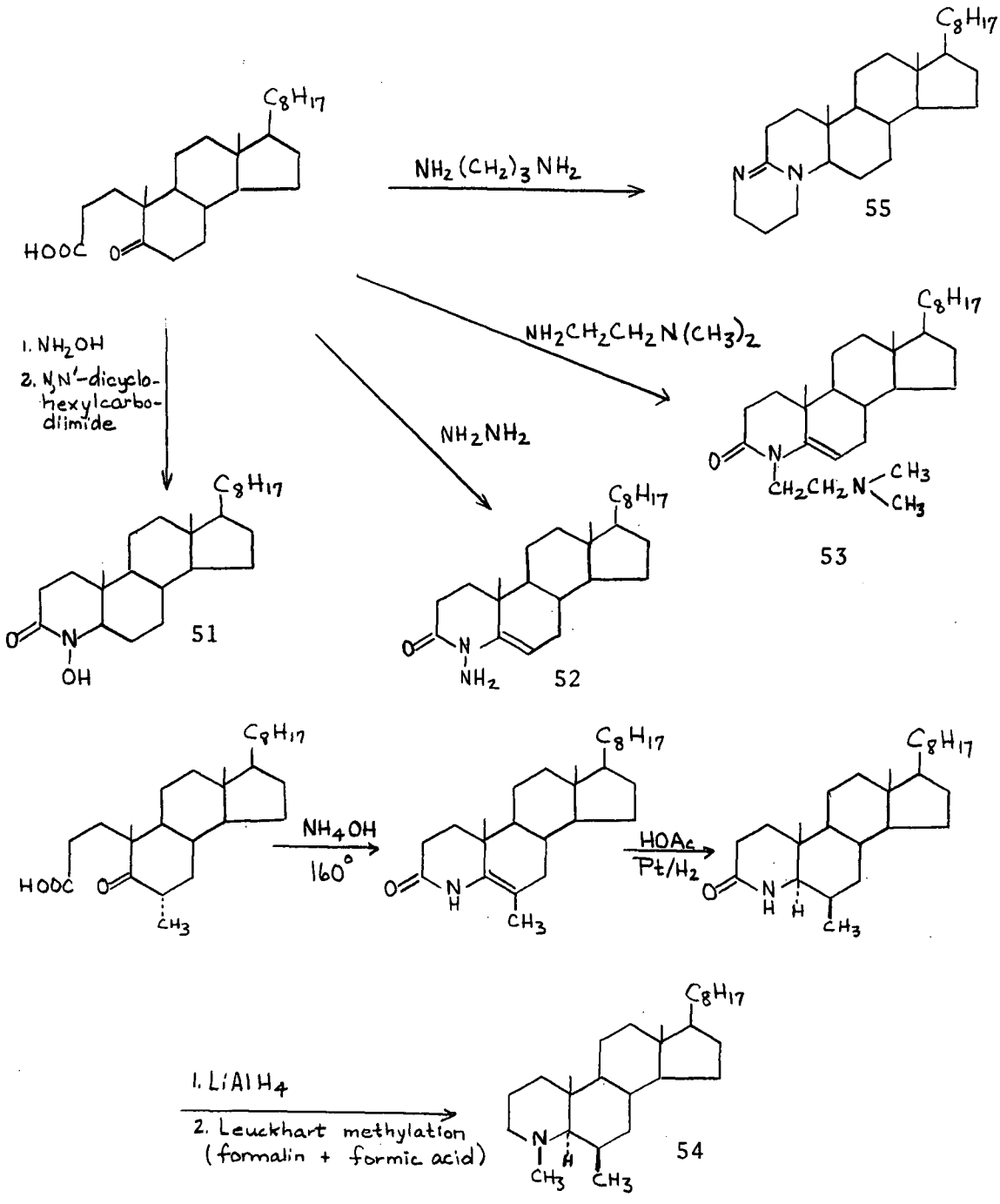


Figure 11. Cyclization of Keto Acids to 4-Aza Steroids.

In our laboratory this method was applied to the synthesis of 6-aza-cholestane (56),[39] 6-aza-androstane (57),[40] and 6-aza-pregnane (58)[41] derivatives (Figure 12) and this, along with a similar one carried out independently elsewhere,[42] provided the first general synthesis of ring B aza steroids. This work was subsequently extended to the synthesis of the

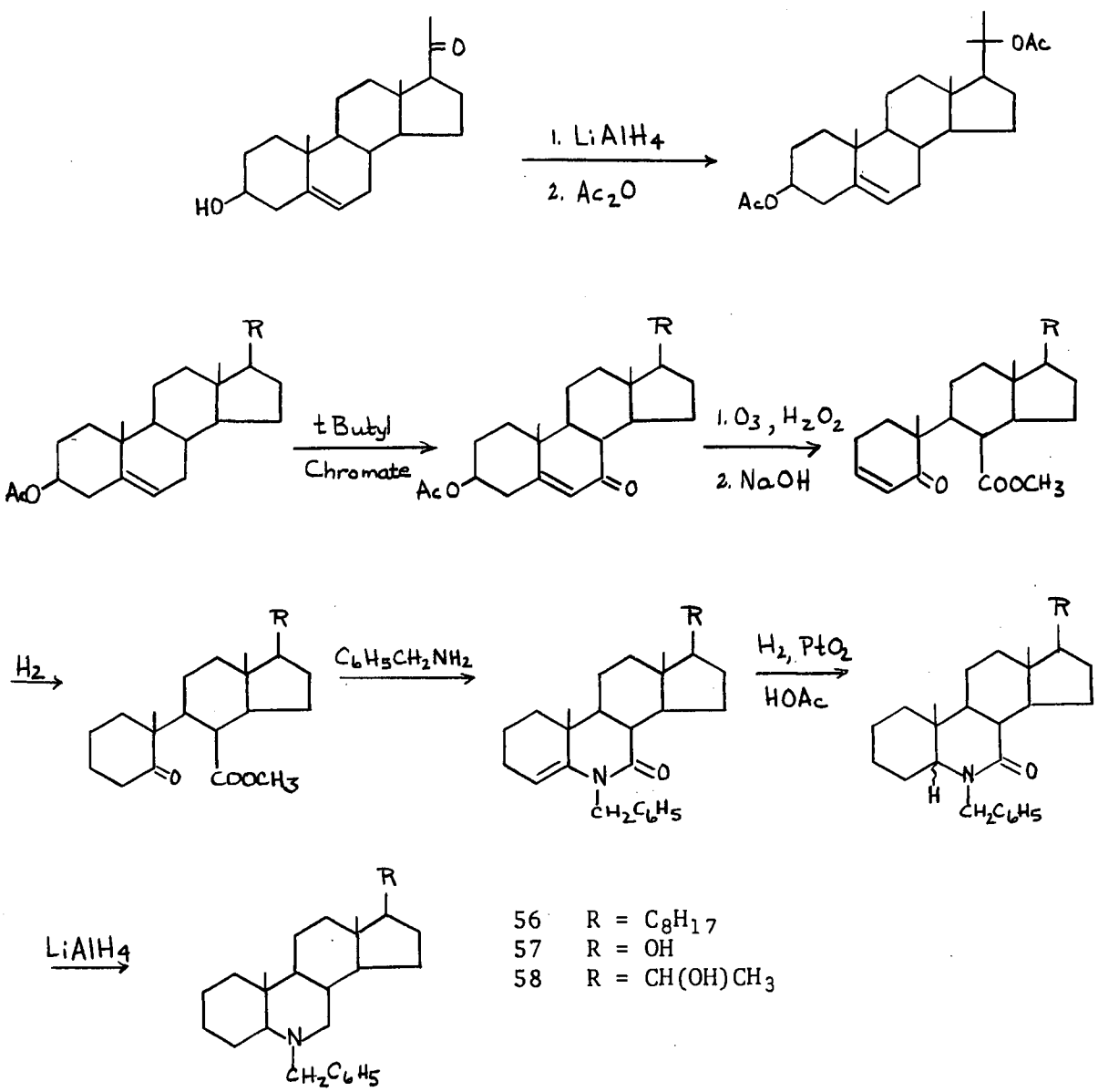


Figure 12. Cyclization of Keto Esters to 6-Aza Steroids.

first 11-aza steroidal sapogenin (59)[43] and finally to an 11-aza pregnane derivative (60).[44] (Figure 13).

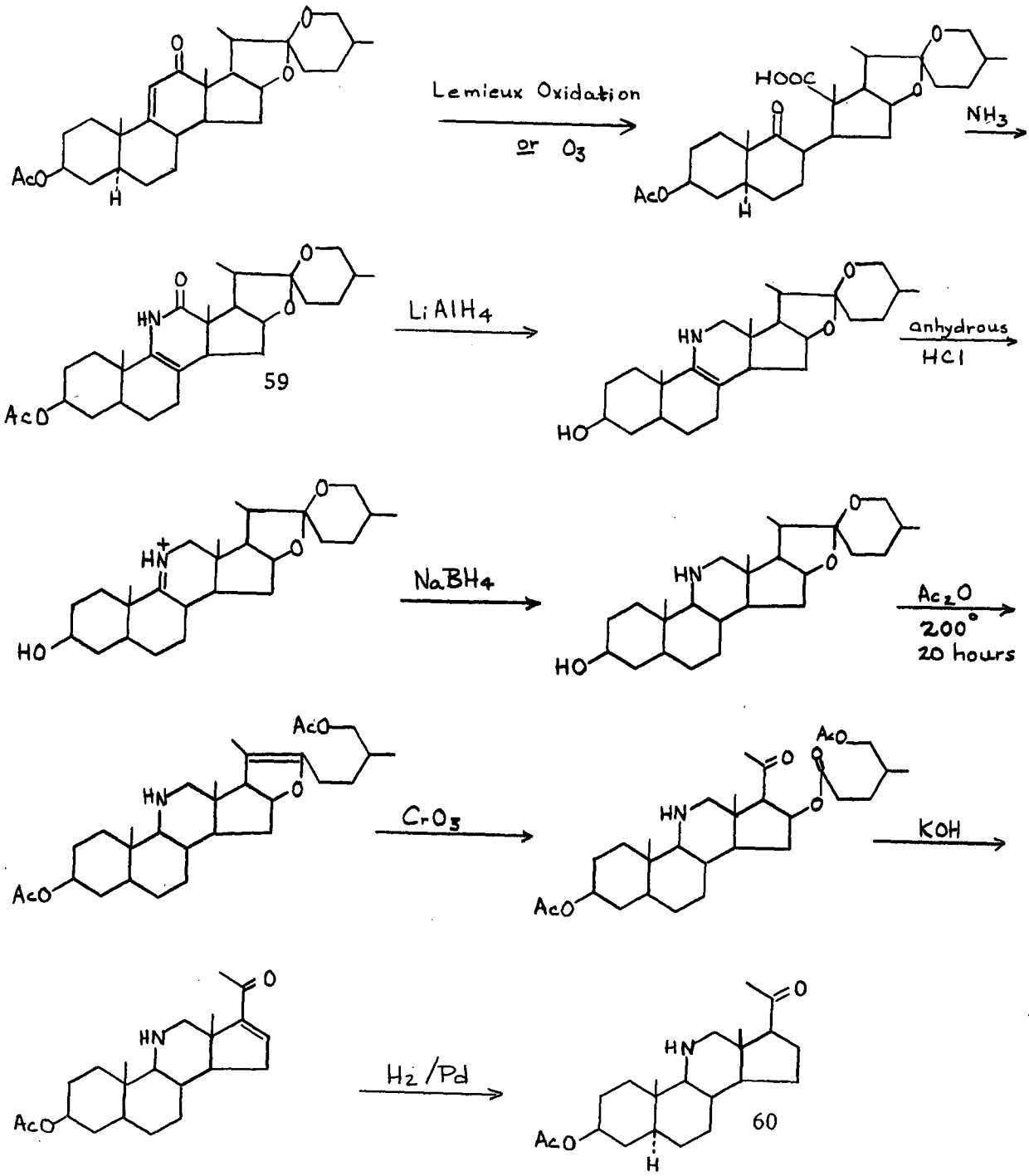


Figure 13. 11-Aza Steroids.

Using the lactam (61) as starting material the 17-aza-pregnane derivative (62) was synthesized[45] and with appropriate modifications, using the lactam (63) as starting material, 17-aza-progesterone (64) was also obtained. (Figure 14).

The Leuckart-Wallach reductive amination of 17-oxoandrostane derivatives with a number of primary and secondary amines has been reported to be a convenient, general, stereospecific method for the preparation of 17β-aminoandrostane derivatives. (Figure 15).[47] 17α-aminoandrostane derivatives have been prepared from the 17β-alcohol tosylate either by direct inversion with secondary amines or via the intermediate 17α-azide and 17α-amine. (Figure 16).[48]

Nitrogen atoms have also been introduced into various positions of the side-chain of cholesterol and this subject has been reviewed recently.[49] The biological activity of some of these derivatives has already been discussed and the syntheses will now be briefly reviewed. The mono-aza analogues were synthesized as shown in Figure 17.[5] Starting from 3β-hydroxypregn-5-en-20-one 3-tetrahydropyranyl ether (65), 22-oxa-25-azacholesterol (66) was obtained[6] which differed from the 20-iso-22-oxa-25-azacholesterol recently synthesized[50] in the stereochemistry at C-20. (Figure 18). Syntheses of 20-oxa-21-nor-25-azacholestan-3β-ol (67) and 20-aza-24-oxa-cholesterol (68), as well as cholesterol analogues with the side chain at C-16 rather than at C-17 (69), were also reported.[6] (Figure 18).

The syntheses described so far have involved modifications of the steroid skeleton. A number of total syntheses of aza steroids have also been reported in the last few years, for example, the recent synthesis of A-nor-2,3-diaza steroid ring systems. (Figure 19).[51] The total synthesis of 6-aza steroids in the estrogenic series has been independently reported

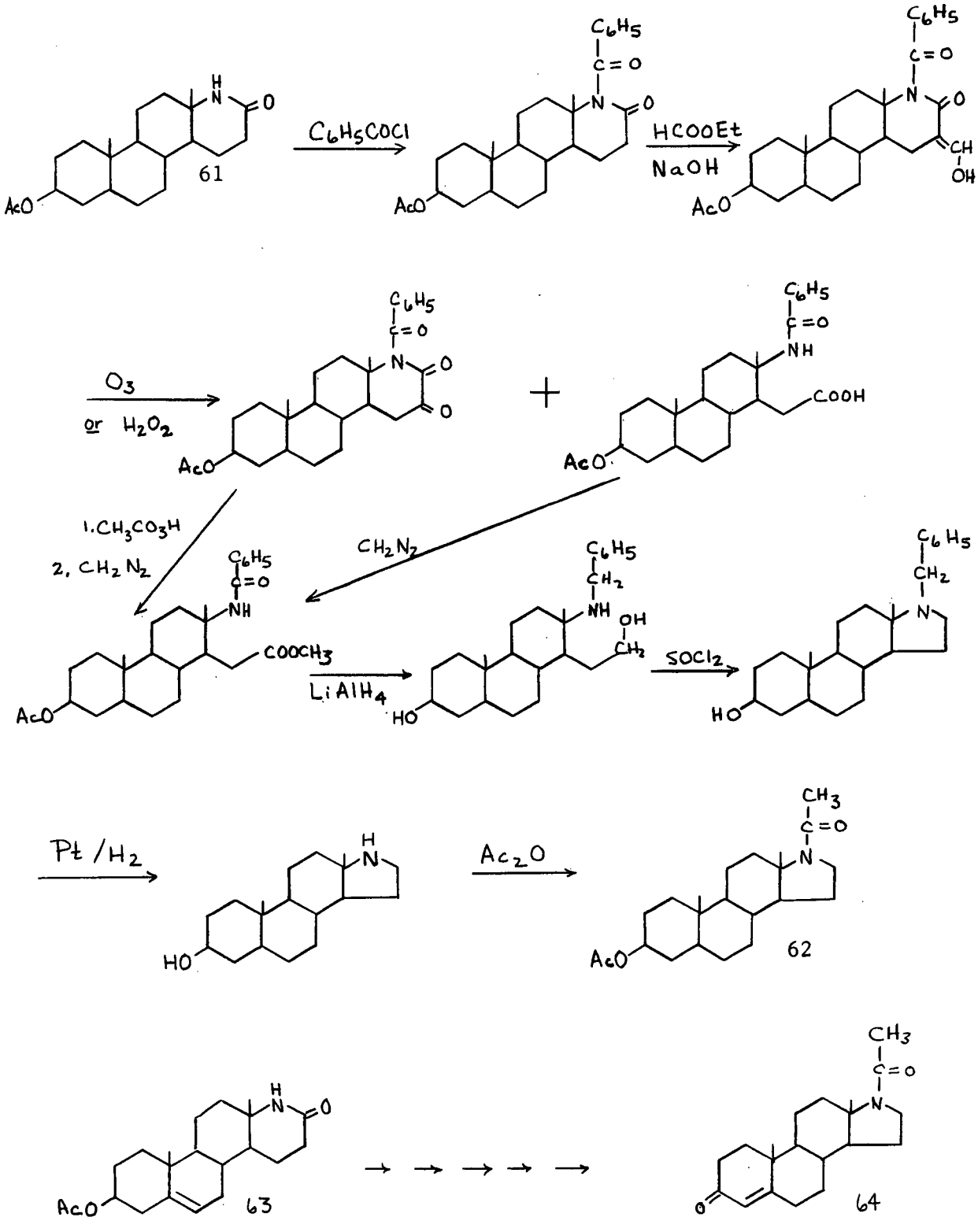


Figure 14. 17-Azapregnane Derivatives.

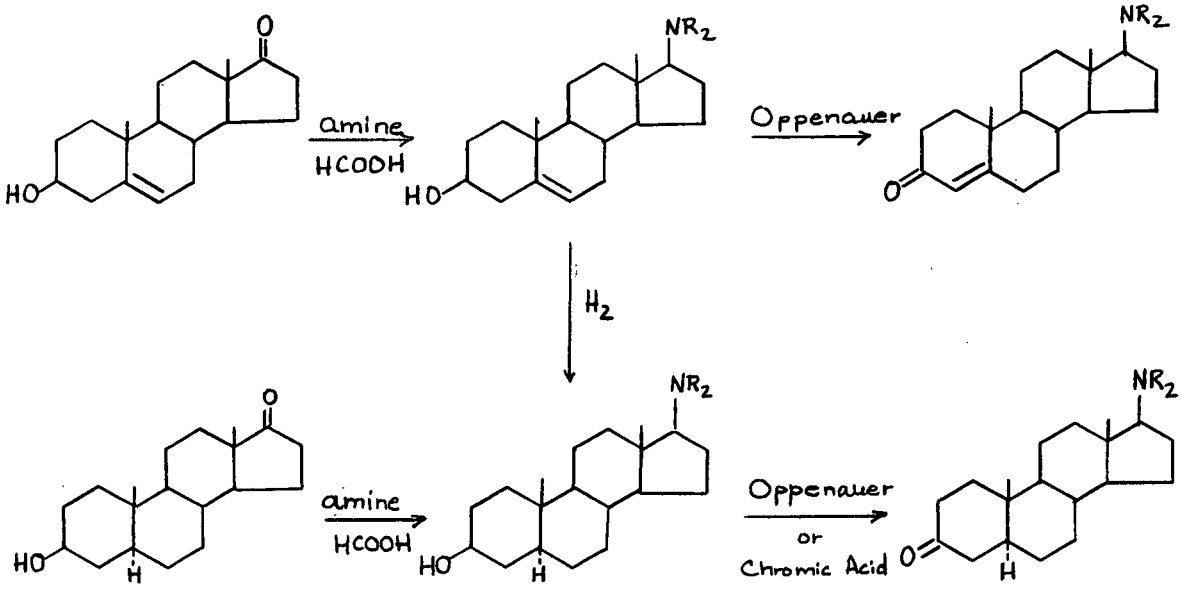


Figure 15. 17β-Aminoandrostane Derivatives.

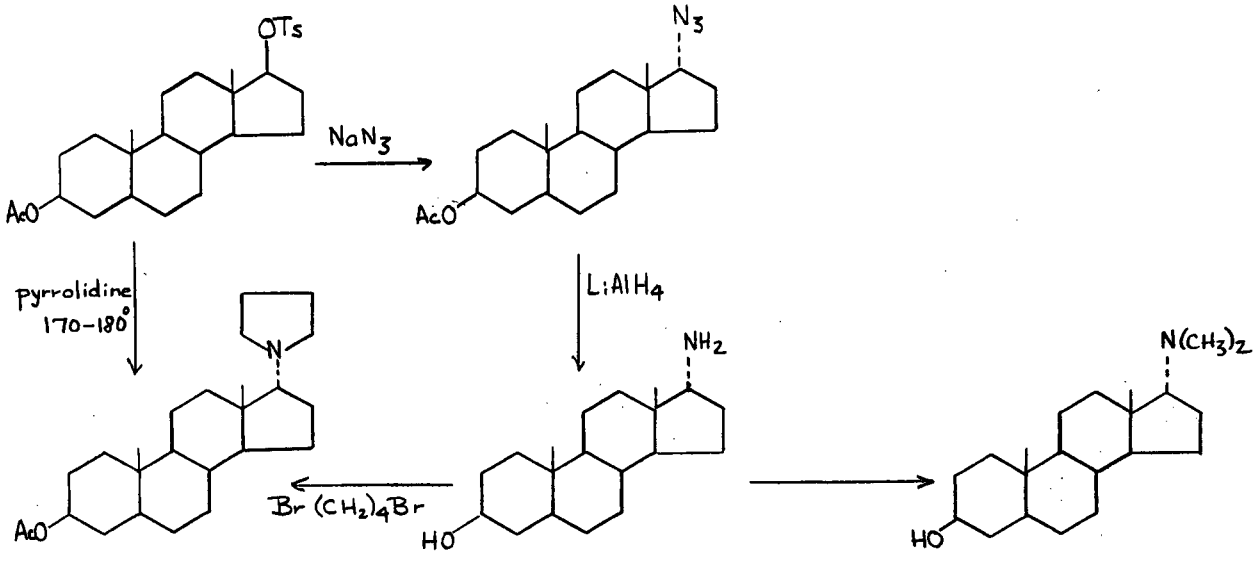
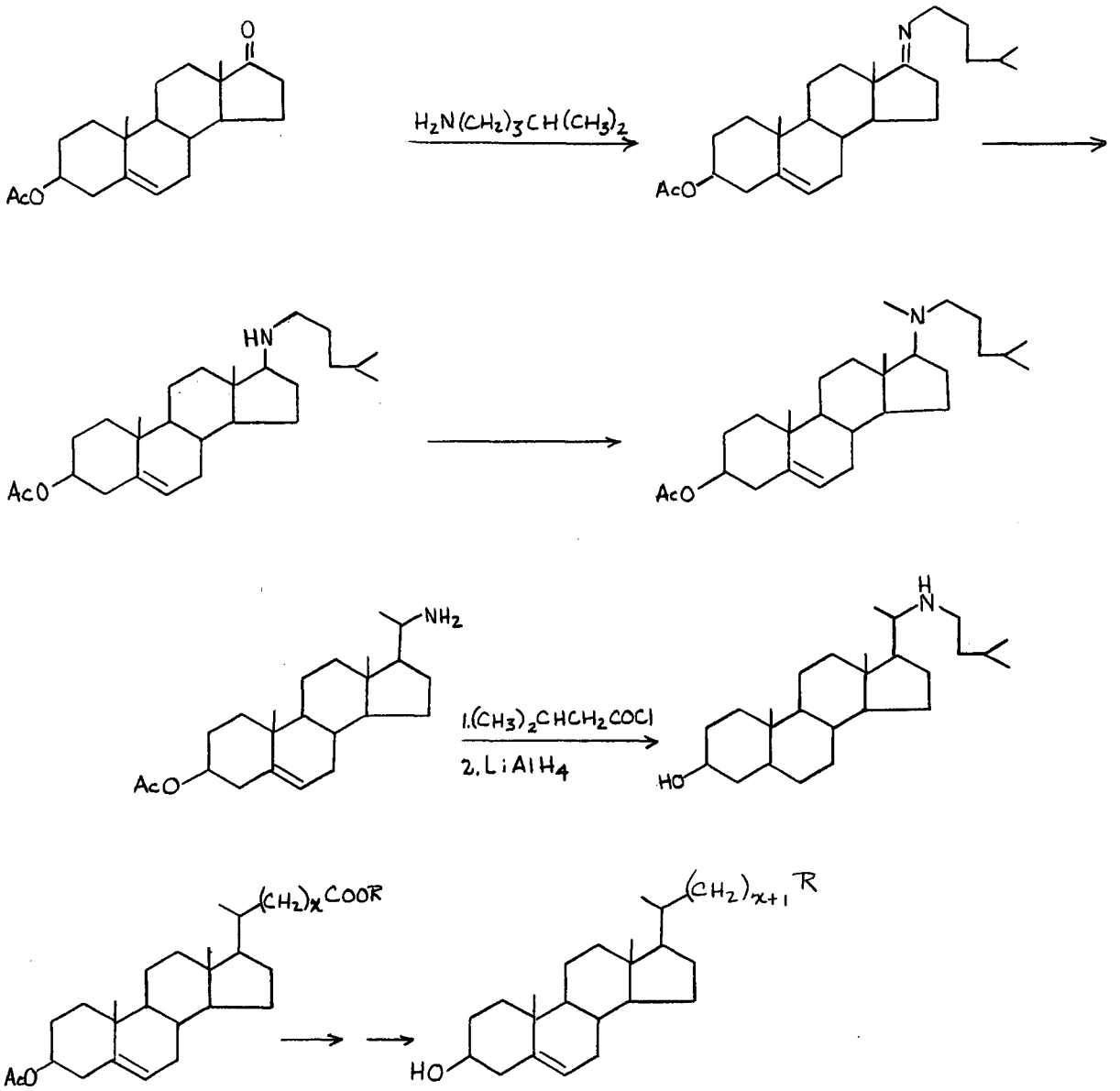


Figure 16. 17α-Aminoandrostane Derivatives.



X = 0	R = $-NHCH_2CH(CH_3)_2$	23-azacholesterol
X = 1	R = $-NHCH(CH_3)_2$	24-azacholesterol
X = 2	R = $-N(CH_3)_2$	25-azacholesterol

Figure 17. Synthesis of 23-, 24- and 25-Azacholesterols.

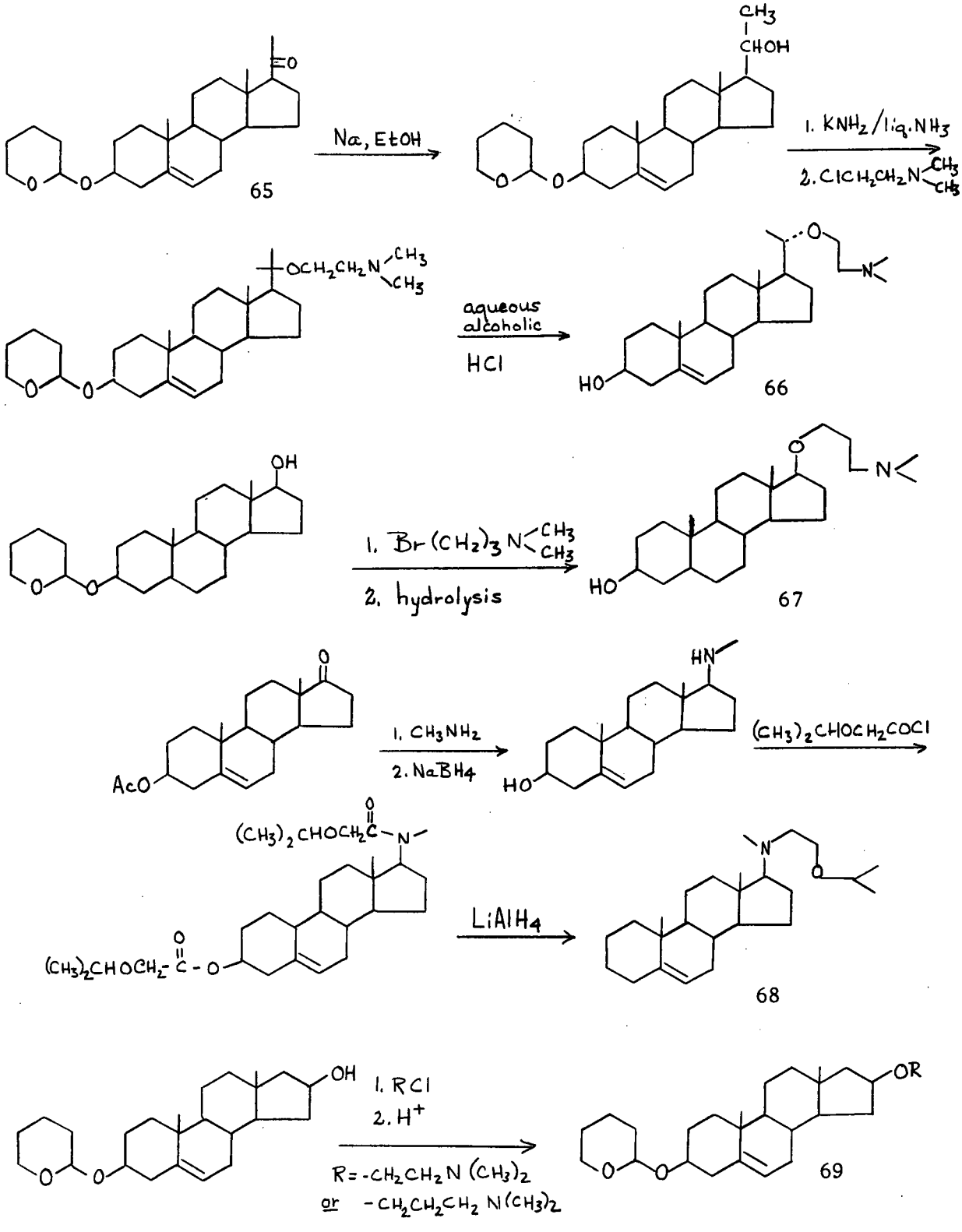


Figure 18. Side-Chain Azacholesterols.

by three groups of workers. One synthesis which led to dl-6-azaequilenin (71) from the ketone (70) is outlined in Figure 20.[52] Another attractive approach to 6-azaequilenin (71) and other 6-azaestrone derivatives has been independently reported. (Figure 21).[53,54] Two syntheses of 8-aza-estrone which have been reported are outlined in Figures 22[55] and 23[56,57] and a synthetic approach to both 8- and 9-aza steroids (74) and (75) respectively is shown in Figure 24.[58,59] Very recently, a three-step total synthesis of DL-8-azaestrone methyl ether (76) and related steroid systems (77) was accomplished in 43% overall yield. Separation of the 14α and 14β isomer was accomplished by fractional crystallization. (Figure 25).[60] Application of this approach to the synthesis of the D-homo-8-aza steroid (78) was unsuccessful.

The synthesis of 13-aza-18-norequilenin methyl ether (79) has been reported independently by two groups of workers. One synthetic sequence is shown in Figure 26 [61] and the other which also leads to 13-aza-18-nor-D-homoequilenin methyl ether (80) in Figure 27 .[62] The synthesis of derivatives of 8,13-diaza-18-norestrone methyl ether (81) and (82) starting with homoveratrylamine (83) and mescaline (84) respectively is outlined in Figure 28.[63] Both series of reactions proceeded in good yield.

A review of the syntheses discussed reveals that very few lead to products which possess a non-aromatic A-ring, the true steroid skeleton and the C-3 oxygen function. The synthesis of 6-aza steroids by the cyclization of an intermediate keto-ester with benzylamine as previously accomplished in our laboratory has already been discussed. (Figure 12). The modification of this work and the reintroduction of the oxygen function at C-3 is described in this part of the thesis.

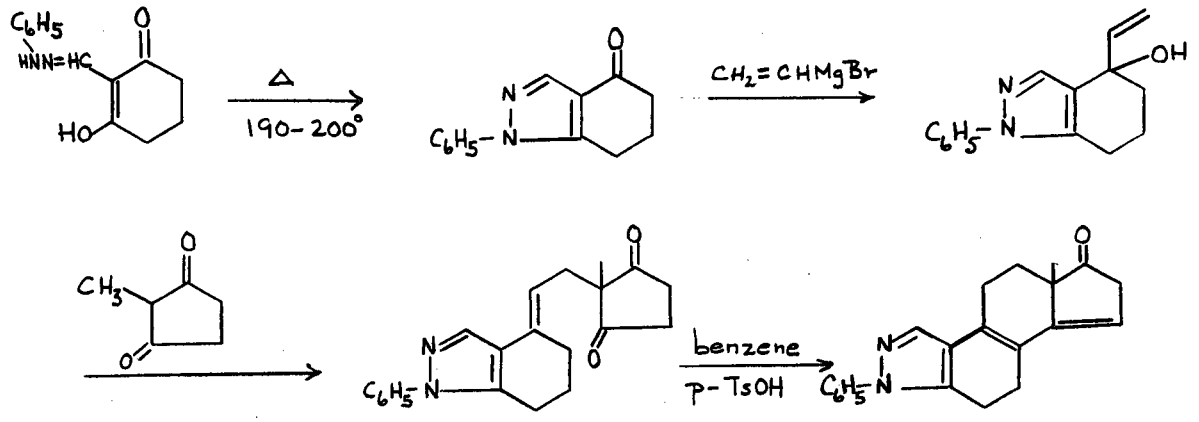


Figure 19. Total Synthesis of A-Nor-2,3-diaza-3-phenyl-oestra-1,5 (10),8,14-tetraen-17-one.

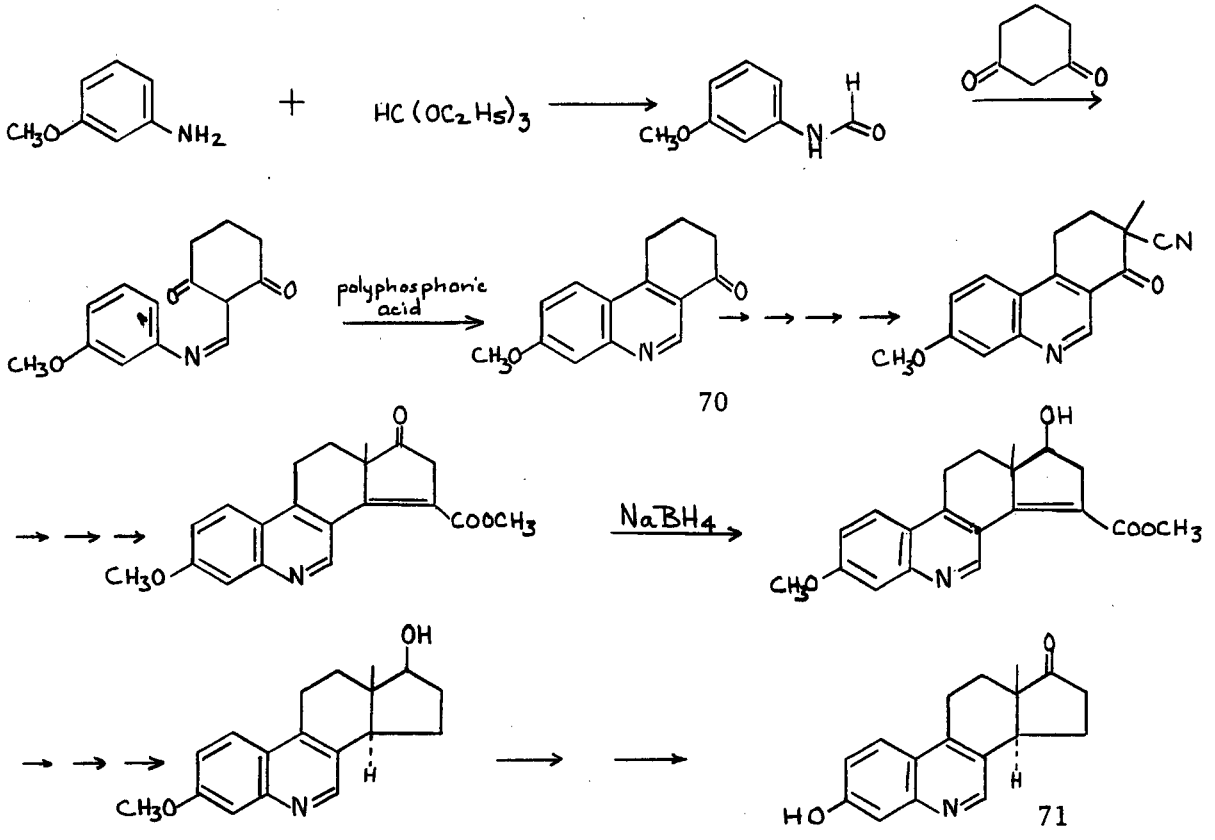


Figure 20. Total Synthesis of dl-6-Aza e quilenin.

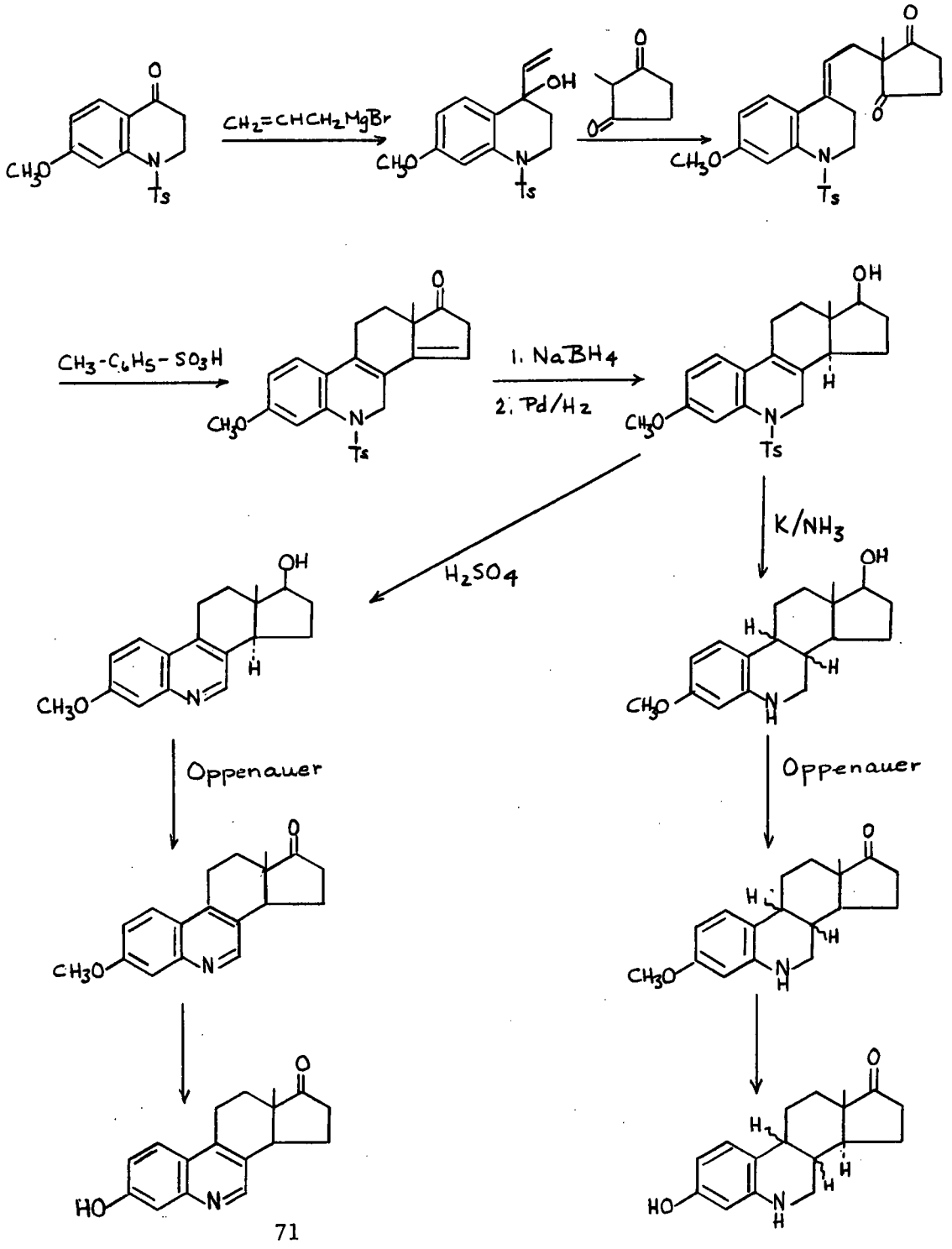


Figure 21. Total Synthesis of dl-6-Aza equilenin and other 6-Aza-estrone Derivatives.

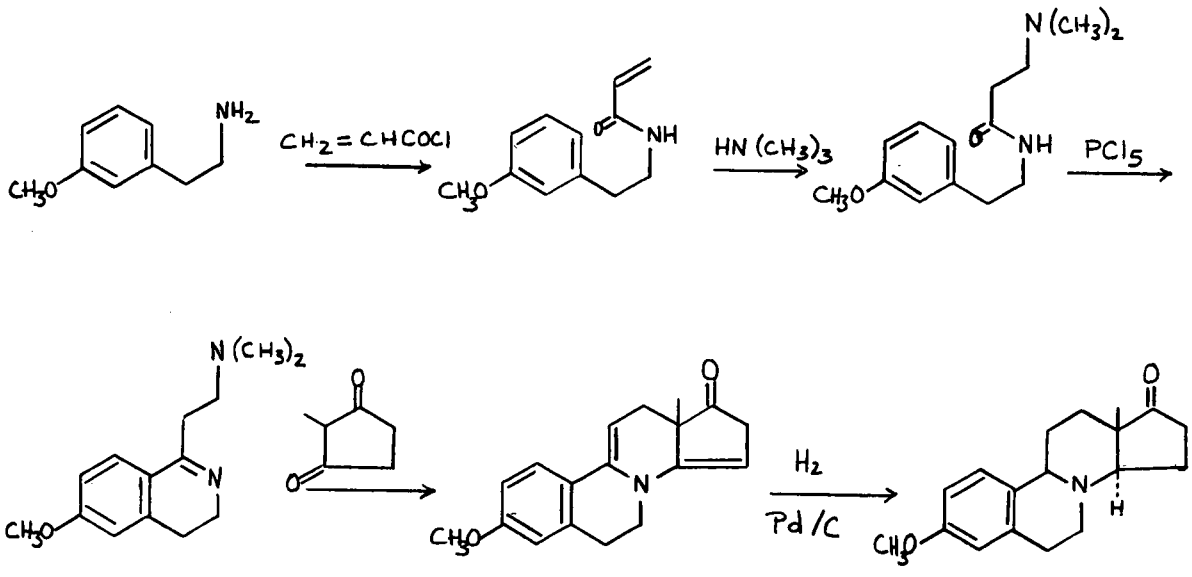


Figure 22. Total Synthesis of 8-Azaestrone

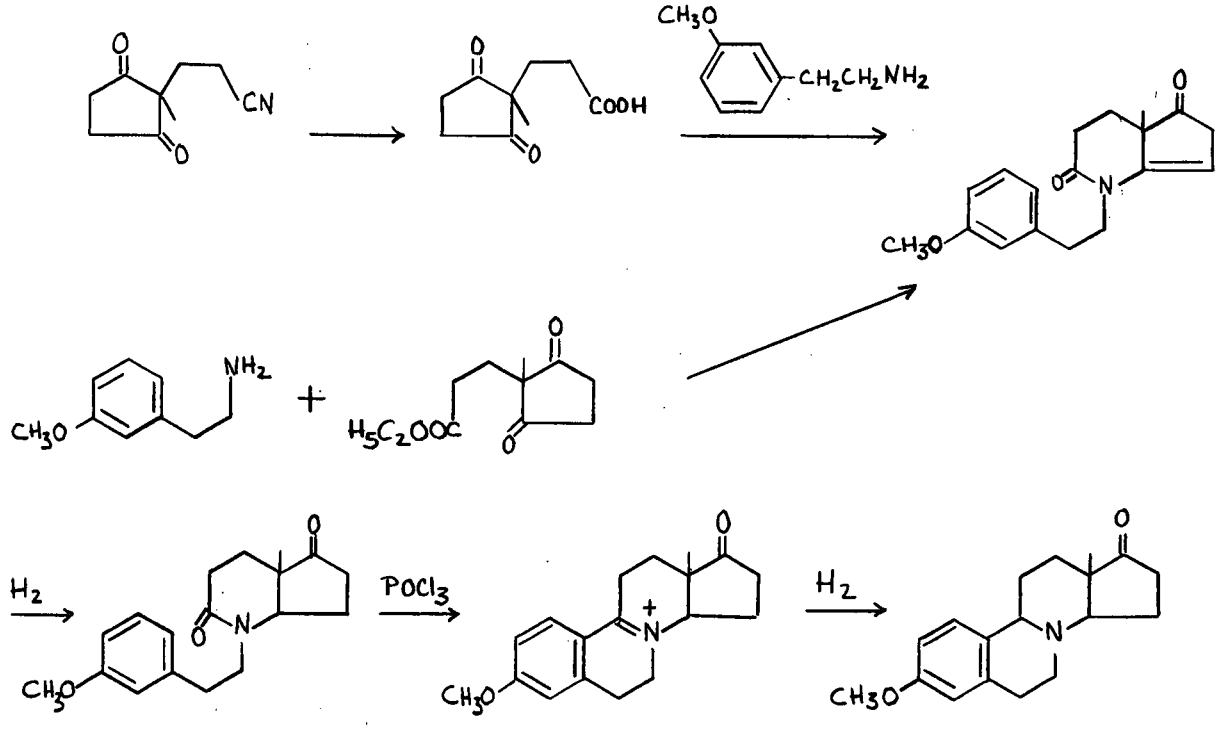
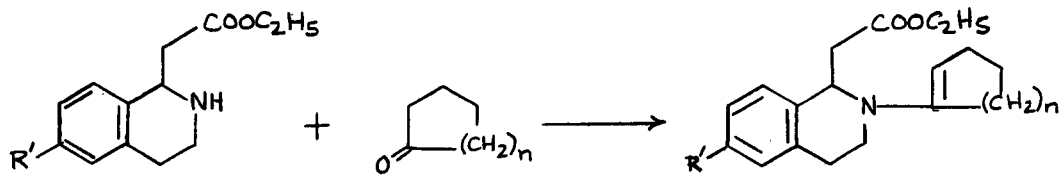
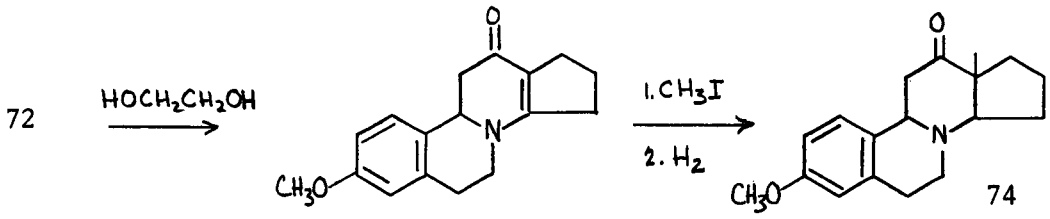
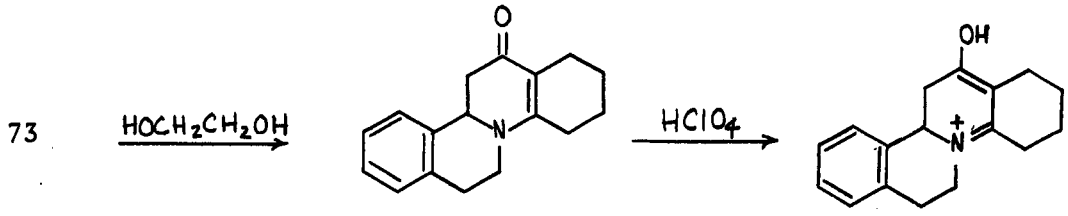


Figure 23. Total Synthesis of 8-Azaestrone







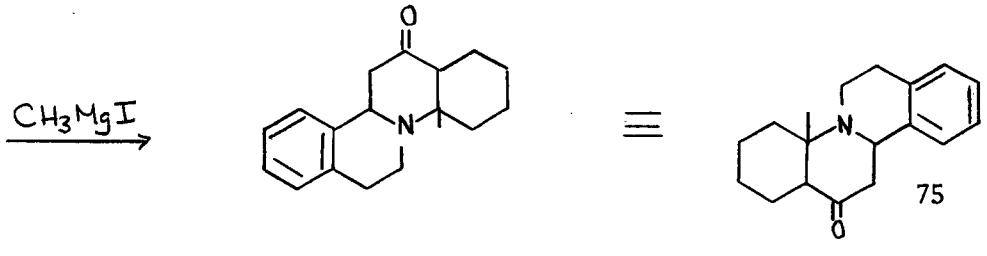


Figure 24. Total Synthesis of 8- and 9-Aza Steroids.

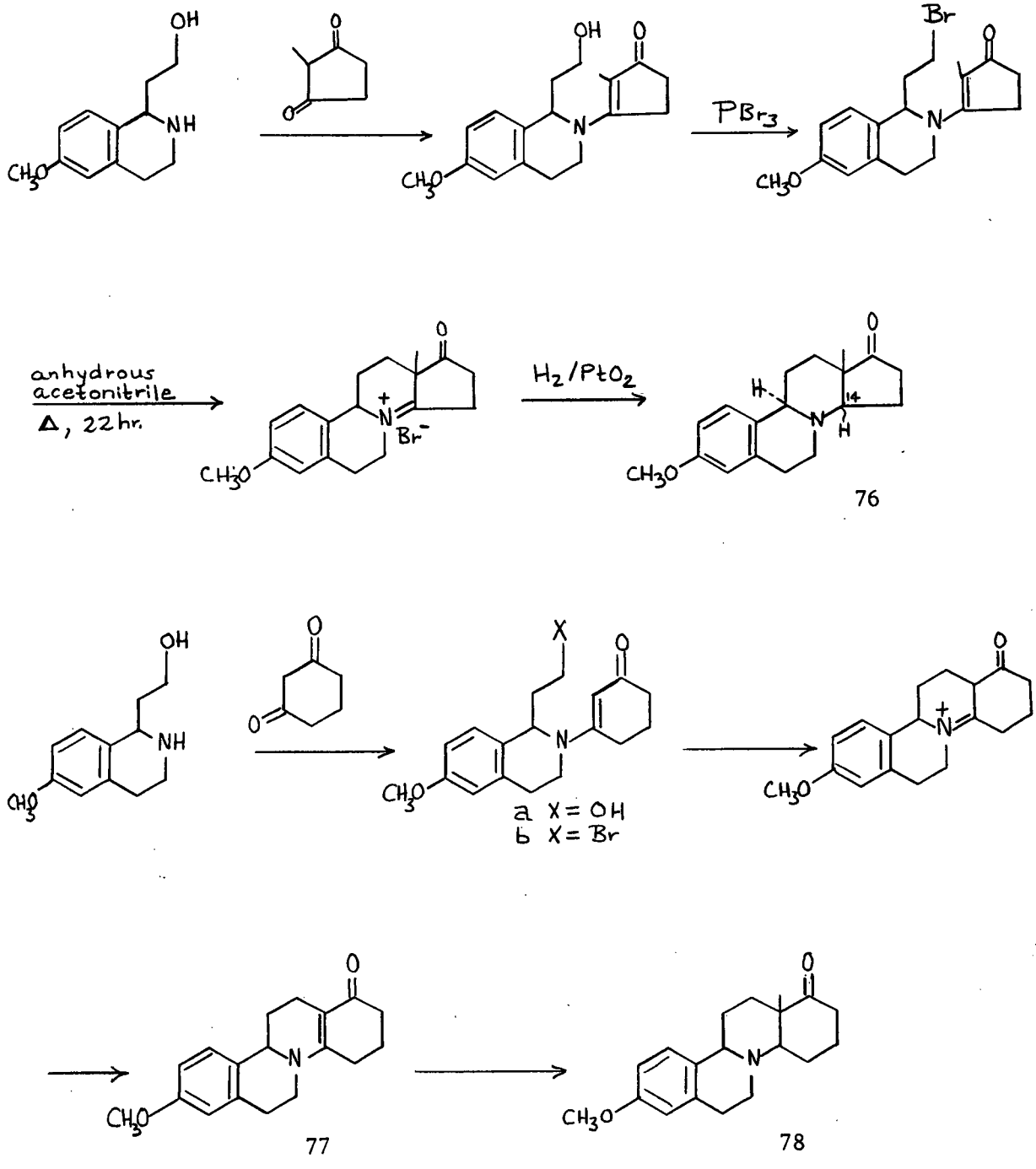


Figure 25. Synthesis of DL-8-Azaestrone Methyl Ether and Related Systems.

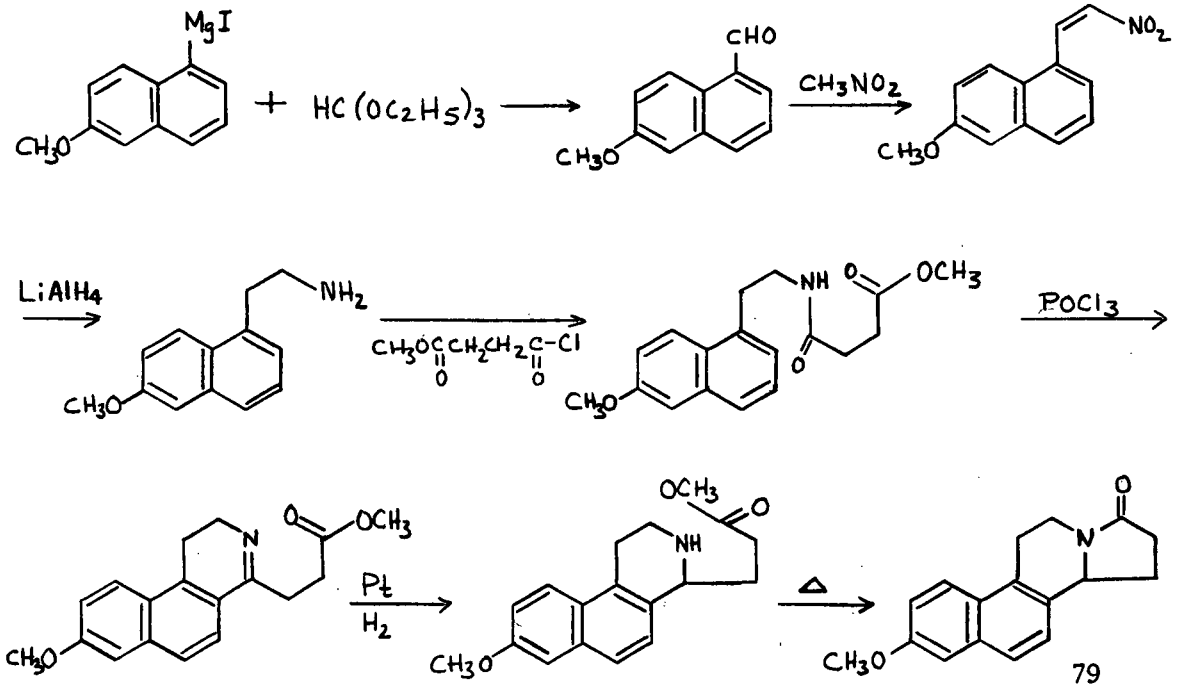


Figure 26. Synthesis of 13-Aza-18-Norequilenin Methyl Ether

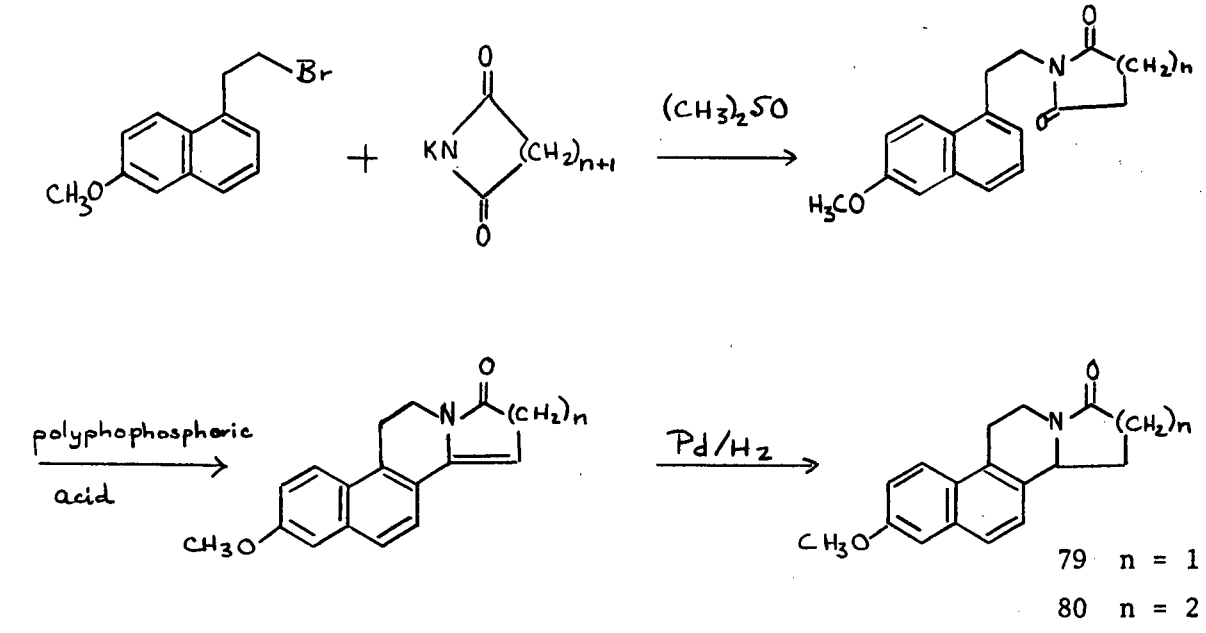


Figure 27. Synthesis of 13-Azaequilenin Derivatives.

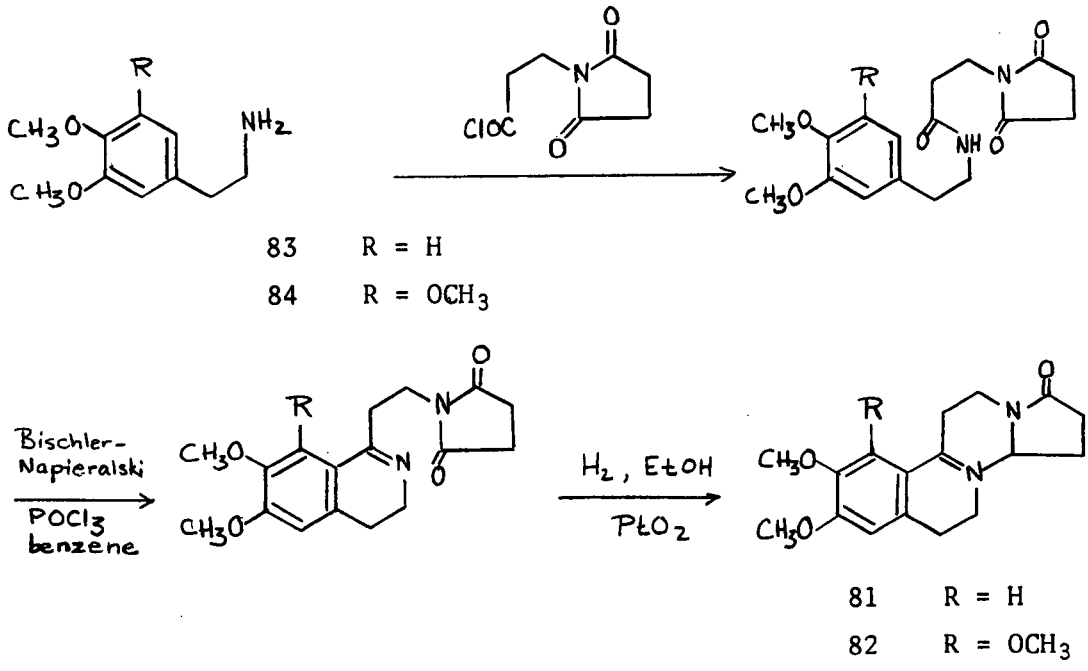
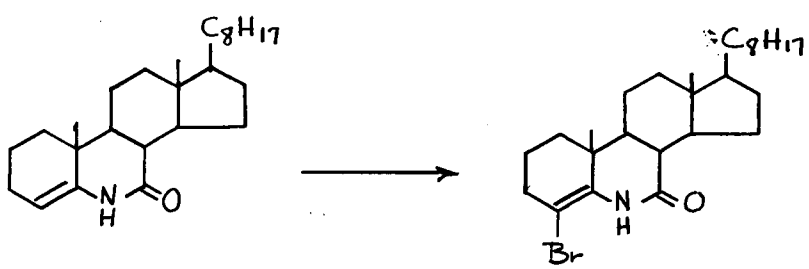


Figure 28. Total Synthesis of 8,13-Diaza-18-noroestrone Methyl Ethers.

DISCUSSION

In previous syntheses of 6-aza steroids of the cholestane,[39,40,42] androstane,[39,40] and pregnane[41] series, the hetero atom was introduced by cyclization of the appropriate seco keto esters with amines. (Figure 12). During the isolation of the keto esters, elimination of the oxygen function at C_3 was normally observed. In cases where elimination was prevented by the exclusion of base from the work-up of the ozonolysis reaction, the subsequent reaction of the keto-ester with the amine resulted in loss of the C_3 function. It was therefore necessary to investigate a possible modification of the above sequence in order to achieve a synthesis of ring A-oxygenated 6-aza steroids.

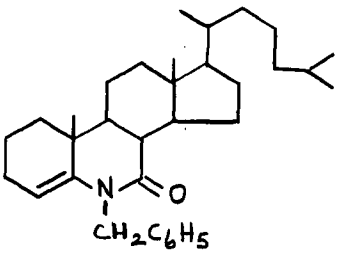
Jacobs and Brownfield[42] investigated re-introduction of an oxygen function at C_3 of the enamine lactam (85). Using conditions suitable for allylic bromination, (N-bromosuccinimide and bromine-carbon tetrachloride)



85 86

a high yield of the undesired vinyl bromide (86) was obtained. Initial investigations in our laboratory were concerned with a method of introducing a functional group at C_3 of the enol lactam (87). This position did not exhibit normal allylic character and all attempts in this direction met with failure.

The possibility of achieving a selective reaction at the 2,3-double



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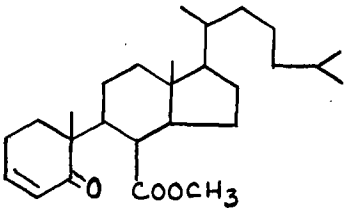

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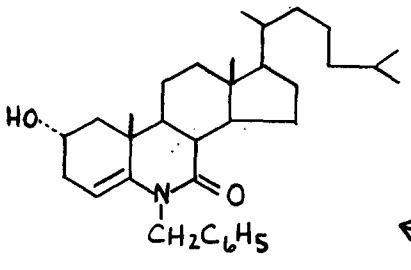

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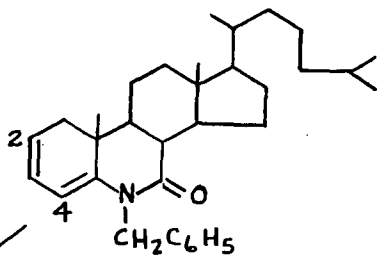

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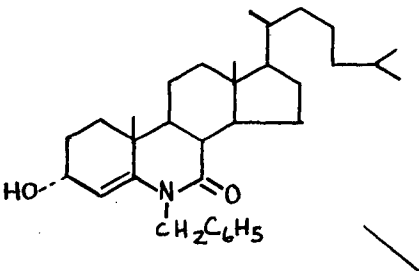

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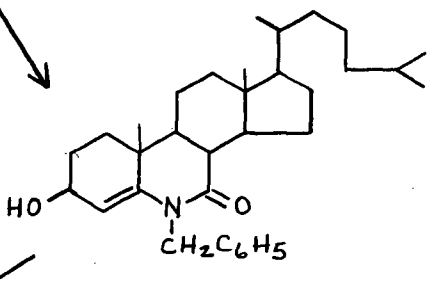

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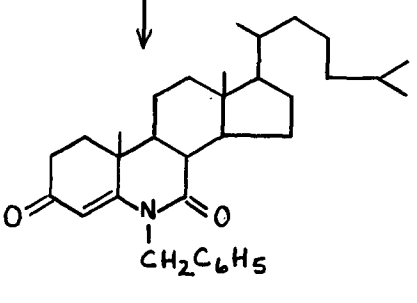
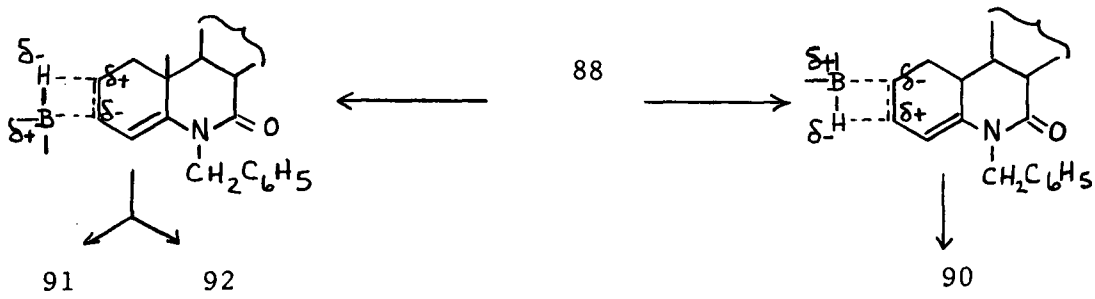


93

bond in the doubly unsaturated lactam (88) was then investigated. The latter compound was prepared in the usual manner[39,40,42] when methyl-5-oxo-5,7-seco-6-nor-3-cholesten-7-oate (89) was reacted with refluxing benzylamine. The ultraviolet spectrum of the resulting N-benzyl-6-aza-2,4-cholestadien-7-one (88) (λ_{max} 297 mμ) was in good agreement with the ultraviolet spectrum reported for the corresponding diene in the -NH series (λ_{max} 299 mμ).[42] The structure was conclusively established when catalytic reduction provided the known enol lactam (87). This reaction also indicated that a selective reaction at the 2,3-double bond would be feasible. An attractive approach was the hydroboration technique developed by H.C. Brown and co-workers.[64]

The hydroboration reaction has been extensively investigated, and it is well established that it provides cis anti-Markownikoff addition to unsymmetrical olefins.[64] Consideration of the electronic and steric factors whichnormally govern the course of the addition reveals several reaction products which might be anticipated in the hydroboration of the unsaturated lactam (88). Thus, the electronic consideration which postulates that the direction of addition is controlled primarily by the polarization of the boron-hydrogen bond would predict that any formal positive charge which may develop in the intermediate would prefer to reside at C_3, as its neutralization is easily accommodated by the adjacent 4,5-double bond. The subsequent conversion of this intermediate (normally considered to be a four-centred transition state)[64] to the final product would yield the 2-hydroxy derivative.

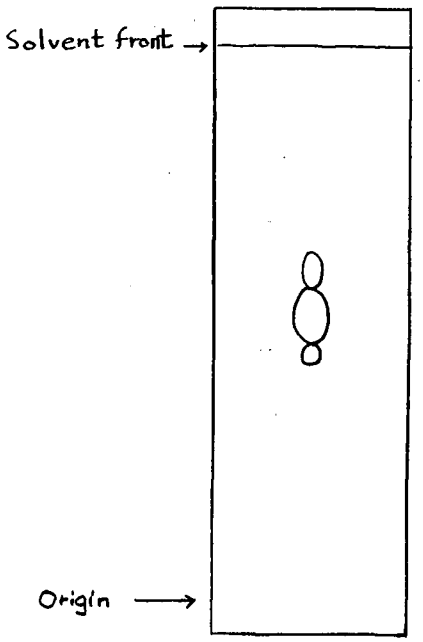
The sterically less favoured β-approach of the reagent and the steric interactions which would exist between the 2β-hydroxyl function and the angular methyl group at C_{10} predict that a predominance of the 2α-hydroxyl derivative (90) would result.



91 92 90

On the other hand, the possibility of the reaction of 88 with the hydroborating reagent in a manner which provides formation of a carbon-boron bond at C_3 is less clear, both from an electronic and steric viewpoint. The electronic consideration provides no real direct evidence, and molecular models reveal that the approach of the hydroborating reagent at C_3 is feasible from either the α or β sides of the molecule, although there is some steric preference for α approach. Therefore, it was reasonable to expect that if any 3-hydroxy analogues were formed both the 3α- and 3β-hydroxy compounds would be isolated (91 and 92 respectively) with the 3 isomer in predominance. Indeed, the experimental results are in reasonable agreement with the above postulates.

Hydroboration of the doubly unsaturated lactam (88) at 0° with diborane in anhydrous diglyme, followed by treatment of the resulting organoborane with alkaline hydrogen peroxide, provided a white solid reaction product, m.p. 133-136°. Thin layer chromatography (TLC) of this product revealed that three components were actually present. Furthermore, TLC indicated that separation into the pure compounds might prove difficult since the R_F values of these substances were very similar. (A typical TLC plate is shown on the left, below). The initial separation of these



compounds by preparative thin layer chromatography was however, successfully accomplished and subsequent investigations showed that careful and extensive column chromatography could be utilized for the separation of larger quantities.

The least polar compound, m.p. 151-153°, obtained crystalline after the above separation, was of interest due to its desirable spectral properties. The ultraviolet spectrum showed the characteristic enol lactam absorption (λ_{max} 237 mμ) indicating that reaction at the 2,3-double bond had occurred. This was confirmed by the infrared absorption bands at 1635 and 1670 cm^{-1} which are characteristic of the enol lactam. That simple hydration of the diene system in ring A had occurred was shown beyond doubt when the molecular formula was established as $C_{33}H_{49}O_2N$ by high resolution mass spectrometry. The NMR spectra for all three compounds will be discussed in detail below.

The second product isolated was crystallized from aqueous methanol, m.p. 144-145.5°. It also exhibited the presence of an enol lactam system (λ_{max} 237 mμ; ν_{max} 1625 and 1670 cm^{-1}), and a simple hydration of the 2,3-double bond was again evident from its molecular formula, $C_{33}H_{49}O_2N$.

The third and most polar compound isolated from the hydroboration reaction was also a crystalline substance, m.p. 176-178°, which again exhibited spectral characteristics in agreement with the desired hydroboration product. It also had the molecular formula $C_{33}H_{49}O_2N$.

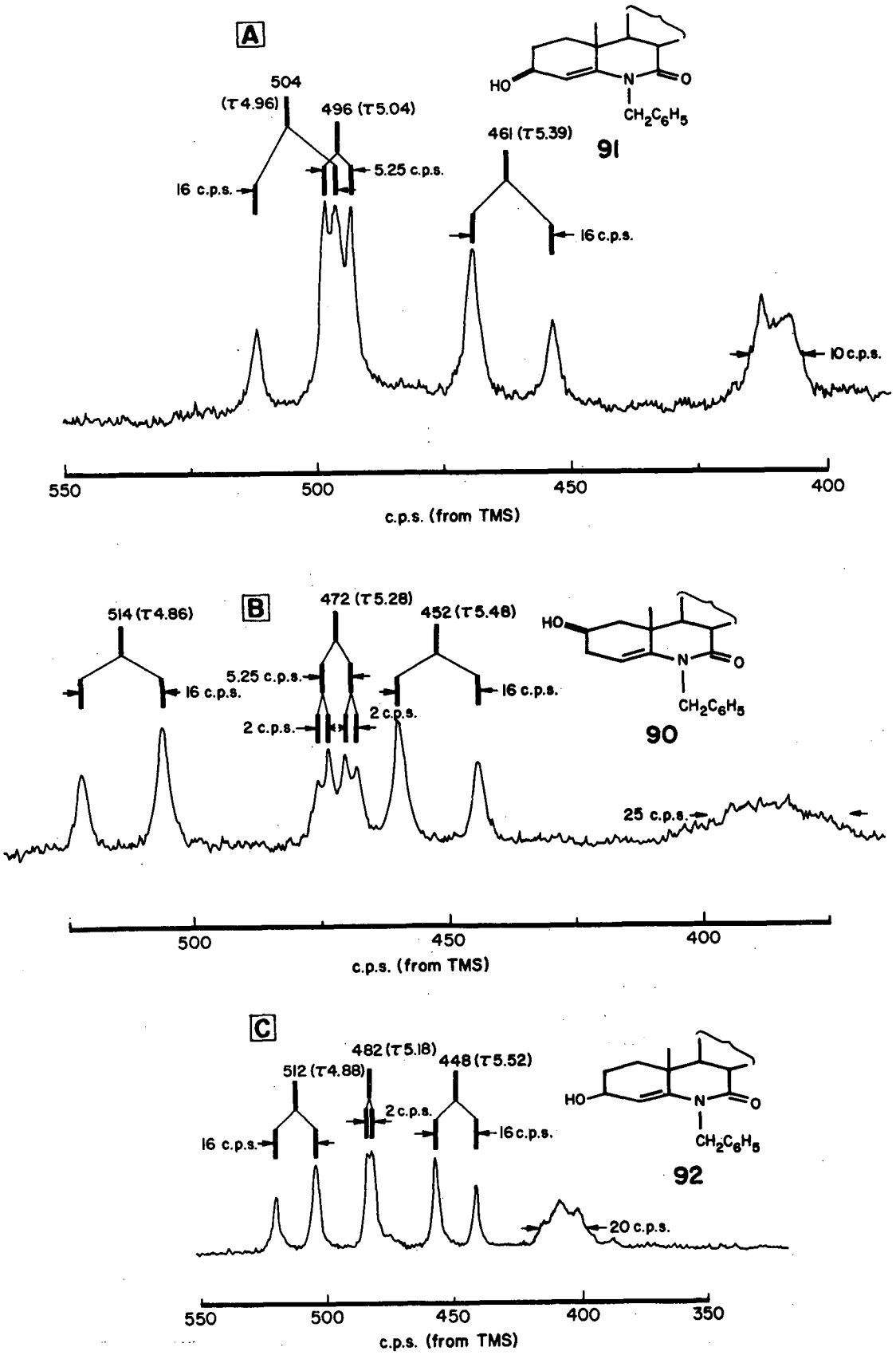
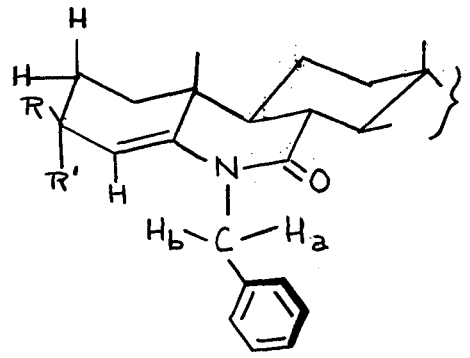


FIG **29** Low Field Region of NMR Spectra of Hydroboration Products.

The NMR spectra obtained for the above three compounds will now be discussed in detail since a comparative analysis of the data allows structural assignments in all three instances. The NMR studies were carried out in considerable detail at 60 and 100 Mc/s so that coupling constants and chemical shifts were readily determined. Figure 29 shows a reproduction of the relevant low field regions of the spectra of these compounds determined at 100 Mc/s. Examination of the splitting patterns reveals several characteristic features. These are: (a) the presence in each instance of a pair of doublets (J = 16 c.p.s.), rather typical of an AB system; (b) an olefinic proton absorption, the splitting pattern differing in each instance depending on the substitution of the adjacent carbon; and (c) a broad multiplet at higher field which is readily attributable to a proton attached to the alcohol-bearing carbon atom. Analysis of sections A, B, and C in Figure 29 can be made in terms of structures 91, 90 and 92 respectively.

If 3α-hydroxy-N-benzyl-6-aza-4-cholesten-7-one is first considered in terms of its conformational structure (91a), it can be readily shown that the presence of the 4,5-double bond transforms ring A into a half-chair conformation, and the dihedral angles involving substituents at C_2 and C_3 are not significantly altered from those encountered in the simple cyclohexene series.



87a R = R' = H

91a R = H; R' = OH

92a R = OH; R' = H

93a R = R' = O

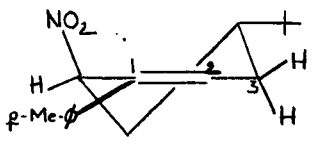
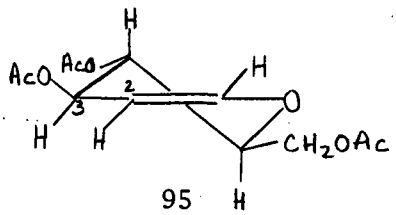
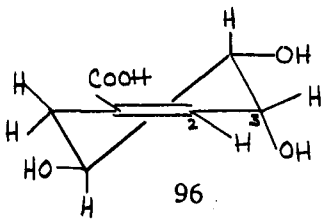
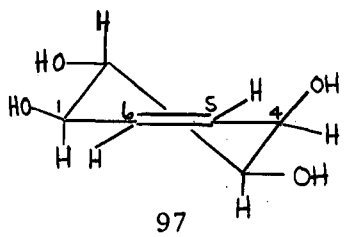
Some detailed studies of vinyl-allylic proton spin couplings have been reported in the literature and the observed values for the cyclohexene derivative (94),[65] D-glucal triacetate (95),[66] shikimic acid (96),[67] and conduritol F (97)[68] are listed in Table 1.

The olefinic proton signal at τ 5.04 in section A, Figure 29, is a doublet. The magnitude of the coupling constant, J = 5.25 c.p.s., is in good agreement with the values of $J_{2,3e}$ and $J_{5,4e}$ observed for (94) and (97) respectively, indicating that the 3α-hydroxyl function is present. Further confirmation of this assignment is available from the width of the C_3 proton signal. Since $J_{2a,3}$ and $J_{2e,3}$ in 91 are expected to be small (usually 2-5 c.p.s.; for example $J_{3,4}$ = 4.4 c.p.s. in 97), and $J_{3,4}$ is shown to be approximately 5 c.p.s., the half-height width of the C_3 proton multiplet should be of the order of 9-15 c.p.s. The observed value is 10 c.p.s. On this basis the least polar hydroboration product was

assigned structure 91.

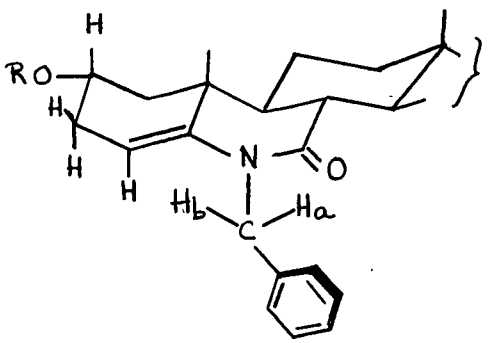
TABLE 1

Vinyl-Allylic Spin Couplings

Compound	J in c.p.s.		Reference
 94	$J_{2,3a}$	2.5	65
	$J_{2,3e}$	5.0	
 95	$J_{2,3a}$	3.2	66
 96	$J_{2,3e}$	4.0	67
 97	$J_{1,6}$	1.9	68
	$J_{4,5}$	5.3	

Section B in Figure 29 represents the low field proton region of the NMR spectrum of the second hydroboration product (m.p. 144-145.5°). The olefinic proton signal in this case is a quartet indicating that the hydroxyl group must be at C_2 rather than at C_3. The coupling constants observed for the substituted cyclohexene 94[65] mentioned above were $J_{2,3a}$ = 2.5 and $J_{2,3e}$ = 5.0. c.p.s. Our observed values of $J_{3a,4}$ = 2 c.p.s. and

$J_{3e,4}$ = 5.25 c.p.s. are in agreement with these. If the proton at C_2 is axial it should appear as a broad multiplet due to its spin coupling with the neighbouring protons at C_1 and C_3 ($J_{a,e}$ = 2-5 c.p.s. and $J_{a,a}$ = 6-12 c.p.s.).[69,70] Although no distinct resolution of the multiplet could be achieved, it was observed as a broad absorption with a half-height width of 25 c.p.s. and structure 90 was assigned.



90a R = H
90b R = Ac

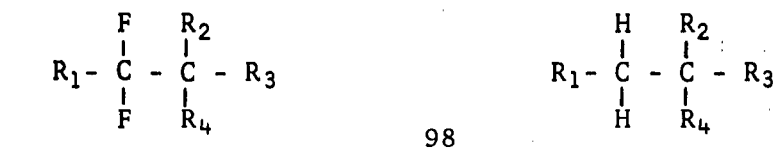
The most polar compound isolated from the hydroboration reaction was also subjected to an extensive NMR study, and section C in Figure 29 illustrates the low field region of the spectrum. One of the striking differences noted in this spectrum is the narrow doublet (J = 2 c.p.s.) observed for the olefinic proton. This situation is immediately reminiscent of the small coupling constant, $J_{2,3a}$ = 2.5 c.p.s., observed in the spectrum of 94.[65] In the case of conduritol F (97)[68] the observed value of $J_{1,6}$ was 1.9 c.p.s. Thus the proton at C_3 was assigned the axial position. Further evidence to support this assignment came from a

consideration of the C_3 proton signal. If it was axially oriented, the electron coupled spin-spin interaction of this proton with the two adjacent protons (axial and equatorial) at C_2, as well as with the C_4 olefinic proton would predict a multiplet with an approximate half-height width of 10-20 c.p.s. The experimental value observed was 20 c.p.s. The NMR data is therefore consistent with the assignment of structure 92 to this product.

As predicted from steric considerations only the 2α-hydroxy alcohol was isolated. A predominance of the 3α-isomer (91) was observed as expected. The relative amounts of 90, 91 and 92 isolated were 5:2:1 respectively.

To complete the discussion of the NMR data it is necessary to consider the pair of doublets (J = 16 c.p.s) which is a characteristic feature in the NMR spectra of all the N-benzyl-6-aza-7-one steroid derivatives synthesized in our laboratory. These are typically shown in Figure 29 and are due to the methylene protons of the benzyl group.

It has been recognized for some time that geminal protons and fluorine atoms attached to any dissymmetric moiety are magnetically non-equivalent and often couple with each other in the NMR spectrum. The earlier work on substituted ethanes (98)[71,72] suggested that this feature



98

was characteristic of the methylene protons (or fluorines) when this group was adjacent to an asymmetric center. Later studies revealed that this requirement was unnecessary. Recent publications from two laboratories[73,74] have reported results on the magnetic non-equivalence of benzylic methylene protons in various heterocyclic bases, while two other groups have presented

data in the phthalimidine[75] and imidazolidinone[76] series which are even more directly pertinent to the present discussion. (Table 2). In the two latter instances, the N-benzyl moiety is attached through the nitrogen atom to an asymmetric centre. In the present investigation a somewhat different system is available as the compounds do not contain an asymmetric centre in the immediate vicinity of the benzylic protons. The data for a variety of N-benzyl-6-aza-7-one steriod analogues is summarized in Table 3.

The difference between the chemical shifts of these two protons is possibly rationalized on the basis of reasonable assumptions about preferred conformations of these compounds. Molecular models reveal that minimum interactions between the phenyl group and the neighbouring atoms occur when this group lies in a plane approximately perpendicular to the plane of the lactam system. As the conformational structures 90a, 91a and 92a indicate, this situation places the two geminal protons (H_a and H_b) in different environments. Clearly H_a would be influenced by the lactam carbonyl group while H_b is in close proximity to the C_4 olefinic linkage in the ring A unsaturated 6-aza compounds. Since the relative effects on these two protons are difficult to ascertain, it is not possible to make definite predictions as to which proton is deshielded.

The NMR data provided good evidence for the structures of the three hydroboration products. Chemical evidence to support the proposed structural assignment was also obtained. The most direct confirmation of the suggested structures would be simple dehydration to the parent diene 88. Although dehydration of 90, 91 and 92 might be a reasonably facile process, it would be expected that the rate of dehydration might differ somewhat. It is well-known that diaxial elimination is a preferred process,

TABLE 2

Coupling Constants for Benzylic Methylene Protons

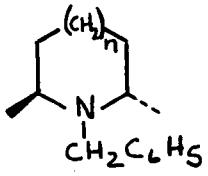
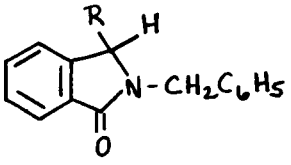
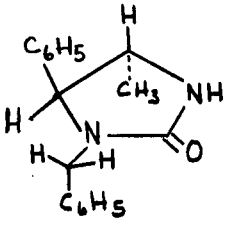
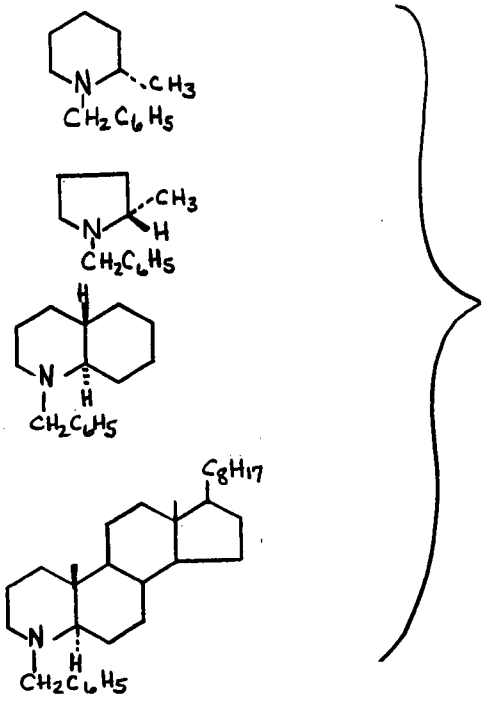
Compound		J (c.p.s.)	Reference
	n = 1 n = 0	14 13.2	73
	R = C_6H_5 R = CH_3	15	75
		15	76
		12-13	74

TABLE 3

Nuclear Magnetic Resonance Data (100 Mc/s) on Benzylic Protons

Compound	Proton	Line Positions (cps separation from TMS)	Chemical Shift τ-scale	Chemical Shift Difference, ppm
87 (see 87a)	a, b	528,512	4.80	0.68
		460,444	5.48	
90 (see 90a)	a, b	522,506	4.86	0.62
		460,444	5.48	
90b	a, b	522,506	4.86	0.57
		465,449	5.43	
91 (see 91a)	a, b	512,496	4.96	0.43
		469,453	5.39	
92 (see 92a)	a, b	520,504	4.88	0.64
		456,440	5.52	
93 (see 93a)	a, b	516,500	4.92	0.32
		484,468	5.24	

and therefore it was reasonable to assume that the 3α-hydroxyl group should eliminate more readily than the isomeric 3β function.

The instability of the axial isomer, 3α-hydroxy-N-benzyl-6-aza-cholest-4-ene-7-one (91), was immediately apparent during its attempted purification. Column or thin-layer chromatography of this compound on alumina immediately led to partial conversion to the diene (88). On standing, pure 91 had a tendency to dehydrate to the starting diene. On the other hand, the equatorial 3β-hydroxy derivative (92) was considerably more stable and its purification was not quite so difficult. Dehydration occurred to a lesser extent and it was possible to purify this compound by chromatographic procedures with only a small loss due to conversion to the diene.

The relative ease of dehydration was again evident in the attempted acetylation of 91 and 92. In both instances, reaction with acetic anhydride and pyridine provided the diene 88 as one of the major components. In each case some acetylation had occurred as indicated by the appropriate carbonyl absorption in the infrared spectrum of the crude reaction mixture, but isolation of the pure compounds was rendered impossible by their constant tendency to revert to the diene.

A difference in the ease of dehydration was also noted in the mass spectra of the isomeric 3-hydroxy compounds 91 and 92 (Figure 30, see also Table 4). The 3α-hydroxy isomer 91 lost water most readily and in both cases the M-18 fragment (m/e 473) formed the base peak. On the other hand, the base peak in the mass spectrum of the 2-hydroxy derivative (90) was the molecular ion (m/e 491) indicating that loss of water does not occur so readily in this case.

The mass spectra of aza steroids have been studied in detail[77] and

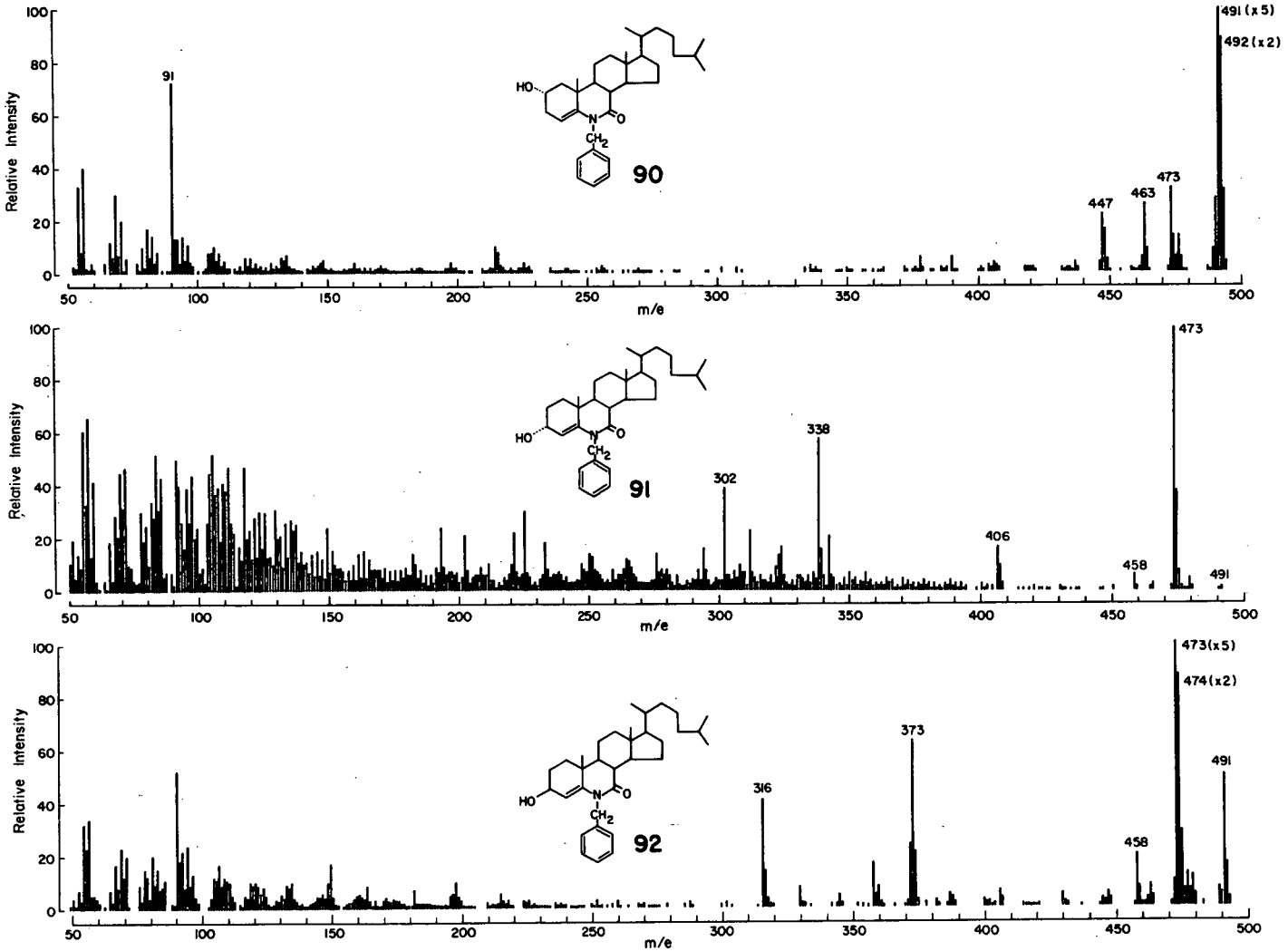
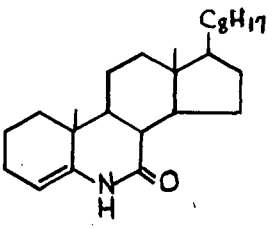


FIG. 30 MASS SPECTRA

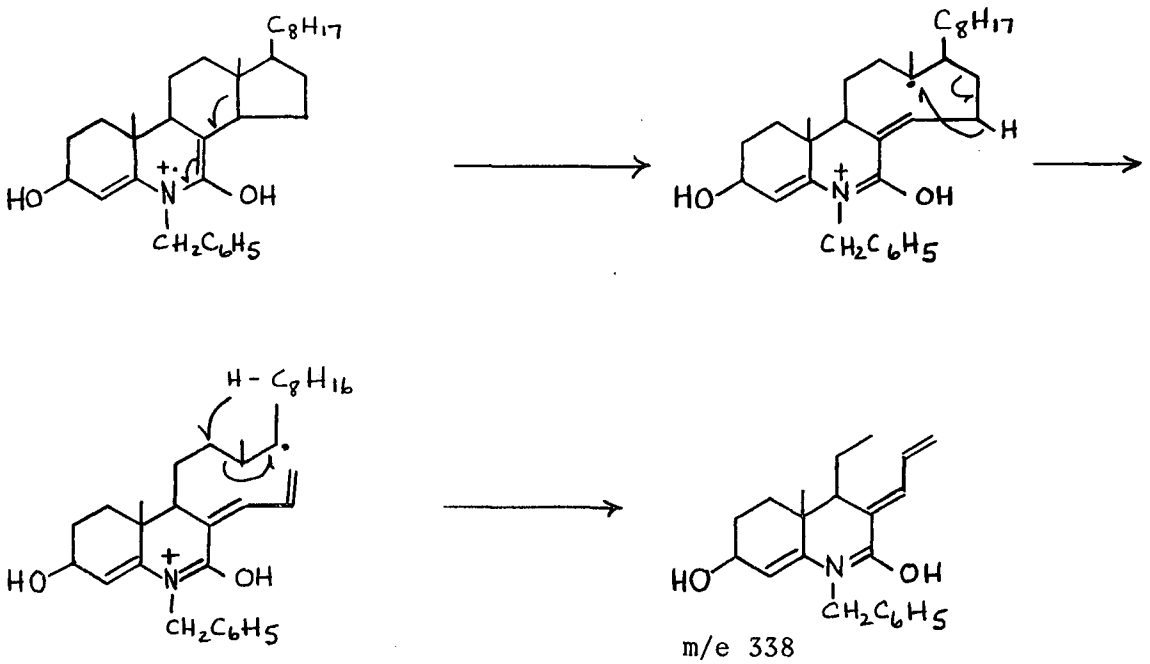
peaks corresponding to M-15, M-28 or 29, M-43 or 44, and, in the case of cholesterol derivatives, M-85 were characteristic of these compounds. The peak at m/e 91 due to the benzyl fragment ($C_7H_7^+$) was common to all the spectra of N-benzyl derivatives and was also observed in the spectra of the three hydroboration products. The peaks at m/e 476 (M-15), 463 (M-28) and 447 (M-44) in the mass spectrum of 90 are characteristic and are probably due to the loss of CH_3, CO, and CHO + CH_3 respectively.

It is interesting to note that the mass spectra of the two 3-hydroxy isomers 91 and 92 are quite different. The peaks at 458 (M-33), 406 (M-85), 373 (M-118), 358 (M-133) and 316 (M-175) in the mass spectrum of 92 were relatively intense. The first two are probably due to the loss of water plus CH_3, and C_6H_{13} of the cholesterol side chain respectively. The last three peaks are 16 mass units greater than the corresponding M-28, M-43 and M-85 peaks observed at m/e 357, 342 and 300 in the spectrum of 99 and are probably due to preliminary loss of the benzyl group followed by the normal fragmentations observed for 6-aza steroids.[77]



99

The peaks at m/e 458 and 406 also appeared in the mass spectrum of 91. The relatively strong peak at 338 probably corresponds to the one observed 16 mass units lower in the spectrum of the enol lactam (87) which was attributed to fragmentation of the C and D rings:[77]

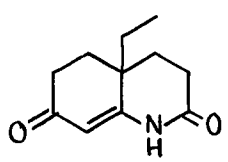


The remainder of the spectrum is complex, and cannot be readily interpreted.

The experimental results indicated that the 2-hydroxy derivative (90) was considerably more stable than the other hydroboration products. Chromatographic purification could be achieved without difficulty, and reaction of this material with acetic anhydride and pyridine provided the corresponding acetate. This compound exhibited the expected spectral features in the infrared and ultraviolet spectra (1740 cm^{-1}, λ_{max} 237 mμ), and the molecular formula, $C_{35}H_{51}O_3N$, was established by high resolution mass spectrometry. Saponification of the acetate regenerated in part the starting material 90, but provided in addition a small amount of the diene (88). This demonstrated that dehydration is also feasible in this system, but somewhat stronger conditions are necessary than in the case of the 3-hydroxy derivatives 91 and 92.

The final step in the synthetic sequence was the oxidation of the alcoholic functions to yield the desired Δ^4-3-keto chromophore in ring A. Several different experiments were carried out and these are discussed in succession.

Oxidation of 92 with chromium trioxide in acetone (Jones reagent) or in pyridine (Sarett reagent) yielded a new crystalline compound, m.p. 173-174.5°, $C_{33}H_{47}O_2N$, which exhibited spectral properties in accord with the 3-keto structure 93. Of particular note was the ultraviolet spectrum which changed on the addition of alkali to the solution (λ_{max}^{EtOH} 284 mμ; λ_{max} 287 mμ 5 minutes after addition of a few drops of 0.1N NaOH; λ_{max} 292 mμ after 30 minutes; and λ_{max} 294 mμ after 3 hours). The bathochromic shift (237 mμ ⟶ 284 mμ) observed in neutral solution and the spectral changes in alkali are in excellent agreement with those reported for the identical chromophore (100), ($\lambda_{max}^{H_2O}$ 284 mμ; λ_{max} 334 after 3 minutes in 0.1N NaOH; λ_{max} 293 mμ after 30 minutes).[78] There was also a marked



100

alteration in the optical rotatory dispersion (ORD) curve of the oxidation product compared to the typical Cotton effect already known for the enol lactam systems[79] and observed for the three hydroboration products. (Figure 31). As the ORD curves of aza steroids will be discussed in Part 2 of this thesis no further comment will be made here.

The olefinic proton signal in the NMR spectrum was a sharp singlet at lower field than in the spectra of the corresponding alcohols 91 and 92. These results established the structure of the oxidation products as 93.

In a similar manner, compound 91 was oxidized to 93. This provided conclusive evidence that the only difference in the structures of 91 and 92 was the stereochemistry at C_3, as had been indicated previously by the NMR

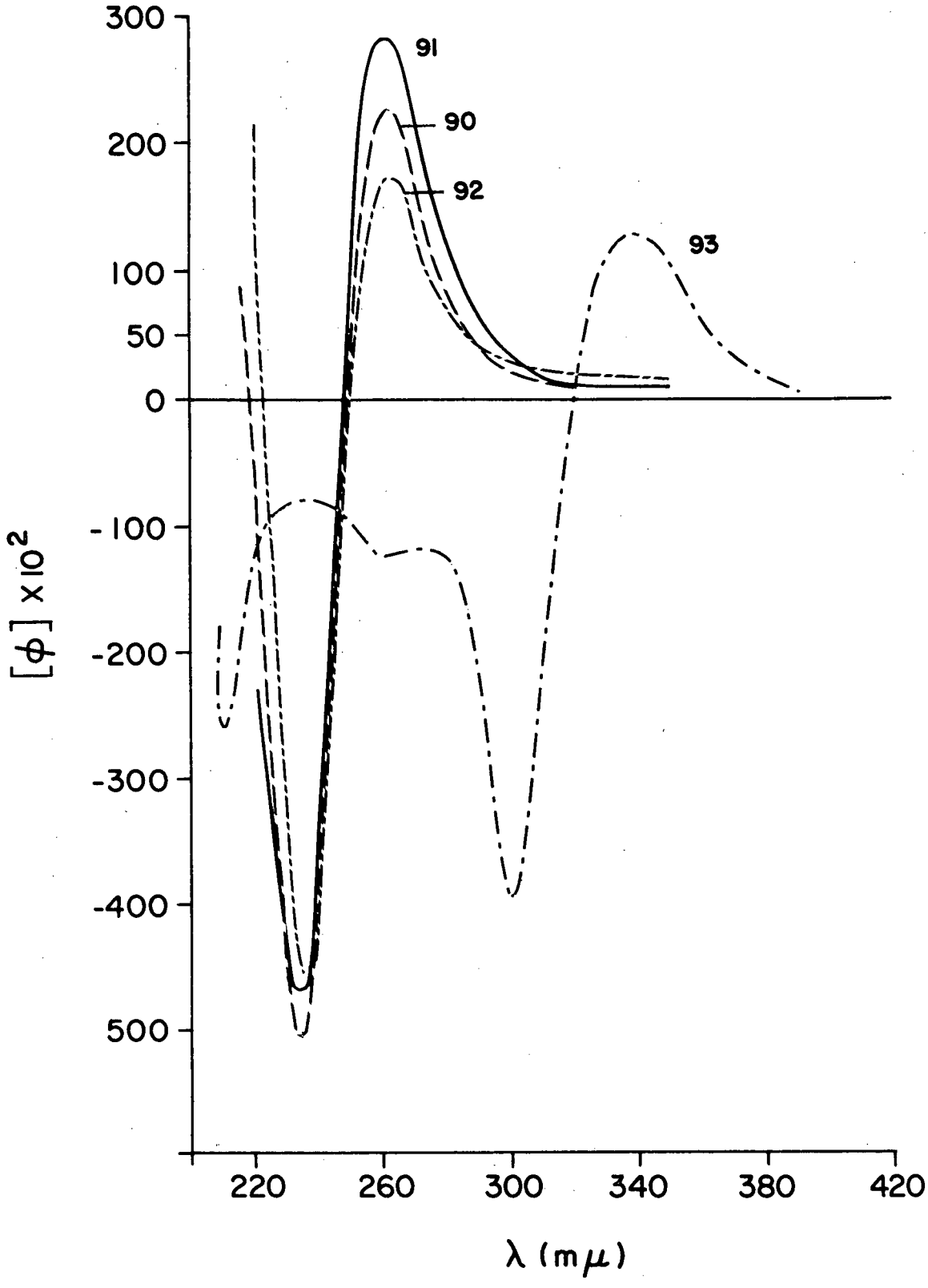


Fig. 31 ORD Curves

data.

In subsequent experiments the total hydroboration mixture was subjected to oxidation and the desired Δ^4-3-keto-6-aza steroid 93 was isolated. In this way better yields of 93 could be obtained.

Since the appropriate unsaturated lactam system required for hydroboration is also available in the androstane and pregnane series this synthesis of ring A-oxygenated steroids could be extended to these series.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Ultraviolet spectra were measured in methanol solution on a Cary 11 spectrophotometer, and infrared spectra were taken as KBr pellets on a Perkin-Elmer Model 21 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded at 60 megacycles/sec. on a Varian A60 instrument and at 100 megacycles/sec. on a Varian HA100 instrument, using deuteriochloroform as solvent; the line positions or centres of multiplets are given in the Tiers τ scale with reference to tetramethylsilane as the internal standard; the multiplicity, integrated areas and type of protons are indicated in parentheses. Only the values obtained at 100 megacycles/sec. are recorded below. The mass spectra were taken on an Atlas CH4 mass spectrometer, using the direct insertion technique, the electron energy being maintained at 70 eV. The high resolution mass spectra for the determination of molecular formulae were obtained on an AEI MS9 mass spectrometer. The optical rotatory dispersion (ORD) curves were taken in methanol solution on a JASCO UV/ORD/CD-5 spectropolarimeter.

In all of our experiments, the thin-layer chromatography plates were prepared from neutral alumina (Woelm), to which 1% by weight of a fluorescent indicator (Electronic phosphor, General Electric Co.) was added. Antimony trichloride in glacial acetic acid or 50% aqueous orthophosphoric acid were used as the spray reagents. In general, the plates were heated for 10 minutes at 100°C after spraying, during which time, the compounds are recognized as blue spots on the chromatoplates. The solvent systems utilized benzene and chloroform, and these are indicated below in parentheses.

For column chromatography, neutral alumina (Woelm) was used in all cases, and deactivation was done by the addition of water. The approximate

activity of the adsorbent utilized in specific experiments is indicated below.

N-Benzyl -6-aza-2,4-cholestadien-7-one (88)

Methyl 5-oxo-5,7-seco-6-nor-3-cholesten-7-oate (89, 2.2 g.) was taken up in benzylamine (5 ml.), and the mixture refluxed for 15 hours under an atmosphere of nitrogen. The cooled reaction mixture was treated with ether, and the latter was washed with dilute aqueous hydrochloric acid to remove excess benzylamine. The separated ether solution was washed with 5% aqueous sodium hydroxide and water, and finally, dried over anhydrous magnesium sulfate. Removal of the solvent in vacuo, initially on a steam bath, and finally, at 200-220°/0.1 mm., provided a yellow-brown glass (2.0 g.). Chromatography of this reaction product on alumina (200 g., activity IV) yielded the desired lactam (88, 1.2 g.) as a colorless viscous oil. All attempts to obtain the product in crystalline form failed, although its purity was established by TLC (benzene); λ_{max}(log ε): 297 mμ (3.73); ν_{max}: 1670, 1638 and 1575 cm^{-1} (diene lactam); NMR: 2.88 (multiplet, 5H, aromatic), 4.24, 4.60, 4.95, 5.09 (multiplets, 5H, olefinic H and $-N\underline{CH_2}C_6H_5$). Calc. for $C_{33}H_{40}NO$: 473.365; found 473.365.

Catalytic Reduction of N-Benzyl-6-aza-2,4-cholestadien-7-one

The lactam 88 (50 mg.) was dissolved in ethanol (10 ml.) , and to this solution, 10% palladium on charcoal (30 mg.) was added. The reduction was allowed to proceed for 30 minutes, during which time, one mole of hydrogen had been absorbed. Removal of the catalyst and evaporation of the solvent yielded the reduction product (45 mg.), which was identical in every respect (IR, mixed melting point, UV, TLC) with the known enol lactam 87.[39,40]

Hydroboration of N-Benzyl-6-aza-2,4-cholestadien-7-one

The lactam (88, 1.2 g.) was dissolved in anhydrous diglyme (24 ml.) and placed in a small three-necked flask. Diborane gas generated in another vessel was then slowly passed into this mixture kept at 0°C over a period of two hours. The reagent was prepared from sodium borohydride (630 mg.) in diglyme (17 ml.) to which a solution of boron trifluoride-etherate (2.8 ml.) in diglyme (12 ml.) was slowly added over a period of two hours.

The reaction mixture was immediately treated with 5% aqueous sodium hydroxide (45 ml.) and 30% aqueous hydrogen peroxide (12 ml.), and after allowing to stand for 5 minutes, it was extracted with ether. The ether extract was thoroughly washed with water and 5% aqueous ferrous sulfate solution. After drying over anhydrous magnesium sulfate, and evaporation of the solvent, a slightly yellow amorphous material (1.1 g.) was obtained. Trituration of the latter with petroleum ether caused some separation of a white solid (m.p. 133-136°). However, for the chromatographic separations mentioned below, the total crude product was always used.

The crude reaction product was initially shown by TLC to be a mixture of three components (chloroform, R_f values of 0.49, 0.50 and 0.60). A detailed investigation of the possibility of utilizing preparative TLC as a method of separation was carried out, and a typical experiment is described.

The crude hydroboration mixture (120 mg.) was placed on an air-dried chromatoplate (60 X 20 cm., 65 g. of adsorbent, 0.4 mm. approximate thickness), and the plate was developed in chloroform. Removal of the zones and extraction with methanol-ether (1:1) provided a fraction (10 mg.) subsequently shown to be 92, another fraction (69 mg., 90), and a third

fraction (22 mg., 91). The recovery (101 mg., 80%) was consistently good in this separation.

For larger scale separations, careful column chromatography could be utilized. For this purpose, alumina (activity IV) was the most desirable adsorbent, and benzene was the eluting solvent. In a typical separation, the crude hydroboration mixture (2.4 g. obtained in another experiment) was chromatographed on alumina (200 g.). Careful elution with benzene yielded a total of 46 fractions (50 ml. each), which were then examined by TLC. Fractions 13-17 (230 mg.) were combined and contained mainly 91 and a trace of 90. Fractions 18-24 (630 mg.) were combined and contained mainly 92 and traces of 90.

Each of these combined portions were rechromatographed (ratio of adsorbent:material of 100:1) to finally yield pure 91 (210 mg., R_f 0.60), 90 (570 mg., R_f 0.50), and 92 (110 mg., R_f 0.49).

In each instance, the column chromatographic separation was complicated by decomposition of the compounds. It will be noted that considerably less than half (only 890 mg. from 2.2 g.) of the desired hydroboration products were recovered. Appreciable dehydration of both 91 and 92 to the lactam 88 was always observed, whereas 90 was stable and could be purified without difficulty.

In general, the TLC technique was superior in overall separation and recovery, although obviously more tedious in its application.

The data obtained for the three hydroboration products is as follows:

3β-Hydroxy-N-benzyl-6-aza-4-cholesten-7-one (92): m.p. 176-178° (crystallized from ether-hexane), λ_{max}(log ε): 237 mμ (4.03); ν_{max}: 3460 (OH), 1667 and 1630 cm^{-1}(enol lactam); NMR: 2.90 (multiplet, 5H, aromatic),

4.88 (doublet, J = 16 c.p.s., 1H, H of -N-$\underline{CH_2}C_6H_5$ group, see 92a), 5.52 (doublet, J = 16 c.p.s., 1H, H of -N-$\underline{CH_2}C_6H_5$ group, see 92a), 5.18 (doublet, J = 2 c.p.s., 1H, olefinic), 5.93 (broad multiplet, 1H, $\rangle\underline{CH}OH$); ORD: (Figure 3, C, 0.0999 mg./ml.), $[\phi]_{350}$ + 1472, $[\phi]_{300}$ + 2945, $[\phi]_{263}$ + 17,700 (peak), $[\phi]_{250}$ 0, $[\phi]_{236}$ - 46,300 (trough), $[\phi]_{218}$ + 21,600. Calc. for $C_{33}H_{49}NO_2$: 491.376. Found: 491.371.

3α-Hydroxy-N-benzyl-6-aza-4-cholesten-7-one (91): m.p. 151-153° (crystallized from ether-hexane), λ_{max}(log ε): 237 mμ (4.01); ν_{max}: 3510 (OH), 1670 and 1635 cm^{-1} (enol lactam); NMR: 2.90 (multiplet, 5H, aromatic), 4.96 (doublet, J = 16 c.p.s., 1H, H of -N-$\underline{CH_2}C_6H_5$ group, see 91a), 5.39 (doublet, J = 16 c.p.s., 1H, H of -N-$\underline{CH_2}C_6H_5$ group, see 91a), 5.04 (doublet, J = 5.25 c.p.s., 1H, olefinic), 5.89 (broad multiplet, 1H, $\rangle\underline{CH}OH$); ORD: (Figure 3, C, 0.0948 mg./ml.), $[\phi]_{350}$ + 1037, $[\phi]_{300}$ + 3630, $[\phi]_{261}$ + 28,500 (peak), $[\phi]_{248}$ 0, $[\phi]_{235}$ - 47,700 (trough), $[\phi]_{220}$ - 23,300. Calc. for $C_{33}H_{49}NO_2$: 491.376. Found: 491.371.

2α-Hydroxy-N-benzyl-6-aza-4-cholesten-7-one (90): m.p. 144-145.5° (crystallized from aqueous methanol), λ_{max}(log ε): 237 mμ (4.01); ν_{max}: 3460 (OH), 1670 and 1630 cm^{-1} (enol lactam); NMR: 2.90 (multiplet, 5H, aromatic), 4.86 (doublet, J = 16 c.p.s., 1H, H of -N$\underline{CH_2}C_6H_5$ group, see 90a), 5.48 (doublet, J = 16 c.p.s., 1H, H of -N$\underline{CH_2}C_6H_5$ group, see 90a), 5.28 (quartet, $J_{3e,4}$ = 2 c.p.s., $J_{3a,4}$ = 5.25 c.p.s., 1H, olefinic), 6.17 (broad multiplet, 1H, $\rangle\underline{CH}OH$); ORD: (Figure 3, C, 0.0973 mg./ml.), $[\phi]_{330}$ + 1010, $[\phi]_{300}$ +2020, $[\phi]_{262}$ + 22,950 (peak), $[\phi]_{248}$ 0, $[\phi]_{234}$ - 51,000 (trough), $[\phi]_{216}$ +8080. Calc. for $C_{33}H_{49}NO_2$: 491.376. Found 491.373.

Acetylation of Hydroboration Products

Attempts to obtain the acetate derivatives of 90, 91 and 92 were only partially successful. The isomeric C_3-hydroxy compounds (91 and 92)

could not be successfully acetylated (acetic anhydride, pyridine), since both compounds led to a mixture of the desired acetates (infrared data only) and the lactam 88. Further chromatographic purification of the acetylation mixture merely provided for further conversion of the acetate to the lactam 88.

The experiment was more successful in the conversion of the 2-hydroxy compound (90) and is described.

The 2α-hydroxy-6-aza steroid (90, 25 mg.) was treated with pyridine (0.5 ml.) and acetic anhydride (0.5 ml.), and the mixture was heated for 25 minutes at 70°C. The cooled mixture was poured onto ice water, and the white precipitate which formed was separated and dried (22 mg.) This material was recrystallized from ether-methanol to yield the pure acetate (10 mg.), m.p. 156-157.5°; λ_{max}(log ε): 237 mμ (4.08); ν_{max}: 1740 and 1245 (OAc), 1670 and 1643 cm^{-1} (enol lactam); NMR: 2.90 (multiplet, 5H, aromatic), 4.86 (doublet, J = 16 c.p.s., 1H, H of -N$\underline{CH_2}C_6H_5$ group, see 90b), 5.43 (doublet, J = 16 c.p.s., 1H, H of -N$\underline{CH_2}C_6H_5$ group, see 90b), 5.26 (quartet, $J_{3e,4}$ = 2.4 c.p.s., $J_{3a,4}$ = 5.8 c.p.s., 1H, olefinic), 5.03 (broad multiplet, 1H, C$\underline{H}$OAc), 8.05 (singlet, 3H, $\underline{CH_3}$CO). Calc. for $C_{35}H_{51}NO_3$: 533.387. Found: 533.383.

Saponification of 2α-Acetoxy-N-benzyl-6-aza-4-cholesten-7-one

The above acetate (5 mg.) was taken up in a mixture of ethanol (1 ml.) and 1N aqueous potassium hydroxide (0.3 ml.), and the mixture allowed to stand for 10 minutes at 20°C. The reaction mixture was made acidic by the addition of 1M acetic acid in ethanol, and then evaporated to dryness. The residue was extracted with chloroform and this extract was placed on a chromatoplate (chloroform). Two zones were eluted from the plate (methanol-ether 1:1). One of these was shown to be the 2α-hydroxy compound

90 (2 mg.), and the other was the lactam 88 (1 mg.).

Oxidation of Hydroboration Products

A series of experiments were performed on the isolated pure products, as well as on the crude hydroboration mixture. Chromium trioxide in acetone (Jones reagent) and in pyridine (Sarett reagent) gave identical results. Several typical experiments are described.

The 3 -hydroxy-6-aza derivative (90, 22 mg.) was treated for 12 hours at room temperature with chromium trioxide (30 mg.) in pyridine (1 ml.). After this time, methanol (0.5 ml.) was added and the reaction mixture was evaporated to dryness. The residue was taken up in chloroform, and the concentrated chloroform extract was placed directly on a thin-layer chromatoplate and separated (benzene-chloroform 1:1). The material which was eluted from the plate with ether-methanol was recrystallized from ether-hexane to provide a pure sample of N-benzyl-6-aza-4-cholesten-3, 7-dione (93, 5 mg.), m.p. 173-174.5°; λ_{max}(log ε): 284 mμ in neutral methanol solution (4.39): 287 mμ, after 5 minutes in the presence of 0.1N sodium hydroxide (4.33); 292 mμ, after 30 minutes in the presence of 0.1N sodium hydroxide (4.06); 294 mμ, after 3 hours in contact with alkali (4.32); 296 mμ, after 20 hours in contact with alkali (4.31); ν_{max}: 1680, 1665 and 1590 cm^{-1} (ketone and lactam carbonyl); NMR: 2.88 (multiplet, 5H, aromatic), 4.92 (doublet, J = 16 c.p.s., 1H, H of $-N\underline{CH_2}C_6H_5$ group, see 93a), 5.24 (doublet, J = 16 c.p.s., 1H, H of $-N\underline{CH_2}C_6H_5$ group see 93a), 4.60 (singlet, 1H, olefinic); ORD: (Figure 3, C, 0.103 mg./ml.), $[\phi]_{400}$ + 950, $[\phi]_{340}$ + 13,070 (peak), $[\phi]_{320}$ 0, $[\phi]_{300}$- 29,400 (trough), $[\phi]_{270}$ -11,880, $[\phi]_{260}$ - 12,360, $[\phi]_{230}$ - 8070, $[\phi]_{208}$ - 18,040. Calc. for $C_{33}H_{47}NO_2$: 489.361. Found: 489.362.

A similar oxidation of the 3α-hydroxy-6-aza steroid (91, 20 mg.) provided 93 (4 mg.). The latter compound was shown to be identical in every respect (mixed melting point, infrared, TLC) with the above oxidation product.

The crude mixture (53 mg.) taken directly from the hydroboration reaction was oxidized with chromium trioxide (85 mg.) in pyridine (2.2 ml.) for 12 hours at room temperature. Addition of methanol (2 ml.), and work-up of the reaction mixture as described above provided the crude oxidation product. The latter was again subjected to TLC separation, and the eluted material (13 mg.) was crystallized from ether-hexane to provide pure 93 (11 mg.).

TABLE 4

Mass Spectra of Hydroboration Products

Compound 91

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
491	2	400	3	358	4	320	6	282	3	244	3
490	1	398	1	357	3	319	3	281	5	243	3
480	2	394	2	356	7	318	4	280	8	242	3
479	5	393	1	355	3	317	4	279	6	241	5
478	1	392	3	354	4	316	6	278	8	240	4
477	1	391	1	353	2	315	3	277	7	239	6
476	2	390	3	352	5	314	5	276	14	238	7
475	8	389	2	351	4	313	7	275	4	237	6
474	38	388	4	350	7	312	23	274	4	236	6
473	100	387	2	349	2	311	2	273	3	235	5
472	2	386	3	348	5	310	7	272	3	234	8
465	3	385	1	347	2	309	7	271	3	233	18
464	2	384	2	346	3	308	10	270	3	232	6
459	2	383	1	345	2	307	5	269	3	231	4
458	6	382	3	344	5	306	7	268	5	230	2
450	2	381	1	343	6	305	4	267	6	229	3
446	1	380	3	342	21	304	6	266	9	228	2
445	1	379	2	341	2	303	6	265	11	227	5
437	1	378	4	340	6	302	39	264	12	226	7
436	1	377	2	339	16	301	4	263	8	225	30
434	1	376	3	338	58	300	4	262	6	224	6
432	1	375	1	337	5	299	5	261	6	223	5
431	1	374	3	336	7	298	3	260	4	222	7
430	2	373	2	335	3	297	2	259	8	221	22
426	1	372	4	334	6	296	4	258	3	220	10
425	1	371	2	333	3	295	8	257	4	219	6
423	1	370	5	332	5	294	16	256	3	218	4
422	1	369	1	331	6	293	4	255	4	217	5
420	2	368	2	330	6	292	8	254	6	216	2
418	1	367	1	329	2	291	4	253	7	215	3
416	1	366	4	328	4	290	4	252	8	214	1
414	1	365	3	327	2	289	3	251	13	213	4
408	3	364	5	326	3	288	4	250	14	212	4
407	10	363	2	325	6	287	2	249	7	211	10
406	17	362	3	324	17	286	2	248	8	210	6
404	2	361	2	323	14	285	2	247	10	209	6
402	2	360	3	322	9	284	6	246	4	208	9
401	1	359	2	321	3	283	2	245	4	207	6

TABLE 4 (cont'd)

Mass Spectra of Hydroboration Products

Compound 91

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
206	6	168	8	130	20	92	40	54	9
205	5	167	8	129	31	91	50	53	14
204	3	166	8	128	10	90	2	52	5
203	8	165	12	127	13	89	7	51	20
202	21	164	5	126	13	87	7	50	11
201	5	163	15	125	30	86	5		
200	3	162	5	124	16	85	43		
199	7	161	14	123	30	84	31		
198	6	160	4	122	13	83	52		
197	7	159	10	121	28	82	28		
196	7	158	4	120	12	81	34		
195	6	157	8	119	23	80	10		
194	9	156	4	118	20	79	25		
193	24	155	8	117	47	78	19		
192	7	154	9	116	12	77	30		
191	9	153	9	115	17	76	3.5		
190	3	152	10	114	2	75	3		
189	6	151	15	113	22	74	4		
188	2	150	7	112	26	73	9		
187	6	149	24	111	47	72	10		
186	3	148	5	110	38	71	47		
185	8	147	12	109	41	70	32		
184	5	146	6	108	19	69	45		
183	10	145	15	107	39	68	21		
182	14	144	6	106	37	67	29		
181	6	143	14	105	52	66	4		
180	6	142	6	104	45	65	19		
179	9	141	11	103	26	64	1		
178	5	140	10	102	3	63	4		
177	8	139	15	101	8	61	1		
176	4	138	11	100	7	60	4		
175	8	137	25	99	24	59	42		
174	4	136	23	98	20	58	13		
173	8	135	27	97	44	57	66		
172	3	134	13	96	26	56	33		
171	9	133	26	95	39	55	61		
170	5	132	9	94	16				
169	8	131	21	93	26				

Compound 90

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
494	0.8	432	0.4	351	0.2	252	0.2	203	0.2	167	0.4
493	6.2	422	0.2	350	0.4	250	0.2	202	0.2	166	0.2
492	35.6	421	0.4	348	0.2	247.	0.2	201	0.2	165	0.4
491	100.0	420	0.4	347	0.2	246.	0.2	200	0.4	164	0.2
490	5.6	419	0.4	346	0.2	245	0.2	199	0.4	163	0.4
489	1.8	418	0.4	340	0.2	245	0.2	198	0.8	162	0.4
488	0.2	408	0.4	339	0.2	243	0.4	197	0.4	161	0.8
487	0.4	407	0.6	338	0.4	242	0.4	196	0.4	160	0.4
479	0.2	406	0.8	337	0.2	241	0.2	195	0.2	159	0.4
478	0.2	405	0.4	336	0.6	240	0.2	194	0.2	158	0.3
477	1.2	404	0.6	334	0.4	239	0.2	193	0.2	157	0.4
476	2.8	402	0.2	310	0.2	238	0.2	192	0.2	156	0.2
475	1.2	401	0.4	308	0.4	237	0.2	191	0.2	155	0.2
474	2.8	400	0.2	302	0.4	236	0.4	190	0.2	154	0.2
473	6.4	392	0.2	297	0.2	229	0.2	189	0.2	153	0.2
472	0.4	391	0.2	296	0.2	228	0.5	188	0.2	152	0.4
465	0.2	390	1.2	286	0.2	227	0.4	187	0.4	151	0.3
464	1.8	388	0.4	285	0.2	226	0.8	186	0.4	150	0.4
463	5.2	387	0.2	284	0.2	225	0.4	185	0.4	149	1.0
462	1.2	386	0.4	279	0.2	224	0.4	184	0.2	148	0.8
461	0.4	383	0.2	275	0.2	223	0.2	183	0.4	147	0.6
460	0.2	382	0.2	274	0.2	222	0.2	182	0.2	146	0.4
459	0.2	379	0.4	273	0.2	221	0.4	181	0.2	145	0.6
458	0.4	378	1.2	272	0.2	220	0.2	180	0.2	144	0.3
454	0.2	377	0.2	271	0.2	219	0.2	179	0.2	143	0.4
450	0.2	376	0.4	270	0.4	218	0.4	178	0.2	141	0.2
449	1.0	375	0.2	269	0.2	217	0.6	177	0.2	140	0.2
448	3.2	373	0.2	268	0.2	216	1.6	176	0.2	139	0.3
447	4.4	372	0.4	266	0.2	215	2.0	175	0.2	138	0.4
446	0.8	364	0.4	264	0.2	214	0.4	174	0.3	137	0.4
438	0.4	363	0.2	260	0.2	213	0.4	173	0.4	136	0.6
437	0.8	362	0.2	258	0.2	212	0.2	172	0.4	135	1.4
436	0.2	360	0.2	257	0.4	211	0.2	171	0.6	134	1.0
435	0.4	357	0.2	256	0.6	210	0.4	170	0.4	133	1.2
434	0.4	356	0.2	255	0.2	206	0.2	169	0.3	132	0.4
433	0.2	352	0.2	254	0.4	205	0.2	168	0.2	131	0.6

Compound 92

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
130	0.4	90	0.2	493	0.8	416	0.2	330	1.6	240	0.3
129	0.8	89	0.2	492	3.4	415	0.3	320	0.3	239	0.3
128	0.2	87	0.2	491	10.0	407	0.8	319	0.3	239	0.4
127	0.5	85	1.6	490	1.1	406	1.3	318	0.8	237	0.3
126	0.3	84	0.8	489	1.5	404	0.3	317	2.8	236	0.4
125	0.6	83	2.8	483	0.4	403	0.2	316	8.2	234	0.2
124	0.4	82	1.2	480	0.8	402	0.5	314	0.2	233	0.2
123	0.8	81	3.4	479	2.4	401	0.4	304	0.2	232	0.2
122	0.4	80	0.4	478	1.4	400	0.6	302	0.5	231	0.2
121	1.2	79	2.0	477	2.6	389	0.4	300	0.3	230	0.2
120	0.6	78	0.2	476	1.4	388	0.9	289	0.2	229	0.2
119	1.2	77	0.8	475	5.8	387	1.0	288	0.5	228	0.3
118	0.2	73	1.2	474	35.2	386	0.3	286	0.3	227	0.2
117	0.6	72	0.2	473	100.0	383	0.3	279	0.3	226	0.6
116	0.2	71	5.0	472	2.0	382	0.6	276	0.2	225	0.5
115	0.4	70	1.4	471	0.4	378	0.4	275	0.2	224	0.6
113	0.4	69	6.0	464	0.9	375	0.6	274	0.2	220	0.2
112	0.4	68	1.2	463	1.7	374	4.2	273	0.2	219	0.2
111	1.0	67	2.4	462	0.7	373	12.6	272	0.2	218	0.6
110	0.6	65	0.8	461	0.3	372	4.8	270	0.4	217	0.3
109	1.6	61	0.3	460	0.3	371	0.5	269	0.3	216	0.6
108	1.0	60	0.8	459	1.6	370	0.2	268	0.4	215	1.1
107	2.0	59	0.3	458	4.0	366	0.2	264	0.2	214	0.4
106	1.6	58	0.4	457	0.3	362	0.2	261	0.2	213	0.3
105	1.6	57	8.0	448	0.7	361	0.5	260	0.6	212	0.3
104	0.4	56	1.6	447	1.1	360	1.6	258	0.3	211	0.2
103	0.2	55	6.6	446	0.5	359	1.0	256	0.2	210	0.5
101	0.2	54	0.4	445	0.7	358	3.4	254	0.3	205	0.2
99	0.6	53	0.6	444	0.2	356	0.2	253	0.2	204	0.2
98	0.8			437	0.2	354	0.3	252	0.6	203	0.2
97	1.2			432	0.3	352	0.3	251	0.2	202	0.2
96	1.0			431	0.5	346	0.4	250	0.3	201	0.2
95	2.8			430	1.0	345	0.6	247	0.2	200	0.6
94	0.8			421	0.3	344	0.3	246	0.2	199	0.8
93	2.6			420	0.2	342	0.3	245	0.2	198	2.0
92	2.6			419	0.2	334	0.2	244	0.2	197	1.1
91	14.4			418	0.2	332	0.4	243	0.2	196	1.0
				417	0.2	331	0.4	242	0.2	195	0.2

m/e	I	m/e	I	m/e	I	m/e	I
194	0.3	156	0.4	117	1.0	70	2.4
193	0.2	155	0.2	116	0.2	69	4.6
192	0.2	153	0.3	115	0.4	68	1.6
191	0.3	152	0.3	113	0.6	67	3.3
190	0.3	151	0.9	112	1.0	66	0.5
189	0.3	150	3.4	111	2.0	65	1.4
188	0.4	149	2.0	110	2.2	63	0.2
187	0.4	148	0.8	109	2.4	61	0.5
186	0.4	147	1.1	108	1.8	60	0.8
185	0.4	146	0.9	107	3.4	59	1.0
184	0.4	145	0.9	106	2.2	58	1.0
183	0.4	144	0.6	105	2.4	57	6.8
182	1.4	143	0.5	104	0.7	56	4.6
181	0.2	142	0.3	103	0.2	55	6.3
180	0.2	141	0.2	99	0.5	54	0.5
179	0.2	140	0.2	98	0.9	53	1.3
178	0.2	139	0.4	97	2.6	52	0.2
177	0.4	138	0.3	96	1.8	51	0.7
176	0.4	137	0.6	95	4.8	50	0.3
175	0.6	136	0.7	94	1.6		
174	0.7	135	1.9	93	4.4		
173	0.5	134	1.6	92	3.6		
172	0.6	133	1.7	91	10.4		
171	0.9	132	0.8	90	0.3		
170	0.6	131	1.1	89	0.4		
169	0.2	130	0.6	86	2.2		
168	0.2	129	0.7	85	1.6		
167	0.7	128	0.3	84	1.4		
166	0.2	127	0.2	83	3.0		
165	0.6	126	0.4	82	1.8		
164	1.7	125	1.0	81	4.0		
163	0.7	124	1.5	80	0.8		
162	0.7	123	1.2	79	3.0		
161	1.1	122	1.8	78	0.5		
160	1.0	121	2.0	77	1.8		
159	0.6	120	1.6	73	0.4		
158	0.5	119	2.0	72	0.2		
157	0.5	118	0.8	71	3.9		

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PART 2

ORD Studies of Lactam and Amide Chromophores

INTRODUCTION

Optical rotatory dispersion, the change in optical rotation with wavelength, was discovered by Biot in 1817 but its widespread application to structural, stereochemical and conformational problems did not begin until 1953. Optical rotatory dispersion (ORD) studies have been performed mainly with saturated and α,β-unsaturated ketones because the $n \rightarrow \pi^*$ transition occurs in the region above 260 mμ to which studies have been restricted until recently. Furthermore, ketones are one of the most common functional groups in organic chemistry, especially if one considers that alcohols are readily oxidized to the corresponding ketones. For saturated ketones the octant rule[1] which permits the prediction of the sign and, semiquantitatively, the intensity of the Cotton effect, has been formulated. This rule has been extended to include α,β- and β,γ-unsaturated ketones.[2,3]

The combination of unequal absorption (circular dichroism) and unequal velocity of transmission (optical rotation) of left and right circularly polarized light in the region in which optically active absorption bands are observed is a phenomenon called the Cotton effect. A plot of the molecular rotation, $[\phi]$, which is proportional to the specific rotation $[\alpha]$, against the wavelength, λ, of the incident light gives a rotatory dispersion (ORD) curve. Optically active chromophores can be classified into two types: (a) the inherently dissymmetric chromophore, and (b) the inherently symmetric, but asymmetrically perturbed, chromophore. Examples of the first class are hexahelicene and twisted biphenyls. The molecular amplitudes of the ORD curves are generally quite high compared to those observed for the second type, a typical example of which is the carbonyl group.[3]

Other chromophores are available which are optically active and absorb in a spectral range convenient for investigation. These chromophores,

usually derivatives of specific functional groups, are listed in Table 1 with the position of their optically active absorption bands. Other non-ketonic chromophores such as biaryls, dienes, aporphines, disulphides, diselenides, trithiones, nitro compounds, azides, thiocyanates, ethylene thioketals, thioacetates, polypeptides, proteins and nucleic acids have also be studied. These subjects have been reviewed recently[3,4] and will not be discussed further here.

Compounds containing the carboxyl group show only plain curves above 270 mμ. The development of automatic recording spectropolarimeters capable of measuring optical rotation down to about 210 mμ has allowed examination of compounds containing the carboxyl chromophore in the region of of their weak absorption band at 225 mμ. Cotton effects have been observed for the carboxyl and related groups in acids,[6] esters,[7] lactones[7,8] and amides.[7] Only the lactone chromophore will be considered in detail. It is of particular interest because of its close relationship to the lactam group.

In connection with previous investigations in the field of azasteroids[9-11] (Figures 12 and 13, Part 1) a series of compounds possessing lactam, enol lactam and amide functions were synthesized. These syntheses involved ring opening followed by cyclization with an amine and reduction of the double bond. Thus the configuration at C_5 in the 6-aza series is

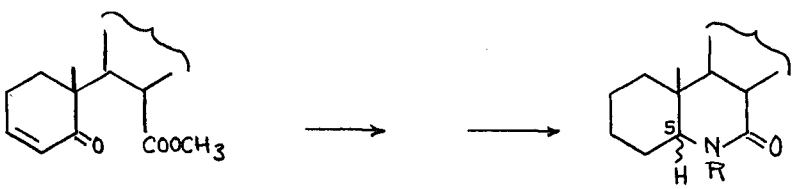
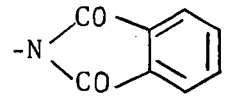
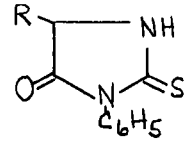
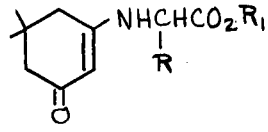
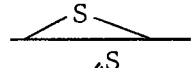
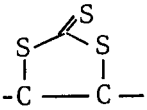
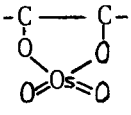


TABLE 1

Chromophoric Derivatives

Functional Group	Chromophoric Derivative	Absorption Maxima (mμ)
$-NH_2$ (and amino acids)	$-NHC(=S)-SR$	330
$-NH_2$ (and amino acids)		300
$\rangle NH$	$\rangle NNO$	370
$-NHCOR$	$-N(NO)-COR$	350-450
$R-CH(NH_2)-COOH$		310
$R-CH(NH_2)-COOH$	$R-CH(COOH)-NHC(=S)OC_2H_5$	280
$RCH(NH_2)-COOH$	$R-CH(COOH)-NHC(=S)C_6H_5$	290, 380
$RCH(NH_2)-COOH$	$R-CH(COOH)-NHC(=S)CH_2C_6H_5$	270, 335
$R-CH(NH_2)-CO_2R_1$		240-320
$-OH$	$-OC(=S)-SR$	350
$-OH$	$-ONO$	325-390
$-OH$	$-O-C(=O)-R$	200-230
$-COOH$	$-CONHC(=S)NR_2$	340
$-COOH$	$-C(=S)NRR$	325-360
$-C=C-$		260
$-C=C-$		235,305,430
$-C=C-$		450, 550

unknown. It was hoped that ORD studies might provide the necessary information for the assignment of configuration to this centre.

In their paper on the synthesis of 6-oxa and 6-aza steroids, Jacobs and Brownfield[12] concluded that the products had the 5α configuration on the basis of molecular rotation differences. (Figure 1). This difference (ΔM_D) is equal to the molecular rotation of the lactone or lactam (M_D) minus the molecular rotation of the parent keto acid (M_D'). The results for compounds prepared by chemical conversions generally assumed to give the most thermodynamically stable products and thus ones of known stereochemistry are shown in Table 2. Since the ΔM_D value reflects only the

TABLE 2

Molecular Rotation Differences

	M_D	M_D'	ΔM_D	C-5
4-oxa-cholestan-3-one	+313	+137.5	+175.5	α
4-oxa-17β-hydroxy -17α-methyl androstan-3-one	+189	+51.2	+137.8	α
4-aza-cholestan-3-one	+170	+137.5	+32.5	α
4-aza-17β-hydroxyandrostan-3-one	+96	-92.5	+188.5	α
4-oxa coprastan-3-one	+71	+137.5	-66.4	β
1	-67	+376	-443	α
2	+81	+376	-295	α

asymmetry about C_5 the configurations of 1 and 2 were assigned on the basis of the observed sign of ΔM_D. It was noted that C_5 in the ring A lactones and lactams used for comparison may be visualized as being epimeric with repsect to C_5 in the B-ring lactone (1) and lactam (2) if the latter compounds

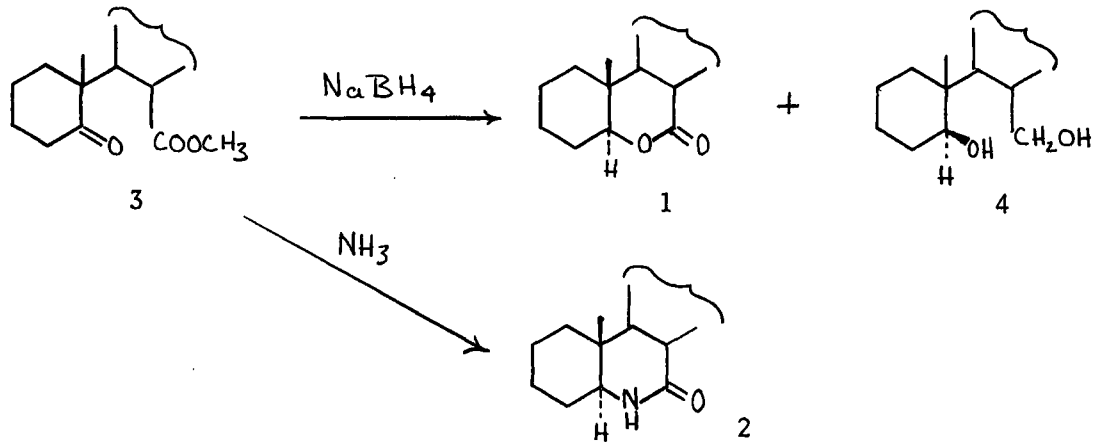
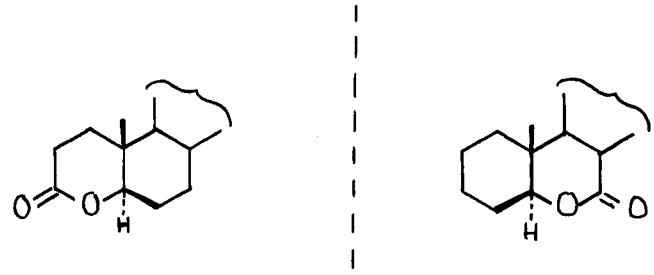


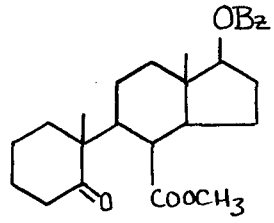
Figure 1. Synthesis of 6-Oxa and 6-Aza Steroids.

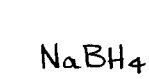
are of the A/B trans configuration. Therefore it was reasoned that if 1 and 2 have this configuration they would exhibit negative ΔM_D values as observed.

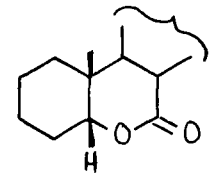


These results were not in accord with those found in the 17β-benzoyloxyandrostane series in which the lactone (5) and hemiacetal (6) were identified.[13] Repetition of Jacobs' and Brownfield's work by Atwater[13] led to identification of the products as 7 and 8 in the 6-oxa series, rather than 4 and 1 respectively. It was also shown that in both the cholestane and 17-keto series large negative rotations (ΔM_D) were observed regardless of the C_5 configuration. (Table 3). The stereochemistry of these lactones was established when it was found that the Baeyer-Villiger oxidation of B-norcoprastan-6-one (11) gave the same lactone (8) that was obtained from the sodium borohydride reduction. Since this oxidative

rearrangement is known to occur with retention of configuration of the migrating centre[14] the A/B cis structure could be assigned to 8. On this basis, no direct evidence was therefore available on the stereochemistry of the 6-aza series.







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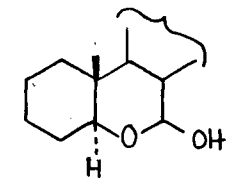

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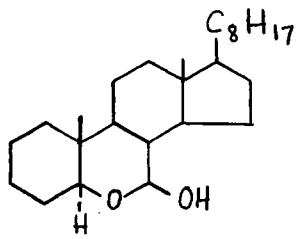

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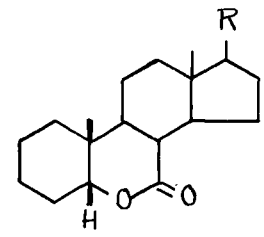
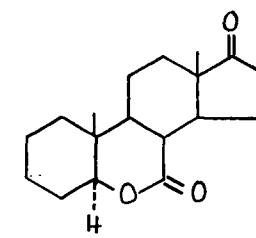


8 R = C_8H_{17}

10 R = O



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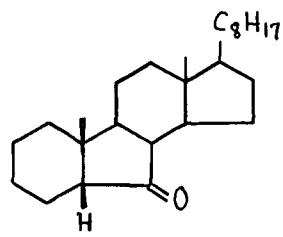

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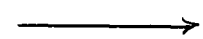

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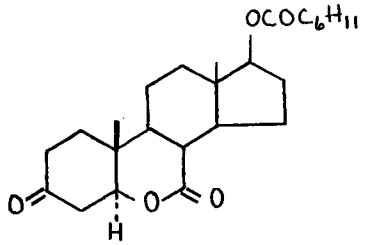
TABLE 3

Molecular Rotations of Oxa Steroids

	ketoester M_D	5β-lactone	5α-lactone	ΔM_β	ΔM_α
Cholestane series (1, 8)	+356	-68	-41	-423	-397
17-keto series (9, 10)	+594	+58	+99	-536	-495

An earlier attempt[13] to resolve this question of stereochemistry based on the difference between the ORD curves of saturated-3-ketones[15] in the A/B cis and A/B trans series was unsuccessful. The ORD of the 5α-lactone (12) was virtually without a Cotton effect while that for 5β-lactone (13) was strongly positive. A positive Cotton effect was also observed for the 5α-compound (14). Similar results have been obtained by other workers and will be discussed below.



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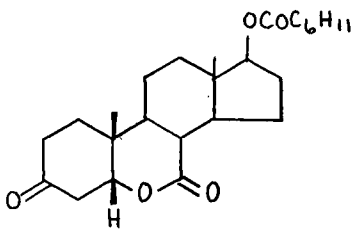

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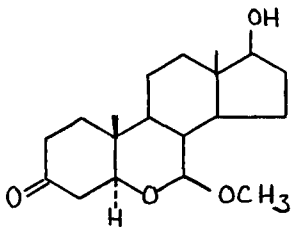
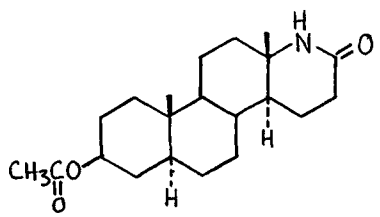


14

Very few ORD studies of the lactam chromophore have been carried out. During the course of our work the ORD curves of lactams (15) and (16) were reported.[16] Positive Cotton effects were observed for both compounds.



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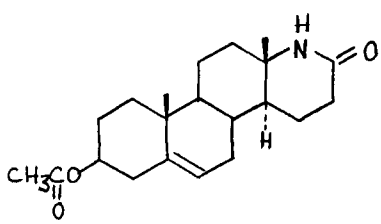
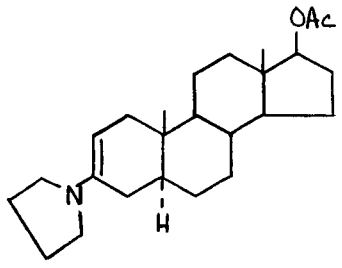


16

The N-substituted Δ^2-steroid enamine 17, and the cyclic enamine 18 were also studied.[18] A very weak positive Cotton effect was observed for 17 and a more intense negative one for 18. The unsaturated amide 19 also showed a



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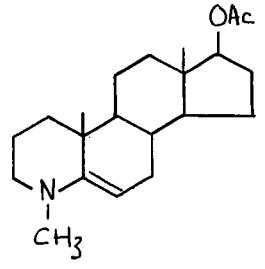

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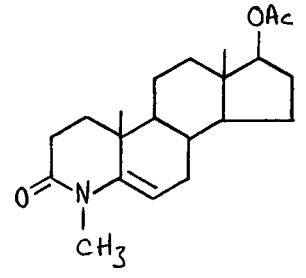


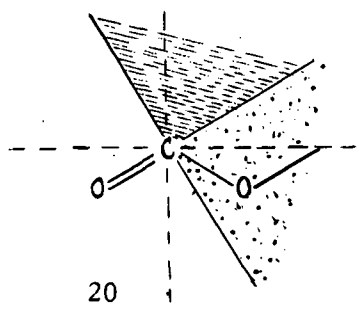
19

negative Cotton effect with the trough and peak at 250 mμ ([ϕ] =-30,000) and 235 mμ ([ϕ] =+60,000) respectively.

Lactones, which are closely related to lactams, have however, been quite extensively studied recently.[7,16-20,22] The sector rule, a semi-theoretical interpretation of the results comparable to the octant rule for ketones, has been proposed.[7] A series of seventy lactones representing nine of the twelve possible stereochemical types were all found to obey this rule.

The lactone group may be considered to be planar according to X-ray studies.[21] To develop the sector rule it is assumed to a crude approximation that the two carbon-oxygen bonds are equivalent and that the plane bisecting the carboxyl angle is a symmetry plane. Each carbon-oxygen bond of the lactone group is considered in turn as a double bond and the signs of the contributions made by the atoms in the far upper octants are allocated according to the ketone octant rule. These appear as 20 and 21 when viewed

from above in projection on the plane of the lactone group. If these two diagrams are superimposed (22A) the signs of the contributions in some sectors cancel in varying degrees while in other sectors the contributions reinforce one another giving a positive contribution in the back upper section E and a negative contribution in the back upper sector B. Atoms near a sector boundary, for example in sector F, near the E/F boundary, will have a small but significant contribution. The signs of the lactone sectors (22B) are the reverse of the signs used in the ketone octant rule.



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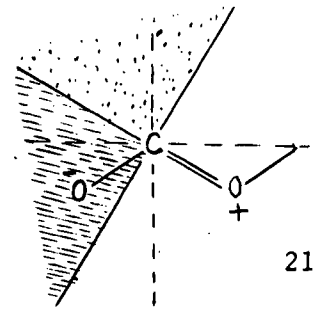

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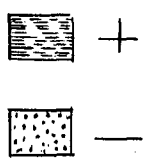
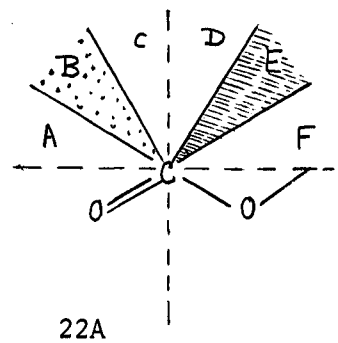
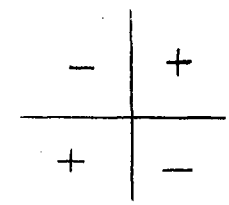




22A

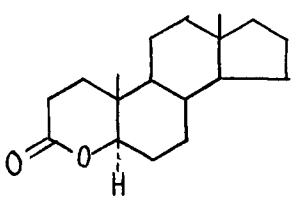


22B

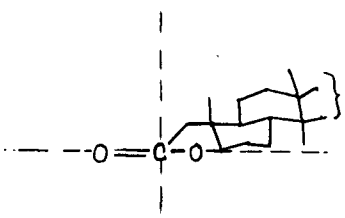
Since the lactone group lies in a true symmetry plane the signs of the back lower sectors, that is those below the plane of the lactone group, are necessarily opposite to those of the back upper sectors. The signs of rotation contributions in the front sectors will presumably be opposite to those of the back sectors but compounds with atoms in near sectors have not yet been considered. In general the immediate neighbourhood of the lactone chromophore determines the sign of the Cotton effect and along any radial line passing through the carboxyl carbon the quantitative effect of a given

substituent will decrease with increasing distance from the chromophore.

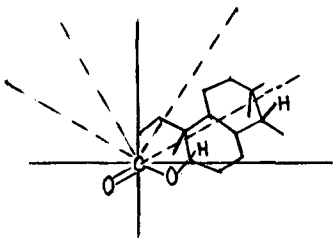
As a convention, when applying the sector rule the hetero ring is drawn to the left of the formula with the upper angular substituent β. In order to predict the sign of the Cotton effect it is necessary to consider two views of the molecule. These are (a) the view along the bisectrix of the O-C-O angle in the plane of the lactone group (23A, 24A, 25A), the usual octant projection and (b) the view of the molecule from above projected onto the plane of the lactone ring (23B, 24B, 25B), the sector projection.



23

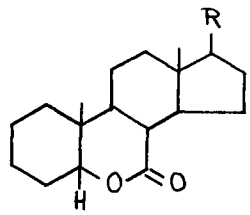


23A



23B

For example, the sector rule predicts a positive Cotton effect for 3-oxo-4-oxa-5α-steroids (23) and a positive Cotton effect is observed. Except for a few exceptions all the compounds considered had terminal lactone rings. Two of these exceptions were the 7-oxo-6-oxa-5β and -5α-steroids, (24) and (25) respectively. Positive Cotton effects were observed for both lactones. In these cases the sector projections (24B and 25B) are complicated by the fact that the lactone group is in the middle ring and no attempt has been made to interpret the sign of the Cotton effect.



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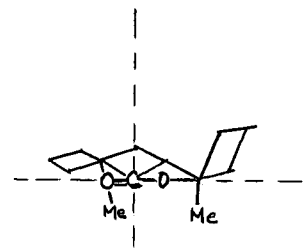
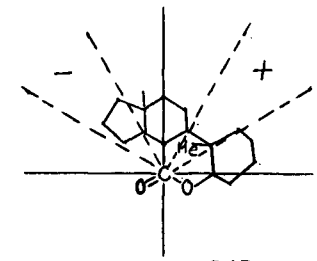
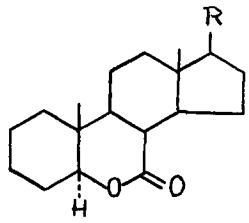


24A



24B



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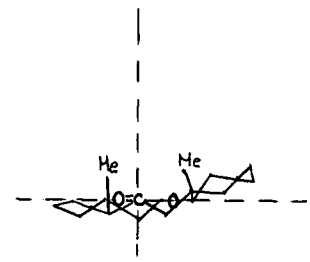
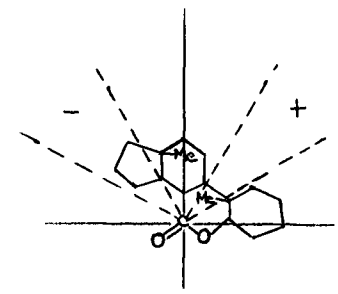
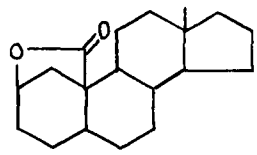


25A



25B

The sector rule has also been successfully applied to a series of bridged ring lactones representing eleven stereochemical types.[17,19] For example, compounds of type 26 have strong positive Cotton effects. This is as would be expected from a consideration of the octant and sector projections which show that the contributions of several pairs of atoms will



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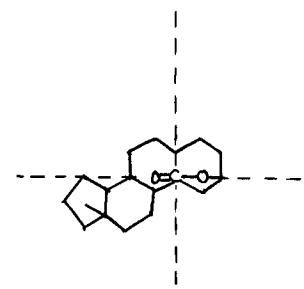
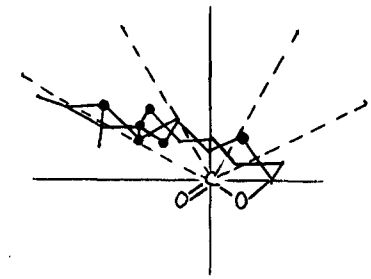
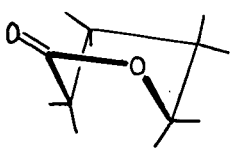


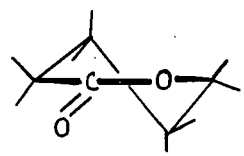


cancel and the remainder lie in the upper right and lower left (atoms marked with a circle) sectors. No exceptions to the rule were found for the lactones studied.

The fact that the lactone group (-C-CO-O-C) is planar (as shown by X-ray analysis)[21a] requires that a δ-lactone ring has either the boat or half-chair conformation. An alternative interpretation of the sign of the Cotton effect of δ-lactones in terms of the conformation of the lactone ring has been suggested by Wolf.[22] In this publication a series of steroid lactones of known conformation were studied and those having either the boat or half-chair conformation, (27) and (28) respectively, showed positive Cotton effects while the enantiomers (29) and (30) showed negative Cotton effects. Of particular relevance to this discussion, may be cited two examples, 6-oxa-5β-cholestan-7-one (31) existing in conformation 27 and possessing a positive Cotton effect while 6-oxa-5α-cholestan-3,7-dione (32) existing in conformation 28 also shows a positive Cotton effect.[7,13]



27



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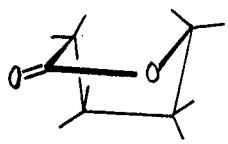

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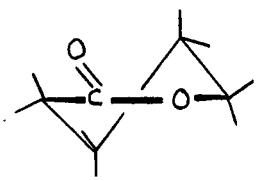

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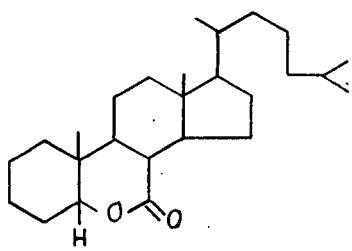

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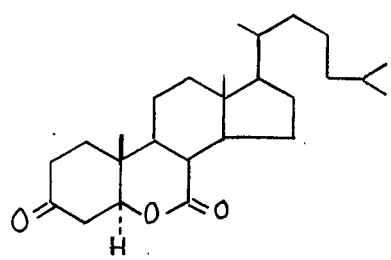
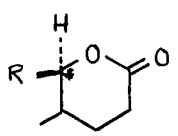


32

The conformation of the saturated δ-lactone ring also influences the position of the Cotton effect. Those compounds having the boat conformation showed the first extremum below 233 mμ while for those with the half-chair conformation it was above 238 mμ.

An early attempt to relate the stereochemistry of a lactone to the sign of its optical rotation was made by Hudson.[23] In his well-known lactone rule he stated that if the hydrogen atom at the alkoxy carbon, C*, in 33 or 34 lies below the plane of the lactone ring the compound is dextrorotatory and, conversely, if it lies above it will have a negative rotation. In the case of a complex lactone with many asymmetric centres, each centre contributes to the total rotation of the molecule. In order to consider only that part due to the lactone formation it is necessary to subtract



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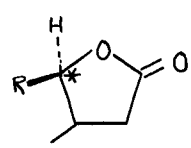

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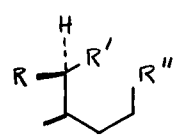
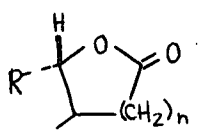


35a R' = OH; R'' = COOH
b R' = OH; R'' = COOMe
c R' = OH; R'' = CH_2OH
d R' = H; R'' = COOH

the rotation of a suitable reference compound (35a-d) containing all the same asymmetric centres as the parent compound. Hudson's original rule was

based on 5- and 6-membered lactones of the sugar series and was later extended to many other lactones including steroids, terpenes and other groups of natural products.[24]

The rotations of some representative lactones and their ring-opened reference compounds have now been compared by means of ORD curves[20] instead of the monochromatic rotations at 589 mμ used by earlier workers. Lactones of general type 36 had negative difference curves (optical rotation of lactone minus optical rotation of the reference compound) as predicted



36

by Hudson's rule and also showed negative Cotton effects. Similarly compounds of type 33 and 34 showed positive difference curves and positive Cotton effects. This agreement between the sign of the difference curve and the Cotton effect would be expected since both are measures of the rotation contribution of the lactone group to the total rotation of the molecule. The direct measurement of lactone Cotton effects is advantageous because it eliminates the necessity of obtaining suitable reference compounds. Hudson's rule is limited to lactones in which the alkoxy-carbon is asymmetric.

ORD curves of carbohydrate lactones have been interpreted so far in terms of the octant rule considering chiefly the effect of a hydroxyl group α to the lactone carbonyl.[8] The sector rule has as yet been concerned entirely with the contributions of alkyl and cyclohexane rings and cannot be extended to α-hydroxy or acetoxyl groups, or halogen atoms.

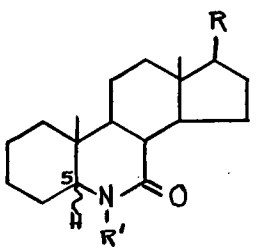
DISCUSSION

The ORD curves of a number of 6-aza and 11-aza steroidal derivatives were measured and the region between 380 and 200 mμ which is of interest will be discussed. For the sake of clarity the results for each series will be considered separately.

The 6-Aza Series

A series of 6-aza steroids in which the basic chromophore is a lactam were studied with the hope of determining the configuration at C_5. The lactam is held in a more or less fixed conformation in a ring and is therefore a suitable group with which to investigate the asymmetric environment of its chromophore. Unfortunately there is not a wide-range of compounds of known stereochemistry available as was the case with lactones and no equivalent of the sector rule for lactones exists at the moment for lactam systems. It was therefore clear at the outset that the results may not be entirely conclusive but they would still be of some interest.

The parent lactam system in the cholestane (37) and androstane (38) series exhibited a positive Cotton effect with the peak in the region between 250 and 260 mμ and the trough about 230 mμ. (Table 4, Figure 2). The



	R	R'
37	C_8H_{17}	H
38	OH	H
39	C_8H_{17}	$-CH_2C_6H_5$
40	OH	$-CH_2C_6H_5$
41	$-CH(OAc)-CH_3$	$-CH_2C_6H_5$

TABLE 4

ORD of 6-Aza Steroid Derivatives

Compound	Concentration in mg/ml	λmμ	$[\phi] \times 10^{-2}$
37	1.099	258 pk 232 tr	+9 -15
38	1.313	252 pk 228 tr	+9.5 -25
39	0.969	260 pk 230 tr	+3 -85
40	1.206	246 pk tr	+29.4 off scale
41	2.346	255 pk tr	+198 off scale
	1.173	255 pk tr	+163.3 off scale
	0.105	254 pk 230 tr	+26 -53.8
43	0.117	257 pk 228 tr	+215 -314
44	0.113	255 pk 228 tr	+118 -305
45	0.103	263 pk 233 tr	+241 -584
46	0.112	262 pk 234 tr	+567 -976
47	0.102	262 pk 235 tr	+253 -435
52	0.096	311 pk 260 tr	+118 -181

pk = peak; tr = trough

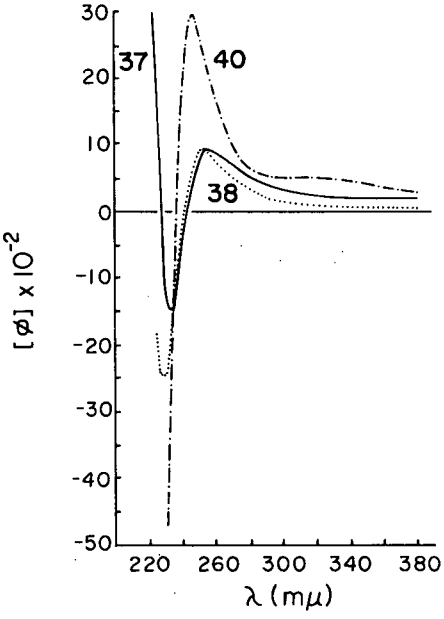


FIG. 2

RD Curves of Compounds 37, 38 and 40 in 6-Aza Steroid Series.

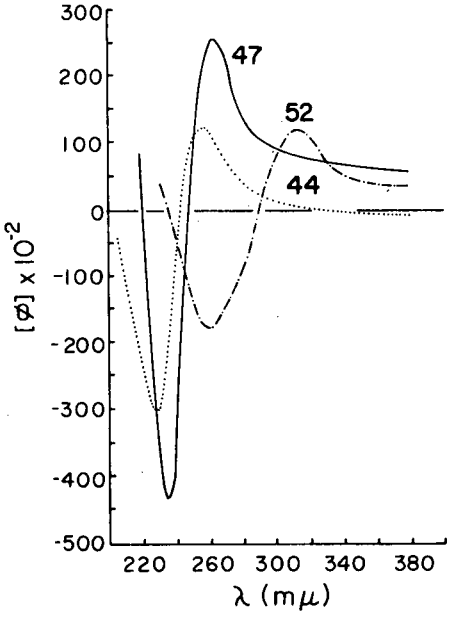


FIG. 3

RD Curves of Compounds 44, 47 and 52 in 6-Aza Steriod Series.

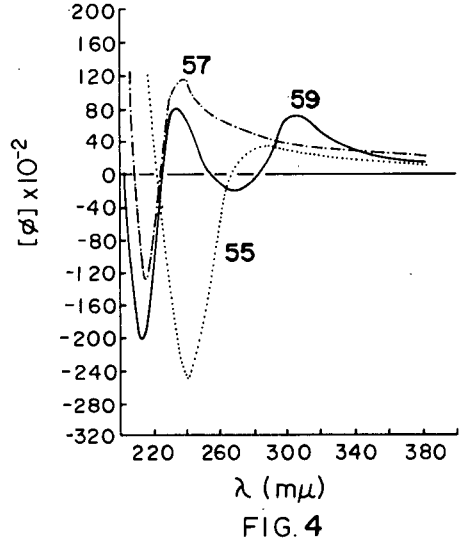


FIG. 4

RD Curves of Compounds 55, 57 and 59 in 11-Aza Steroid Series.

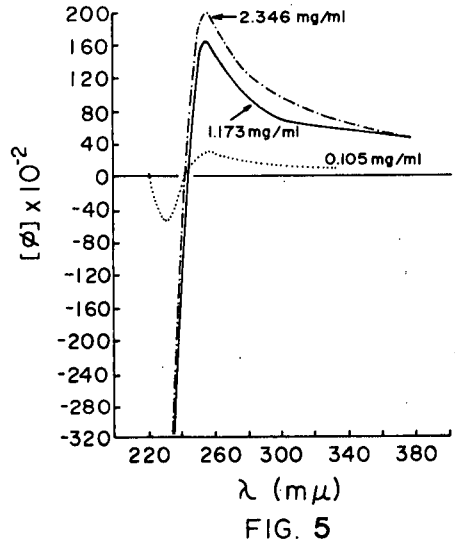
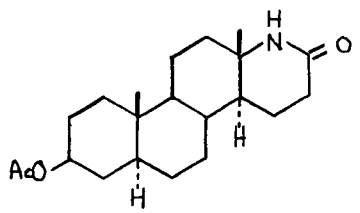


FIG. 5

RD Curve of Compound 41 Showing Effect of Concentration.

application of the lactone sector rule to these compounds may or may not be a valid extension; however, for the sake of comparison an attempt was made to apply it.

First, the saturated lactam (15) was considered. The sector rule, (15A) and (15B), predicts a negative Cotton effect when in fact a weak



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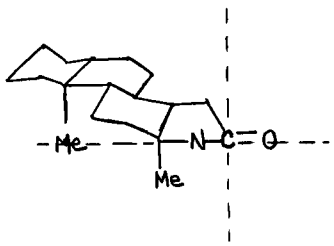
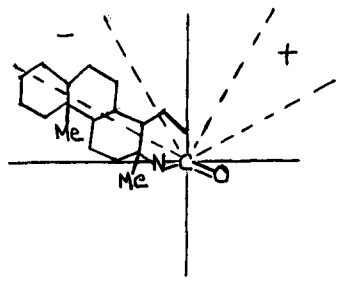
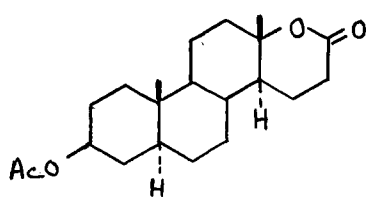


15A



15B

positive one superimposed on a negative background was observed by Wolf.[16] There is some disagreement about the sign of the Cotton effect associated with the corresponding lactone (42). Wolf[16] observed a very weak positive



42

curve superimposed on a strongly negative background. This was confirmed by the positive circular dichroism curve. On the other hand, Klyne[25] reported a negative Cotton effect in agreement with the sector rule prediction. In view of this controversy and the fact that lactam (15) is the only saturated one studied by other groups[16] it is not possible to draw any conclusions about the validity of extending the sector rule to lactams. Similarly nothing can be said about the use of the octant rule in this case

except to mention that if it is applied to the lactam a negative Cotton effect is predicted.

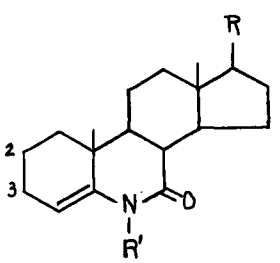
It should be noted that the ORD curves of carbohydrate lactones have been interpreted in terms of the octant rule.[8]

If the sector rule could be applied to lactams it would be difficult to interpret the results for 6-aza steroids as the projections would be similar to those for lactones (24) and (25) which Klyne[7] does not attempt to interpret. A further complication is that both the A/B cis (24) and A/B trans (25) lactones gave positive Cotton effects, the latter of lower intensity. It is reasonable on this basis to suspect that the 6-aza steroids might give positive Cotton effects regardless of the configuration at C_5.

If one applies Wolf's theory[22] that the sign of the Cotton effect is determined by the conformation of the lactone ring positive Cotton effects are predicted, in both cases in accord with observations. If, however, his results can be extended to lactams the position of the first extremum of the ORD curves of the 6-aza steroids may be important. It has been noted[21a] that in view of the planar nature of the latter group, it is probably reasonable to assume that the lactam group is also planar. On this basis ring B would have the half-chair comformation (28) if the configuration at C_5 was α, and the boat conformation (27) if C_5 was β. Wolf observed that the first extremum occurred above 238 mμ when the lactone ring had the half-chair conformation and below 233 mμ when it was in the boat conformation.[22] The fact that our lactams showed the first extremum between 250 and 260 mμ might be some indication that these compounds have the 5α configuration. The intensities observed were low (Table 4) and it is of interest to note that Klyne[7] observed weaker Cotton effects for the 6-oxa-5α-lactone (25) than for the corresponding 5β-compound (24).

Although the results on the 6-aza steroids are suggestive, they certainly do not provide conclusive evidence for the configuration at C_5 in these compounds. In view of the problems associated with the interpretation of the ORD curves of lactams and amides further results will be presented without any attempt to explain the sign of the Cotton effect observed.

The effect of substitution on the nitrogen atom was investigated for the N-benzyl series (39-41). In general, the effect of this substitution was not appreciable. The sign of the Cotton effect remained unchanged but the intensity varied. (Table 4). In the series 39-41 these intensity differences must be due to the C-17 substituent. Similar results were obtained in the enol lactam series (43-47). (Table 4).

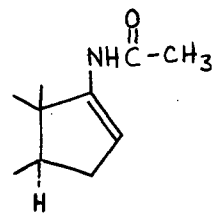


	R	R'
43	C_8H_{17}	H
44	OH	H
45	C_8H_{17}	$-CH_2C_6H_5$
46	$-\underset{\underset{OAc}{|}}{CH}-CH_3$	$-CH_2C_6H_5$
47	OH	$-CH_2C_6H_5$
52	OH	$-CH_2C_6H_5$

+ additional double bond at C_2-C_3.

The various enol lactams (43-47) gave much more intense Cotton effects than the corresponding saturated lactams (37-41) (Figures 2 and 3). The relative positions of the peaks were shifted slightly if at all by the presence of the double bond. In the case of the parent lactams the Cotton effect is due to the weak $n \rightarrow \pi^*$ transition of the carbonyl group which is

not usually observed in the ultraviolet spectrums under routine measurements (above 220 mμ). On the other hand, the enol lactams showed an ultraviolet absorption at 237 mμ (log ε about 4) which is probably due to the $\pi \rightarrow \pi^*$ transition of the conjugated system, $\diagdown C{=}C{-}N{-}C{=}O$. Similar ultraviolet spectra have been observed for related systems, for example (48) and (49),[26]



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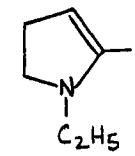

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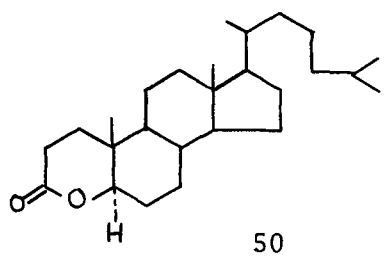
the former having λ_{max} 240 mμ (log ε 3.8), the latter, λ_{max} 238 mμ (log ε 3.9). A similar effect was observed by Wolf[16] in comparing the lactone (50) and enol lactone (51) but in contrast to our results the sign of the Cotton effect was reversed. (Figure 6).



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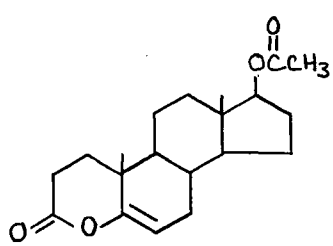

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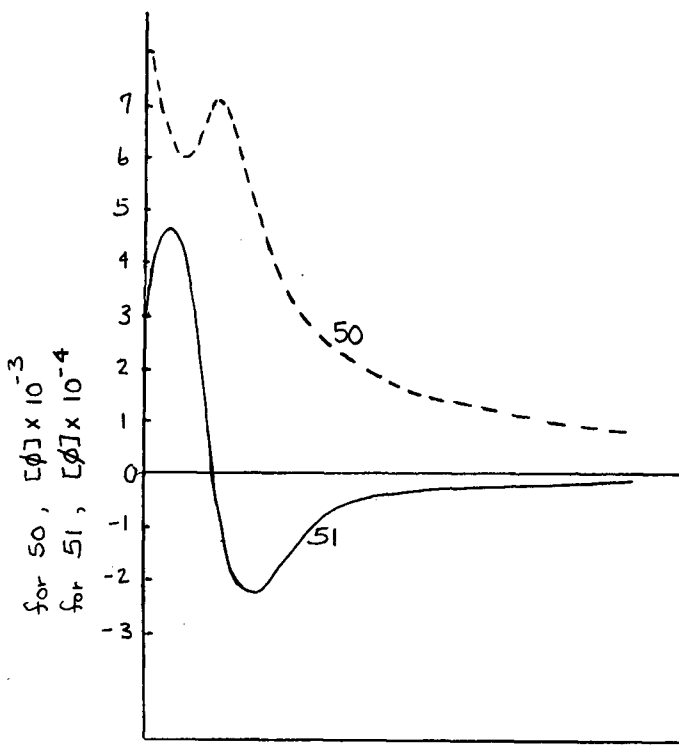
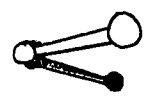


Figure 6. ORD of Steroid Lactones

The presence of an additional double bond which extends the conjugation of the unsaturated lactam would be expected to have an appreciable effect on the ORD curve. In the case of 17β-hydroxy-N-benzyl-6-aza-2,4-androstadien-7-one (52) the curve is shifted to higher wavelength with the peak and trough at 311 mμ and 260 mμ respectively (Figure 3). From a model, the expected chirality of the diene is that of a right-handed helix (53) and on this basis a positive Cotton effect would be expected if the



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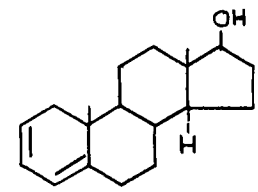

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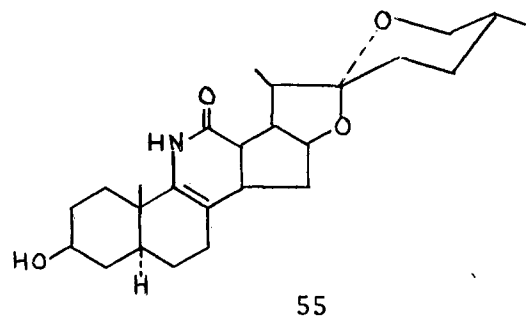
rules developed for the diene chromophore[27] can be applied to this compound. The analogous diene (54) exhibited a positive Cotton effect with the peak at 300 mμ ([ϕ] = 8700) normally attributed to a $\pi \rightarrow \pi^*$ transition.[27]

The 11-Aza Series

The ORD study of aza steroids was extended to the 11-aza series with the study of the unsaturated lactam (55). It was already known from previous



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results,[9,10] that this chromophore with the double bond in the same ring as the lactam system absorbs in the ultraviolet spectrum at a higher wavelength (255 mμ) than the corresponding enol lactams in the 6-aza series (237 mμ). It was therefore expected that the ORD curve would similarly exhibit a bathochromic shift. In accord with expectation the peak was observed at 285 mμ with the trough at 240 mμ. (Table 5, Figure 4). Unfortunately the corresponding saturated system was not available for study.

TABLE 5

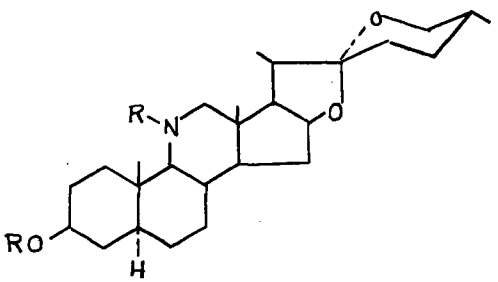
ORD of 11-Aza Steroid Derivatives

Compound	Concentration (mg/ml)	λmμ	$[\phi] \times 10^{-2}$
55	0.118	285 pk	+36.2
		240 tr	-246
56	plain negative dispersion curve		
57	0.107	238 pk	+117
		215 tr	-126
58	0.101	355 pk	+79
		297 tr	+3.6
		238 pk	+378
		212 tr	-742
59	0.112	306 pk	+71.8
		270 tr	-18.0
		233 pk	+82.7
		212 tr	-201

pk = peak; tr = trough

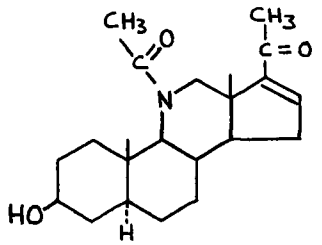
It is well-known from work on polypeptides and proteins[3] that the amide chromophore can be optically active. Thus the N-acetyl-11-aza derivatives (57), (58) and (59) would be expected to have anomalous

dispersion curves.



56 R = H

57 R = -C-CH$_3$ (with O)



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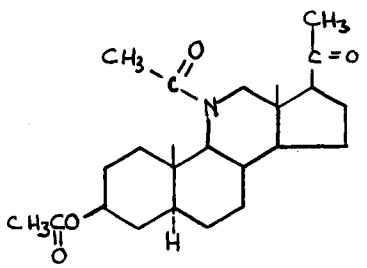

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The parent 11-aza compound (56) showed a plain negative curve. On the other hand, the N-acetyl derivative (57) showed an anomalous Cotton effect with the peak and trough at 238 mμ and 215 mμ respectively, which could be attributed to the amide n → π* transition. (Figure 4). The corresponding compounds from the 11-aza pregnane series (58) and (59) also exhibited similar anomalous dispersion in this region in addition to the expected Cotton effects due to the n → π* transition of the C_{20} ketone group. (Table 5).

It is well known[2] that changes in concentration of some optically active substances can affect the rotation appreciably. This effect is often already noticeable at the sodium D line and may be enhanced in rotatory dispersion measurements. For example, with (+)-3-methylcyclohexanone the specific rotations in methanol at the peak (307.5 mμ) were found to be 910°, 840° and 720°, corresponding to concentrations of 0.132, 0.103 and 0.029 g. per 100 cc.[2] A similar concentration dependence was observed in the case of some of the aza steroids studied. The data for the N-benzyl-6-aza derivative (41) is included in Table 4. The molecular

rotations were found to be 19800, 16330, and 2600° corresponding to concentrations of 2.346, 1.173 and 0.105 mg. per ml. respectively and the curves are shown in Figure 4.

Observation of anomalous Cotton effects associated with amide and lactam functions may allow the extension of ORD studies to stereochemical problems in alkaloid chemistry. Aromatic alkaloids have been successfully studied by ORD recently.[3,28] Other classes of alkaloids can often be readily converted into the N-acetate or N-benzoate derivatives and provided the asymmetric environment is favourable ORD studies might yield useful information.

EXPERIMENTAL

Optical rotatory dispersion curves were measured in methanol on a JASCO Model ORD/UV-5 Spectropolarimeter (l = 0.05 dm; t = 20-25°; c = 1 mg/ml or 0.1 mg/ml).

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PART 3

INVESTIGATION OF THE SPORES OF *EQUISETUM TELMATEIA*

Fertile Branches of Equisetum telmateia Ehrh.

INTRODUCTION

With the fossilized forms of dinosaurs and other prehistoric animals perfectly preserved specimens of giant horse-tails are found. They grew in dense forests attaining a height of sixty to ninety feet and a diameter of three feet and disappeared from the fossil record 150 million years ago. Today there are about twenty-five species of descendants of these giants which range from a few inches to three or four feet in height with the exception of E. giganteum. This species is native to tropical South America and has a stem one inch in diameter and sixty feet tall, clambering somewhat like a vine on other vegetation.

Horsetails contain large amounts of siliceous compounds and were used in pioneer times for scouring pots and pans. They were called scouring rushes. Equisetum species have been known to medicine for centuries. The principle use of the herb is as a diuretic but it has also been recommended for haemoptysis, haemorrhoids, varicose ulcers and tuberculosis. The Indians boiled it in water for a drink and used it for horse medicine. A novel use for horsetails has been found by Dr. Hans Lundberg[1] who operates gold farms in Indiana and Illinois. In areas containing gold deposits small traces of the metal get into undergound streams. Horsetails soak up this water, gold traces and all, and because the gold is foreign to their systems, the plants try to eject it through their leaves. As a result the gold appears in tiny capsules on the leaf tips. In an early experiment near Timmins, Ontario, Dr. Lundberg burned a ton of horsetails and extracted four ounces of gold.

The horsetails have both fertile and sterile branches which arise from a subterranean stem. The fertile branches appear early in spring and, since they lack chlorophyll, draw upon the underground stem for food. They

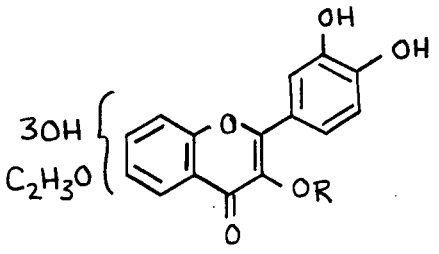
bear the spores in cones at the top of the stems and are short-lived. As soon as the spores are disseminated multi-branched, green sterile branches appear.

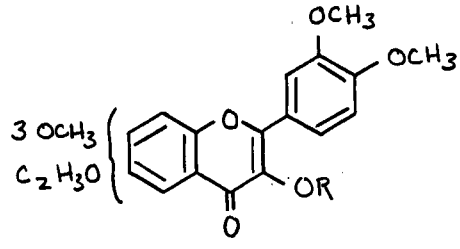
Horsetails are of interest chemically because they have remained essentially unchanged since prehistoric times. The spores of Equisetum maximum Lam., also known as Equisetum telmateia Ehrh., were investigated by a French chemist, Sosa nearly twenty years ago.[2] As the structures he proposed were incomplete it was of interest to re-examine the spores of this species.

When Sosa extracted the spores with ether he obtained a colourless substance which melted at 127.5°. It showed no ultraviolet absorption and did not saponify on heating with potassium hydroxide. This compound was acidic and he named it equisetolic acid, assigned the empirical formula, $C_{37}H_{72}O_5$, and suggested that it was a monohydroxylated aliphatic diacid.

After the spores had been extracted with ether they were treated with ethanol. A glycoside which he called equisporoside separated from this extract and on purification of this substance in water he found that with it there was a second glycoside, equisporonoside. Equisporoside (1) was hydrolyzed with acid to one molecule of d-glucose and equisporol, $C_{17}H_{12}O_9$. To determine the position of the sugar, the glycoside was methylated and subsequently hydrolyzed to give penta-methyl equisporol (2). This compound melted at 250° and gave a negative ferric chloride test. Sosa therefore concluded that glucose was attached at the C_3 position.

Equisporol was methylated and the resulting hexamethyl ether (3) submitted to alkaline degradation. The products were an acid identified as veratric acid (4) and an unidentified phenol, m.p. 112°. On this basis the partial structure 5 was proposed for equisporol.



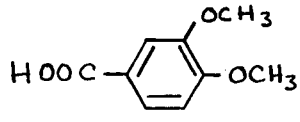


1 R = Glucose

5 R = H

2 R = H

3 R = CH_3



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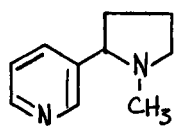
Equisporonoside, which was not soluble in hot water but soluble in ethanol, melted at 293° and its acetate melted at 237°. On hydrolysis it gave a sugar which Sosa thought to be glucose and equisporonol. The colour reactions of equisporonoside and equisporonol were very similar to those of equisporoside and equisporol respectively.

The spores which had already been treated with ether and ethanol were extracted with acetone for two days. A small amount of the pigment was extracted along with a very light colourless solid which melted at 410°. It was very soluble in organic solvents and did not give positive phenol or sapogenin reactions. Finally, Sosa extracted the spores with a mixture of equal amounts of methanol, benzene, and ethyl acetate and obtained more equisporoside.

The sterile green shoots of Equisetum telmateia have been reported to contain very small amounts of nicotine,[3] a saponin probably identical with equisetonin from E. arvense,[4] and 4.4 to 11.3% silicic acid.[5] The fertile brown shoots were reported to contain dimethylsulphone.[6]

Other species of Equisetum have also been investigated. Equisetum arvense has been studied by several groups. Nicotine (6),[3,6,7] 3-methoxy-

pyridine,[8] traces of palustrin,[6] dimethyl sulphone, equisetonin,[4,9,10] aconitic acid (7), oxalic acid and a lipid have been isolated. The flavone mixture known as flavequisetin[11] was further investigated and equisetroside (kaempferol-7-diglucoside) (8), isoquercitrin (9) and luteolin-5-glucoside (10) were identified.[12] Manganese was found in determinable amounts (6.5mg.% of the dry weight of the plants).[13] The fertile brown shoots yielded



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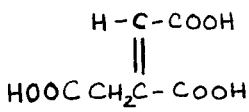

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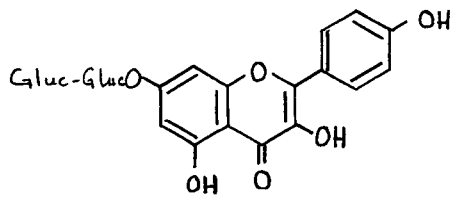

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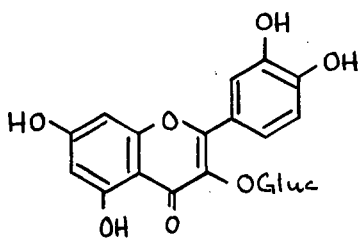

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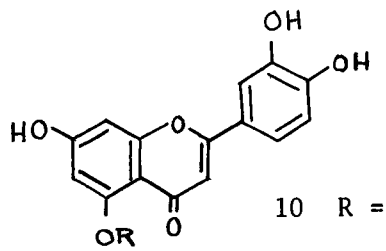


10 R = Glucose

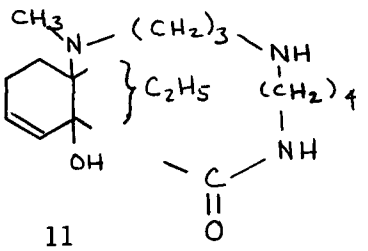
15 R = H

articulatin, $C_{21}H_{22}O_3.2H_2O$, and isoarticulatin. The corresponding aglycones, articulatidin and isoarticulatidin both showed anthraquinone-type reactions.[14] The silicic acid content was lower than in the sterile shoots; 3.21% compared to 16-18%.[5] β-sitosterol has also been isolated from this species.[15]

Very little work has been done on Equisetum sylvaticum L. It contains a small amount of nicotine[3] and a saponin which exhibits different properties to those of equisetonin from E. arvense.[4]

Apart from E. arvense the most work has been done on Equisetum

palustre. The chief alkaloid was isolated in 1936 and named palustrin.[16] Further work was done in 1953[17] and some years later the partial sturcture 11 was proposed.[18] Nicotine, a xanthophyll, lutein, and palustridin were



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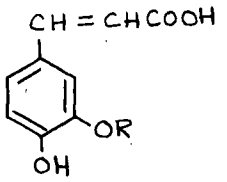
also found.[17] Equisetin and equisetonin isolated by other workers are identical to palustrin and palustridin respectively.[19] A hydrocarbon, $C_{21}H_{42}$,[16] thymine, dimethylsulphone,[20] kaempferol 3-rhamnosylglucoside-7-glucoside,[21] and a partly characterized kaempferol-3,7-diglycoside[21] have also been reported.

Equisetum fluviatile L. is very variable in habit. The non-branched form is known as E. limosum L. and the branched form as E. fluviatile. Aconitic acid was isolated as its magnesium, calcium, and sodium salts.[22,23] A saponin with the same properties as equisetonin was also found.[4]

From 17.5 kg. of dry Equisetum hiemale L. small amounts of dimethylsulphone, 12 mg. of a reactive acid, a water soluble substance, small amounts of nicotine, and ferrulic (12) and caffeic acids (13) were isolated.[6] Palustrin was not found.

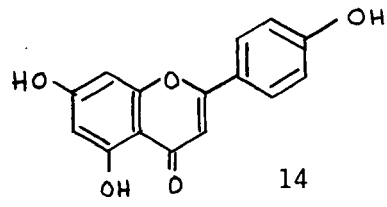


12 R = CH_3

13 R = H

Two flavonoids, apigenin (14) and luteolin (15) were identified in the methanol extracts of Equisetum ramosissimum Desf.[24] and nicotine has been

identified in Equisetum debile.[25]



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It is obvious that very little is known about the chemistry of Equisetum and much work remains to be done.

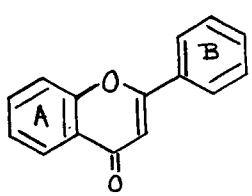
DISCUSSION

Equisporoside

Equisporoside which had been assigned the partial structure (1) by Sosa[2] was isolated from the methanol extracts of the spores. A small amount was purified by paper chromatography and this was used for preliminary spectral studies. (Table 1). Quantitative ultraviolet and visible spectra were done later using a sample of equisporoside which had been crystallized from aqueous acetic acid. (Figure 1).

Ultraviolet and visible spectra of flavonoid compounds have been extensively studied[26-29] and can be used to determine various structural features of these compounds. The spectrum of equisporoside, λ_{max} 388,348 mμ (Band I) and λ_{max} 277, 261 mμ (Band II) immediately suggests several points about the structure of equisporoside.

Flavones (16) and flavonols (17) generally exhibit high intensity absorptions in the 320-380 mμ (Band I) and 240-270 mμ (Band II) regions. The position and intensity of each of these bands varies with the relative



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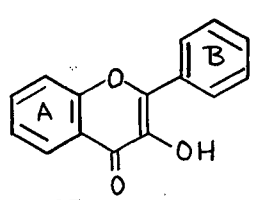

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resonance contributions of the benzoyl (18), cinnamoyl (19), and pyrone (20) groupings to the total resonance of the flavone molecule. Although these groupings undoubtedly interact, the spectra of substituted flavones and flavonols in neutral and alkaline solutions suggest that Band I is associated chiefly with the cinnamoyl grouping (19) and Band II with absorption in the

TABLE 1

Ultraviolet Spectra of Equisporoside

Reagent Added	λ_{max} (mμ)			
	Band I		Band II	
	a	b	a	b
Ethanol solution	388	348	277	261
Aluminum chloride	455	383	275	
Sodium Acetate	394	351	280	266
Boric Acid-Sodium Acetate	404		266	250
Sodium Ethoxide	390		274	

TABLE 2

Ultraviolet Spectra of Equisporol

Reagent Added	λ_{max} (mμ)			
	Band I		Band II	
	a	b	a	b
Ethanol solution	384	341	276	263
Aluminum chloride	450	380	287	
Sodium Acetate		328	263	249
Boric Acid-Sodium Acetate	412	357	285	274
Sodium Ethoxide	387 (low intensity)		287	

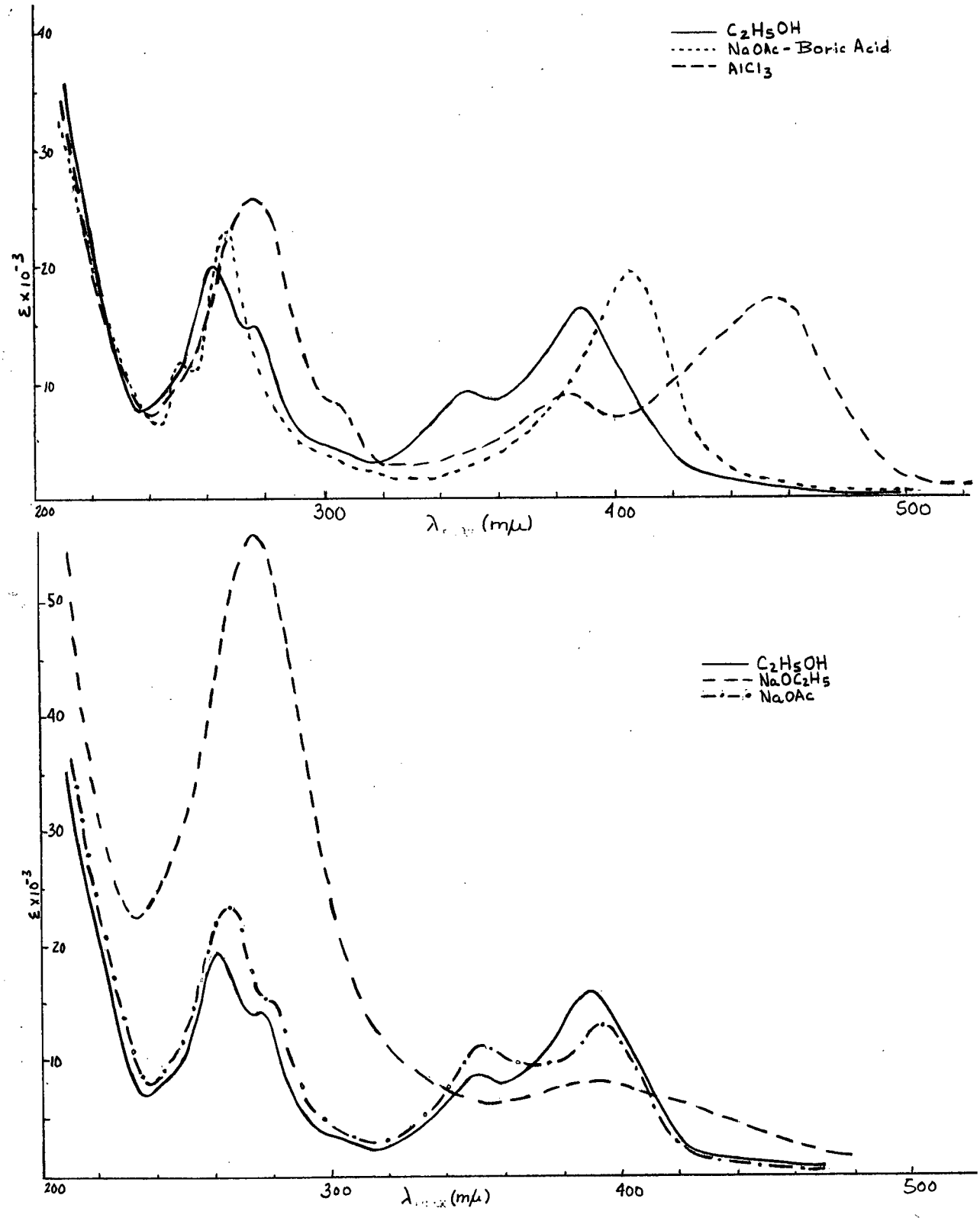
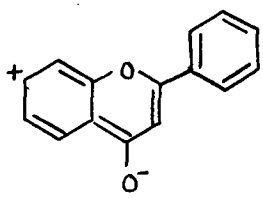


Figure 1. Ultraviolet Spectra of Equisporoside.



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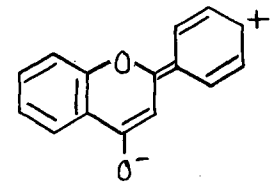

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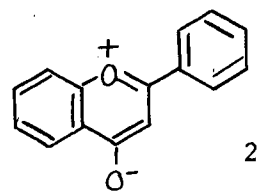
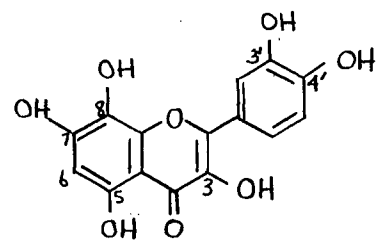


20

benzoyl grouping (18).[26] Thus, the introduction of electron donating groups such as hydroxyl into ring B increase its relative resonance contribution and consequently produce considerable bathochromic shifts of Band I. Introduction of hydroxyl or methoxyl groups into the A ring, on the other hand, primarily increases the resonance contribution of this ring and tends to increase the wavelength and intensity of Band II.

The position of Band II at 261 and 277 mμ suggested that equisporoside was a gossypetin (21) derivative for which λ_{max} 250-260 mμ and 270-280 mμ have been reported.[30] The position of Band I, 348 and 388 mμ



21

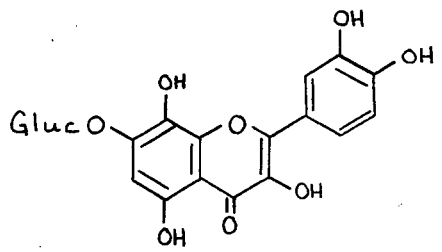
indicated that it was probably a flavonol rather than a flavone; the former usually have Band I between 340 and 380 mμ and the latter between 320 and 350 mμ.[31]

Band II of flavones and flavonols which have only a 4'-substituent

in the B-ring has a single, well-defined peak. For flavones and flavonols which have hydroxyl or methoxyl substituents in both the 3'- and 4'-positions Band II shows two definite peaks or one peak and a pronounced inflection. When three substituents are present in the B-ring, Band II has only a single peak.[32] Thus, the fact that Band II in the spectrum of equisporoside has a peak and a pronounced inflection indicates immediately the presence of 3'- and 4'- substituents in ring B as suggested by Sosa.[2]

The value of spectral data in the identification and structural analysis of flavonoid compounds is increased considerably by the addition of certain reagents which produce shifts in the maxima in accordance with the location of various functional groups in the molecule. Addition of aluminum chloride[27] caused a bathochromic shift of 67 mμ in Band Ia (Table 1, Figure 1) which is reliable evidence for the presence of a free 3-hydroxy group.[27,33] Sodium acetate is sufficiently basic to ionize hydroxyl groups located at positions 7, 3, and 4' of the flavone nucleus. Hydroxyls located elsewhere are unaffected. Ionization of 3- and 4'-hydroxyl functions produces bathochromic shifts of Band I but does not affect the position of Band II. Since Band II is associated mainly with absorption in the A ring, however, ionization of a 7-hydroxyl group results in a pronounced (8-20 mμ) bathochromic shift in this band.[26,33] Addition of sodium acetate[26] resulted in a shift of 6 mμ in Band Ia indicating again the presence of 3- and 4'-hydroxyl groups. Band II remained essentially unchanged and this provided initial evidence for the attachment of the sugar at the 7-position. The presence of one sugar in equisporoside was clearly shown in the NMR spectrum (100 Mc/s of the trimethylsilyl ether (Figure 2).

In the presence of sodium acetate, boric acid chelates with phenolic



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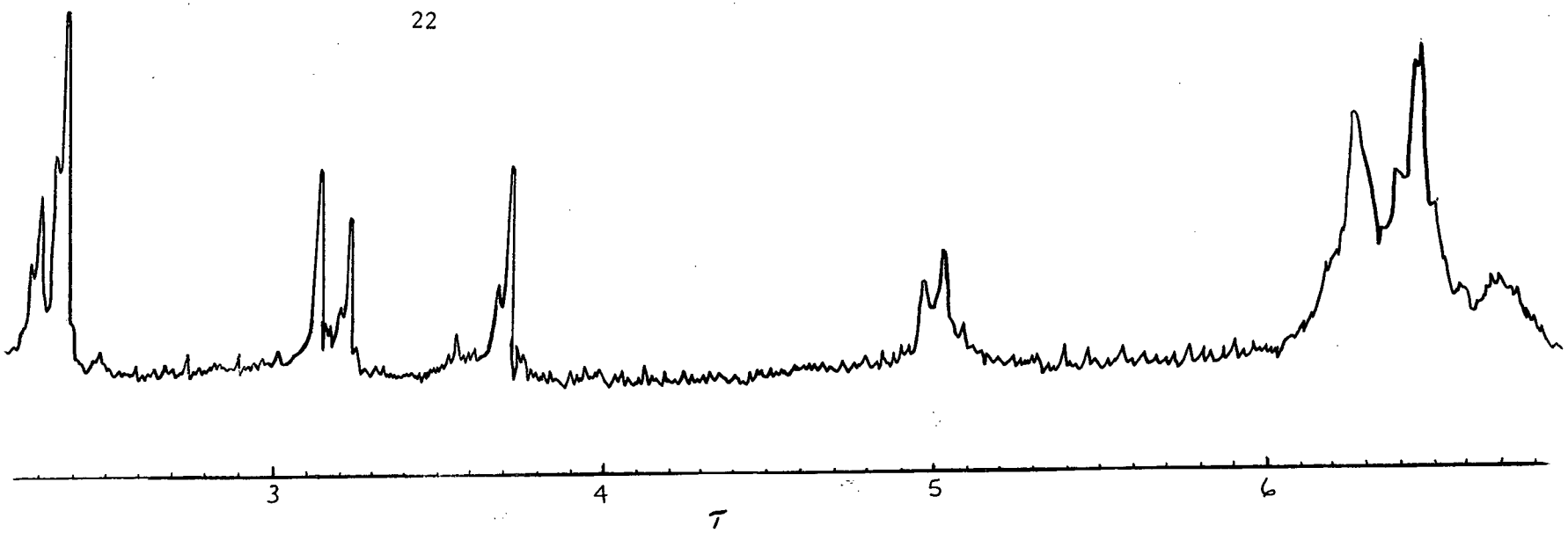
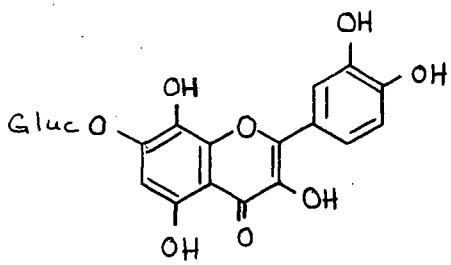


Figure 2. NMR Spectrum of Equisporoside.

compounds containing o-dihydroxyl groups.[28] Addition of these reagents caused a bathochromic shift of 16 mμ in the position of Band Ia in good agreement with the 15-20 mμ shift reported for flavones and flavonols having o-dihydroxyl groups. The spectra of compounds which do not contain such a group are not appreciably affected.[34] The gossypetin system is one of the few exceptions which decomposes in sodium ethoxide regardless of whether the hydroxyl at C_3 is protected. In this case, therefore, alkali instability which is usually characteristic of 3,4'-dihydroxyflavonols[26,34] cannot be used as evidence for the presence of these substituents.

Thus, the spectral evidence is in agreement with equisporoside being a gossypetin derivative. Addition of p-benzoquinone to an ethanol solution of equisporoside gave a positive gossypetone reaction (red-brown precipitate)[35] indicating the presence of para-hydroxyl groups. Equisporoside was subsequently identified as gossypitrin (22), the 7-glucoside of gossypetin. Evidence for this assignment will be given below.



22

Hydrolysis of equisporoside with dilute aqueous acid gave the corresponding aglycone and a sugar which was identified as glucose by paper chromatography in three different solvent systems. A typical paper which was developed with ethyl acetate-pyridine-water, 8:2:1, is shown in Figure 3.

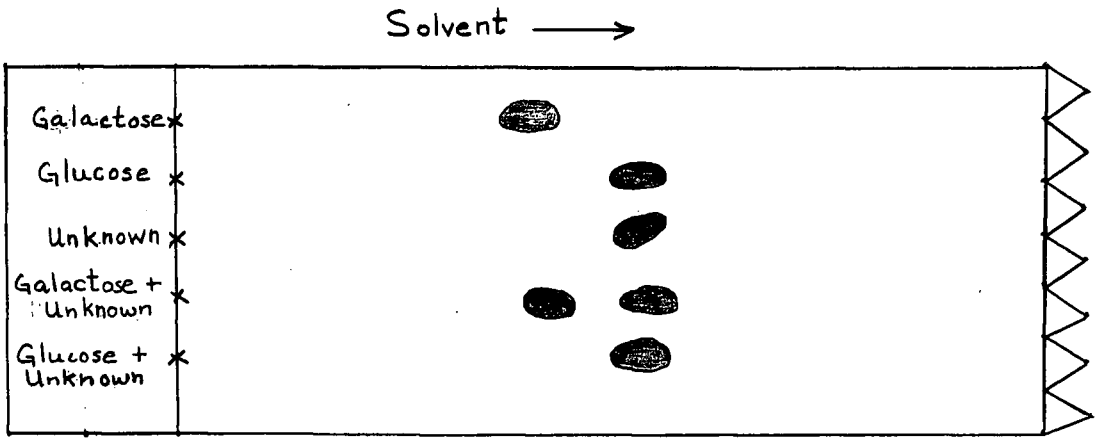


Figure 3. Paper Chromatography of the Sugar.

The aglycone which Sosa[2] named equisporol was crystallized from aqueous acetic acid, m.p. 302-304° (decomposition). The ultraviolet spectrum, λ_{max} 384, 341, 276 and 263 mμ agreed with that reported for gossypetin: λ_{max} 386, 341, 278 and 262 mμ. The NMR spectrum (100 Mc/s, Figure 4) showed the expected splitting pattern for the ring B aromatic protons, plus a singlet which integrated for one proton, indicating the presence of only one unsubstituted position on the A-ring. There were no methoxyl protons.

The derivatives of equisporol also corresponded to those of gossypetin. The melting point of the hexacetate, 226-230°, agreed with that reported for gossypetin hexaacetate.[35,36] The melting point of equisporol hexamethyl ether was undepressed on mixing with a sample of gossypetin hexamethyl ether. This sample was shown to be impure by thin layer chromatography (alumina, chloroform) and the infrared spectrum was not quite as well resolved as that of equisporol hexamethyl ether; however, the two were essentially superimposable. The ultraviolet spectrum, λ_{max} 351,

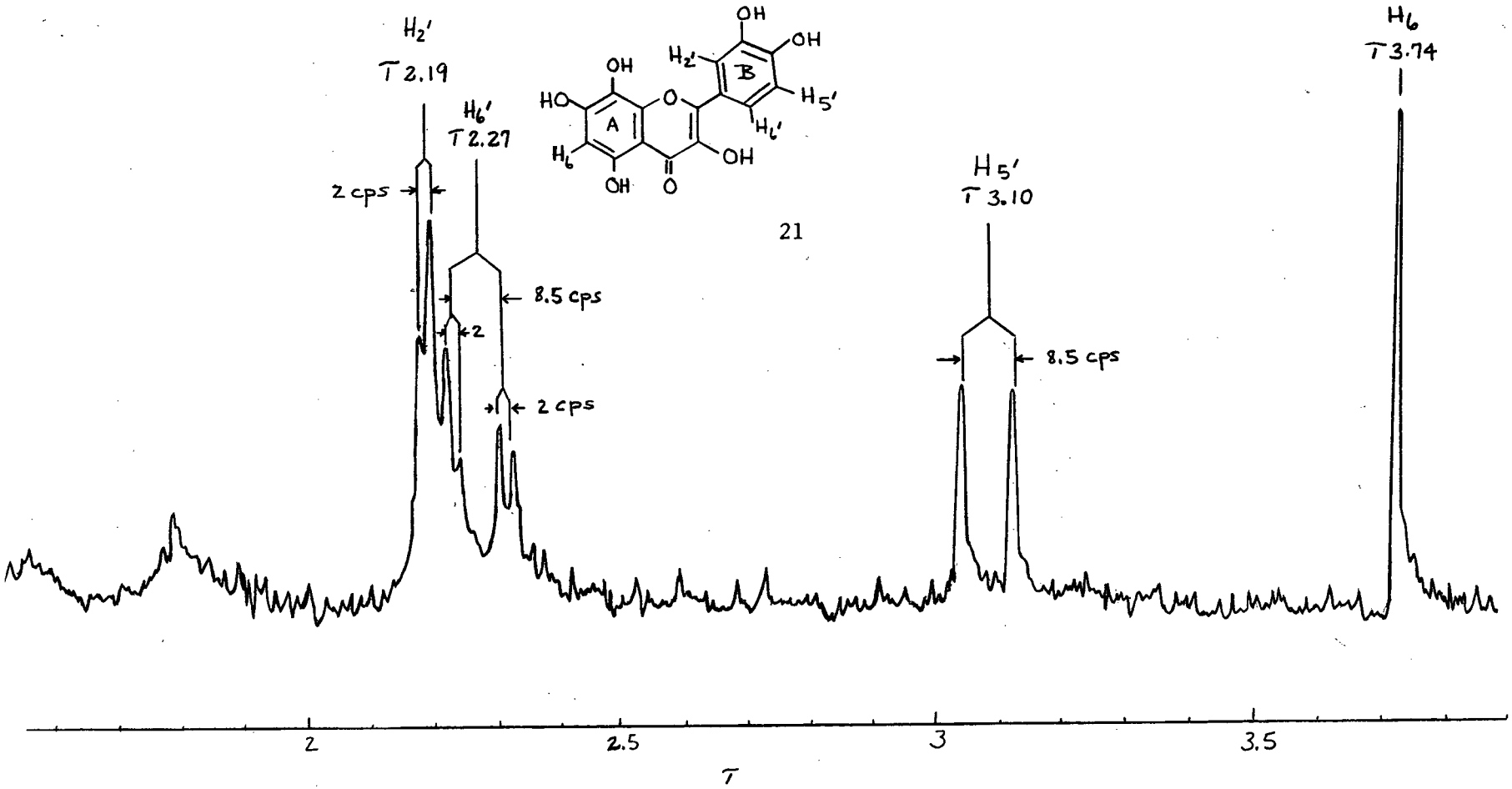


Figure 4. NMR Spectrum of Equisporol.

272 , and 252 mμ was the same as that reported for hexamethyl gossypetin.[36]

Finally, comparison of equisporol with gossypetin showed the mixed melting point was undepressed and the infrared spectra were superimposable. Equisporol was therefore identified as gossypetin (21).

The remaining question was the position of attachment of the sugar residue. Ultraviolet studies of equisporoside had already indicated that this was likely at the C_7 position. In order to definitely establish the position equisporoside was methylated and the product hydrolyzed with dilute sulphuric acid to yield the pentamethyl ether, m.p. 249-252°, the acetate of which melted at 166-168°. The melting points of various pentamethyl ethers of gossypetin and the corresponding acetates are listed in Table 3. From this it can be seen that equisporol pentamethyl ether must be 7-hydroxy-3,3',4',5,8-pentamethoxy flavone. The ultraviolet spectrum, λ_{max} 253 , 272 and 349 mμ agreed with that reported by Geissman,[36] λ_{max} 251 , 270 and 351 mμ, for this compound. Addition of sodium ethoxide caused the expected bathochromic shift in Band II to 282 mμ (29 mμ with a 34% decrease in intensity) due to ionization of the 7-hydroxyl group. The ultraviolet spectrum and this shift were in agreement with those observed by Geissman[36] for 7-hydroxy-3,3',4',5,8-pentamethoxy flavone; however the position of Band I (387 mμ) in the presence of sodium ethoxide differed from the 368 mμ reported by Geissman.[36]

As the aglycone had been identified as gossypetin and the position of attachment of the sugar found to be at the 7-hydroxyl it followed that equisporoside must be the 7-glucoside of gossypetin which is called gossypitrin. Comparison of equisporoside with a sample of gossypitrin (22) confirmed this proposal. The infrared spectra were superimposable and the mixed melting point was undepressed. The structure proposed by Sosa[2]

(1) is therefore incorrect.

TABLE 3

Pentamethyl Ethers of Gossypetin

	m.p.	Acetate m.p.
7-hydroxy-3,5,8,3',4'-pentamethoxyflavone[36]	250-251°	164-168°
8-hydroxy-3,5,7,3',4'-pentamethoxyflavone[37]	196-198°	215-216°
3-hydroxy-5,7,8,3',4'-pentamethoxyflavone[38]	228-230°	207-208°
5-hydroxy-3,7,8,3',4'-pentamethoxyflavone[39]	166-168°	
Equisporol pentamethyl ether	249-252°	166-168°

Equisetolic Acid

Equisetolic acid was isolated from the ether extracts of the spores, m.p. 127-129°. The infrared spectrum showed the presence of a hydroxyl (2623 and 959 cm^{-1}) and a carbonyl group (1693 cm^{-1}). It possessed no ultraviolet absorption in the region above 220 mμ. High resolution mass spectrometry established the molecular formula to be $C_{30}H_{58}O_4$ rather than $C_{37}H_{72}O_5$ as proposed by Sosa.[2] It will be noted from the mass spectrum (Figure 5) particularly in the region below m/e 378, that a regular fragmentation pattern in which the fragments differ from each other by 14 mass units, is observed. This result suggested immediately that equisetolic acid may possess a long hydrocarbon-like chain with carboxylic acid groups attached to both ends. On this basis structure (23) was an attractive possibility.

An attempt to saponify equisetolic acid with potassium hydroxide

$$ROOC(CH_2)_{28}COOR$$

23 R = H

24 R = CH_3

resulted in recovery of the unchanged acid. Due to the insolubility of equisetolic acid an attempt to determine its equivalent weight by titration was unsuccessful.

Acetylation of equisetolic acid using the procedure reported by Sosa[2] to give the monoacetate yielded a product, m.p. 119-123°. (Sosa reports m.p. 119°). However further investigation of this product indicated that in fact no acetylation had occurred. The infrared spectra of equisetolic acid and the latter compound were superimposable. It was therefore clear that Sosa's "acetate" was probably impure starting material.

Methylation of equisetolic acid with diazomethane gave a methyl ester, m.p. 84-86°. The carbonyl absorption was now observed at 1748 cm^{-1} in the infrared spectrum. The NMR spectrum (Figure 7) showed a peak due to the methoxyl protons at τ 6.4, a triplet at τ 7.7 attributable to the methylene protons adjacent to the carbonyl group, a broad multiplet at τ 8.39 which is probably due to the neighbouring methylene protons, and an intense singlet at τ 8.7 representing the remainder of the methylene protons in the molecule. Since the previous results had already established the molecular formula, $C_{30}H_{58}O_4$ for the acid, it is clear that the ester must possess either the molecular formula, $C_{31}H_{60}O_4$ (monoester, m/e 496) or $C_{32}H_{62}O_4$ (diester, m/e 510). Mass spectrometry on the above compound indicated that the diester formulation was correct. Confirmatory evidence is available from the NMR spectrum in which the integrated areas are as

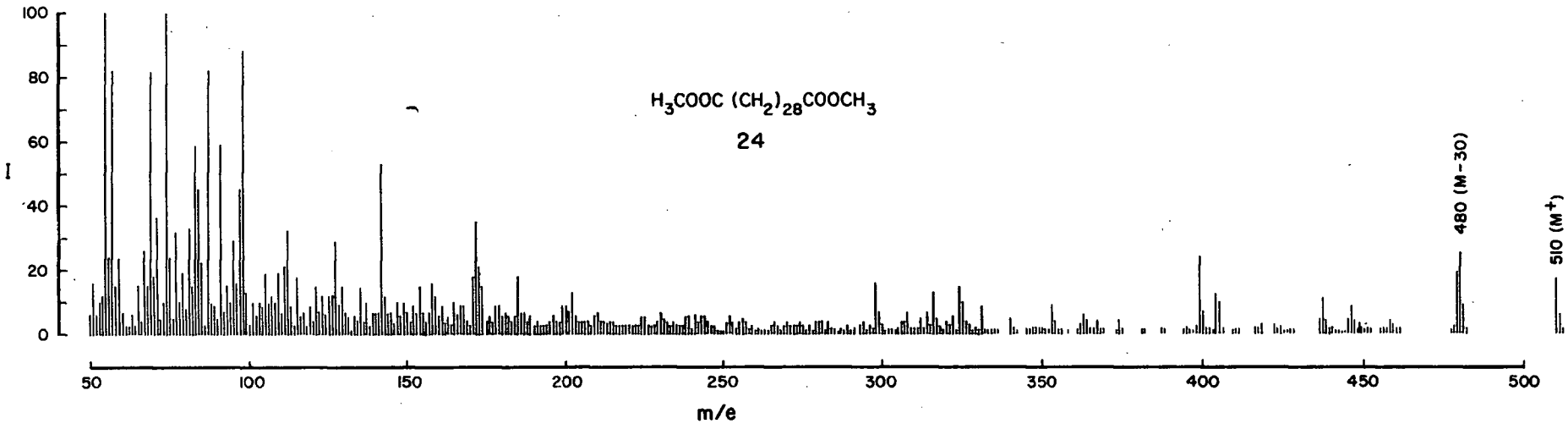


Figure 6. Mass Spectrum of Equisetolic Acid Methyl Ester

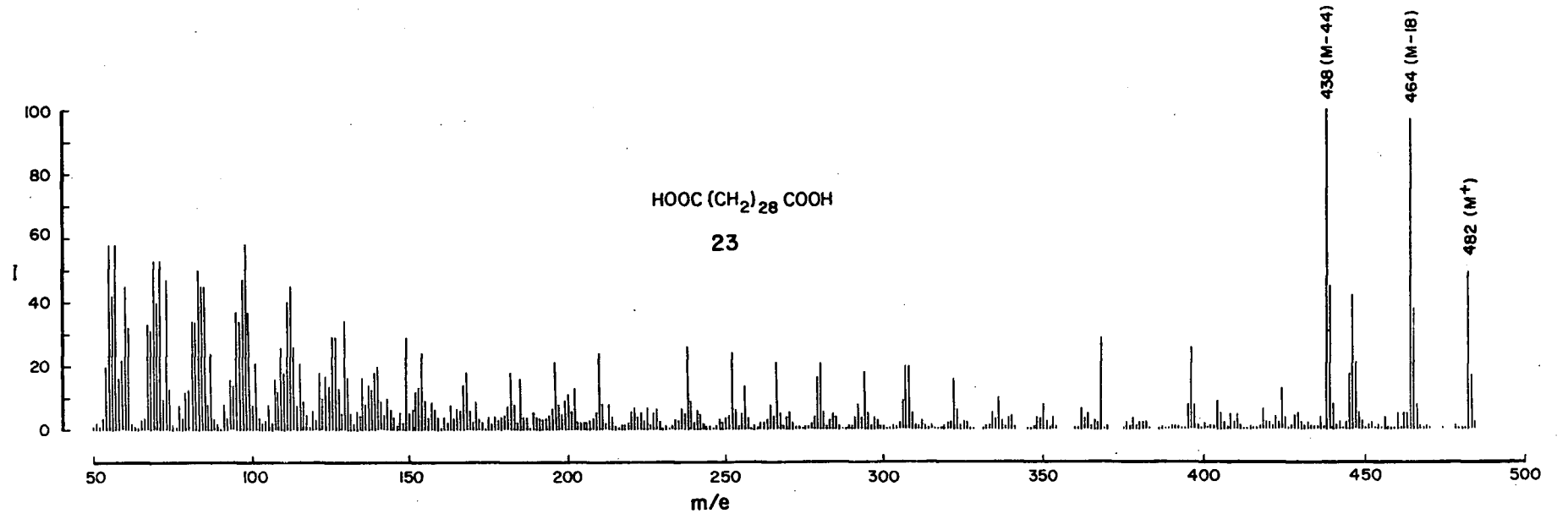


Figure 5. Mass Spectrum of Equisetolic Acid

$H_3COOC(CH_2)_{28}COOCH_3$

24

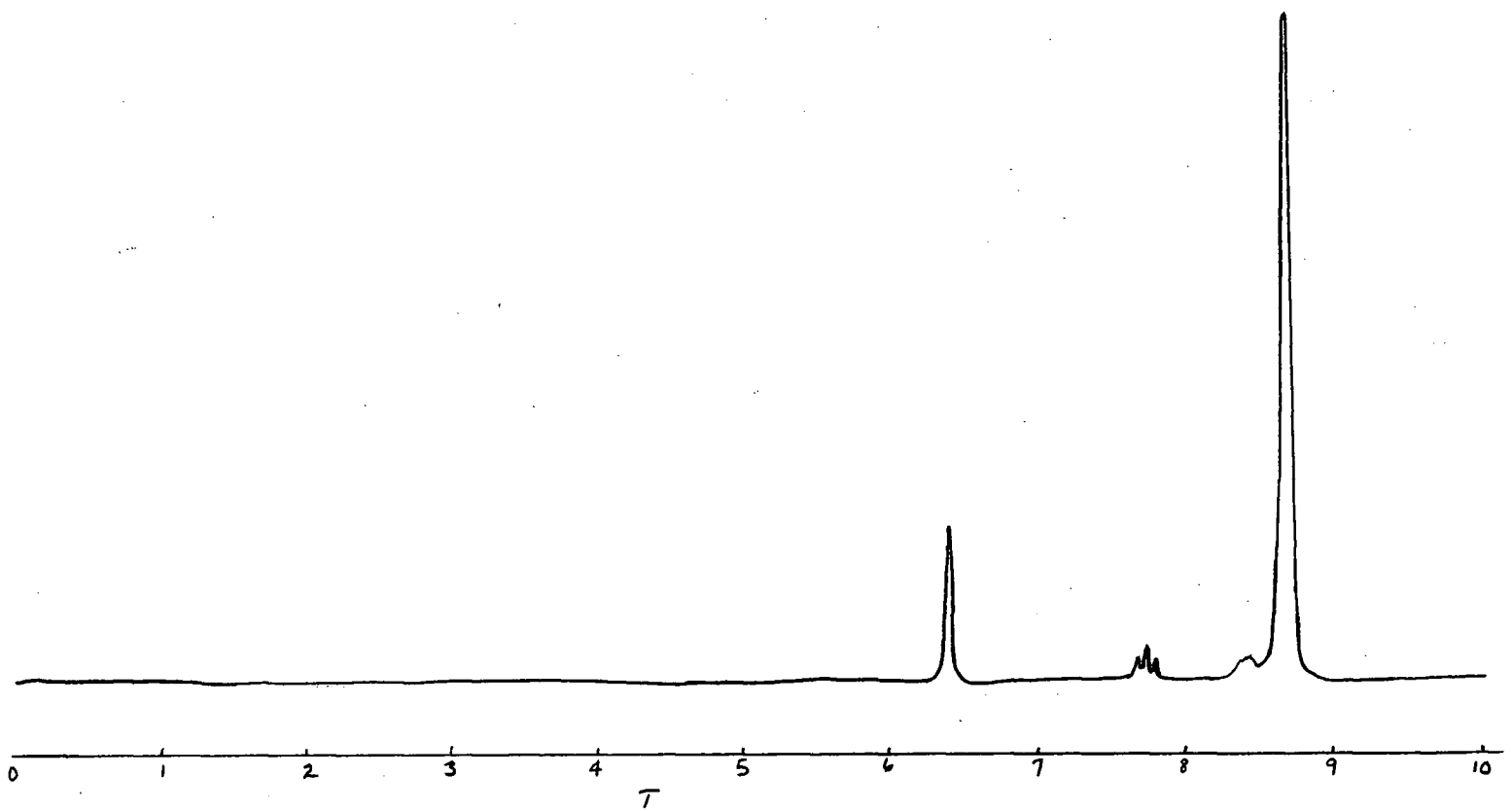


Figure 7. NMR Spectrum of Equisetolic Acid Methyl Ester.

follows: OCH_3 (6 H); $-\underline{CH_2}-\underset{\underset{O}{\|}}{C}-$ (4 H); $-\underline{CH_2}-CH_2-\underset{\underset{O}{\|}}{C}-$ (4 H) and $-CH_2-$ (48 H).

It is now concluded that equisetolic acid has the structure 23 and its methyl ester is 24.

The same acid has been isolated from the spores of Equisetum arvense and is being studied by Bonnett and co-workers.[41]

Other Constituents

An attempt to isolate alkaloids from the methanol extracts yielded only an insignificant amount of material. This was not unexpected in view of the very low alkaloid content reported in the literature for other Equisetum species.[3,6,7]

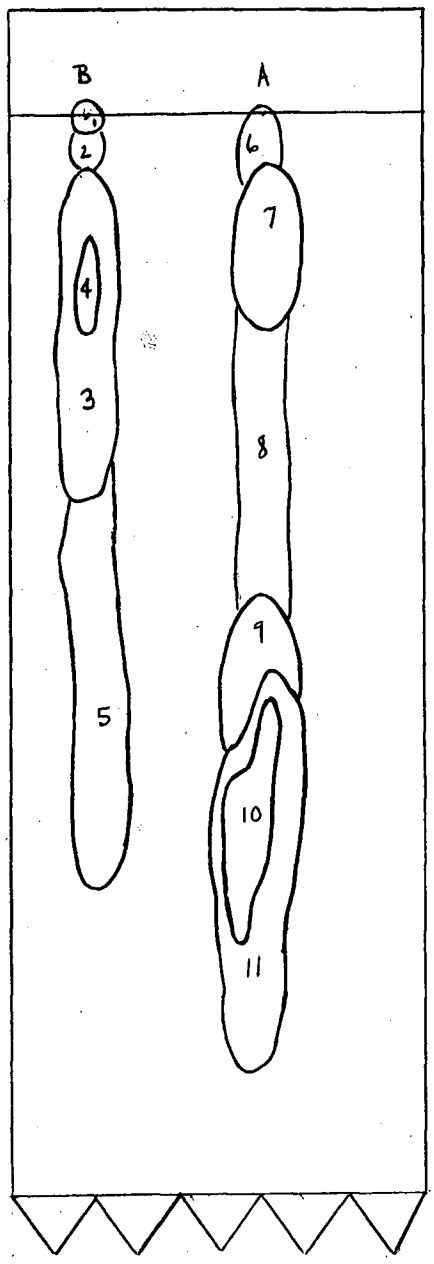
Towers[40] and co-workers extracted Equisetum telmateia spores with water and obtained large amounts of sucrose. This has not been identified yet in our extracts and it is probably in the aqueous layers.

These aqueous layers from which equisporoside separated contained more equisporoside and several other components as shown by paper chromatography in butanol-acetic acid-water, 4:1:5. (Figure 8). Some separation was achieved on a polyamide column but no pure substances were isolated. The fractions all gave positive ferric chloride tests and undoubtedly contained phenolic compounds. The ultraviolet spectra of various mixtures were not very informative.

Investigation of the other constituents of Equisetum telmateia spores is continuing in our laboratory and should prove very interesting.

A - Methanol extract after addition of ether and removal of equisporoside (Procedure A, Experimental).

B - Aqueous extracts of methanol-petrol layer after separation of equisporoside (Procedure B, Experimental).



Spot	Colour	
	Visible	Ultraviolet
1	brown	yellow
2	brown	yellow
3	yellow	blue
4	yellow	brown
5	pale yellow	blue
6	pale yellow	yellow
7	pale yellow	blue
8	pale yellow	blue
9	pale yellow	blue
10	green	brown
11	green	red

4 = equisporoside

Figure 8. Other Constituents of Equisetum telmateia Methanol Extracts.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Ultraviolet and visible spectra were measured in ethanol on Cary 14 and Cary 11 spectrophotometers. Infrared spectra unless otherwise reported were taken as KBr pellets on a Perkin-Elmer Model 21 spectrophotometer. Nuclear magnetic resonance spectra were recorded at 60 megacycles per second on a Varian A 60 instrument and at 100 megacycles per second on a Varian HA 100 instrument using the solvents indicated; the values obtained at 100 megacycles per second are reported here. The line positions or centres of multiplets are given in the Tiers τ scale with reference to tetramethylsilane as the internal standard. The multiplicity, integrated areas and type of protons are indicated in parentheses. The mass spectra were recorded with an Atlas CH4 mass spectrometer using the direct insertion technique, the electron energy being maintained at 70 ev. High resolution mass spectra for the determination of molecular formulae were obtained using an AEI MS9 mass spectrometer. The analyses were performed by the microanalytical laboratory, University of British Columbia.

Collection of Spores

The strobili (cones) of Equisetum telmateia were collected in the Spring (April 14-18, 1966) in Vancouver and Squamish, B.C. After allowing the strobili to dry for 3 or 4 days at room temperature, the spores (4.407 kg.) were shaken out.

Isolation of Equisetolic Acid

The spores (4.407 kg.) were extracted (Soxhlet) with ether for approximately eight hours (until the extracts were colourless). Upon concentration of the combined ether extracts a white solid (8 g.) separated,

which after one crystallization from ethyl acetate melted at 127-129°; ν_{max} (nujol): 2623 and 959 (OH), 1693 cm^{-1}; no ultraviolet absorption. Calc. for $C_{30}H_{58}O_4$: C, 74.70; H, 12.02; O, 13.28. Found: C, 75.16; H, 12.20; O, 13.10. Calc. for $C_{30}H_{58}O_4$: 482.433. Found: 482.437.

Acetylation of Equisetolic Acid[2]

Equisetolic acid (97.9 mg.) in anhydrous pyridine (3.6 ml.) and acetic anhydride (1.2 ml.) was heated on a steam bath for 1 hour. The mixture was poured into ice-water and the product filtered (90 mg.), m.p. 119-123°. The infrared spectrum of this product showed no ester carbonyl absorption and merely indicated that it was impure equisetolic acid. Subsequent comparison by infrared showed these compounds to be identical.

Methylation of Equisetolic Acid

Equisetolic acid (519.4 mg.) was dissolved in a mixture of hot ether (250 ml), benzene (250 ml) and methanol (480 ml) and this solution was allowed to cool slowly to 0°. An ethanolic-ether solution of diazomethane (3.0 g., 0.71 moles) was poured into this solution and it was left to stand overnight. Concentration of the solution caused the product to separate. It was crystallized from chloroform-methanol (465 mg.), m.p. 84-86°; ν_{max} (chloroform): 1748 cm^{-1} (carbonyl); no ultraviolet spectrum; NMR (deuteriochloroform): 6.4 (singlet, 6 H, $-O\underline{CH_3}$), 7.7 (triplet, J = 6 c.p.s., 4 H, $RCH_2\underline{CH_2}COOH$), 8.4 (broad multiplet 4 H, $R\underline{CH_2}CH_2COOH$), 8.7 (singlet, 48 H, methylene protons). Mass spectrum: (Figure 6).

Isolation of Equisporoside

The spores which had already been extracted with ether were submitted either to procedure A or B below:

A. The spores (721 g.) were extracted with methanol (Soxhlet) for four days, the extracts concentrated to a small volume and ether added. A yellow gum

which separated was removed from the solution by decantation, and then dissolved in water. The filtered solution was allowed to stand, during which time a light brown solid (2.49 g.) separated.

B. The spores (750 g.) were extracted (Soxhlet) for ten hours with methanol and the extract evaporated nearly to dryness. The residue was taken up in petroleum ether and extracted with water. After concentration of the combined aqueous layers, yellow-brown needles (2.17 g.) separated. Extraction of the spores with methanol for a further ten hours followed by the same work-up gave a further 1.10 g. of yellow-brown needles.

Paper chromatography using Whatman No.3 paper and 5% aqueous acetic acid or butanol-acetic acid-water (4:1:5) showed that the yellow-brown solids were almost pure but the aqueous solutions from which they separated were complex mixtures of up to six components. In the former solvent, equisporoside scarcely moved from the baseline while in the latter it had and R_F value of 0.43.

Purification of Equisporoside

Equisporoside (4.1 mg.) in a minimum amount of methanol was spotted on Whatman No.3 paper and developed with butanol-acetic acid-water (4:1:5). Equisporoside (R_F 0.43) and the minor, faster running impurity (R_F 0.62) appeared as brown bands under ultraviolet light. The band corresponding to equisporoside was cut out and the material was removed by allowing the paper to remain in contact with methanol-water (1:1, 100 ml) for 2 hours. The resulting solution was filtered and solvent removed to give pure equisporoside (2.6 mg.). Elution of the minor component gave 0.3 mg. A detailed investigation of the ultraviolet spectra of equisporoside was carried out and the results were as follows: λ_{max}^{EtOH} (log ε): 388 (4.21), 348 (shoulder), 277 (shoulder), 261 mμ (4.30); after adding 1 drop of a 2% ethanolic aluminum

chloride solution λ_{max} (log ε): 455 (4.24), 383 (shoulder), 275 mμ (4.41); after addition of excess fused sodium acetate to both the sample and solvent cells λ_{max} (log ε): 394 (4.12), 351 (shoulder), 280 (shoulder), 266 mμ (4.38); 20 minutes after addition of saturated ethanolic boric acid solution (2 ml.) to the ethanol solution of equisporoside (2 ml.), dilution to 10 ml. and addition of excess anhydrous sodium acetate λ_{max} (log ε): 404 (4.29), 266 (4.36), 250 mμ (shoulder); after addition of an 0.03 sodium ethoxide solution (2 ml.) to the ethanol solution of equisporoside (2 ml.) and dilution to 10 ml. λ_{max} (log ε): 390 very broad peak (3.95), 274 (4.75). Gossypitrin λ_{max} (log ε): 388 (4.20), 350, 278, 262 mμ (4.33).[36]

When p-benzoquinone was added to an ethanol solution of equisporoside, a dark red-brown colour developed and a precipitate formed indicating a positive gossypetone reaction.[35]

Equisporoside was dark orange in aqueous sodium hydroxide turning to brown on standing. In concentrated sulphuric acid it was a very intense yellow, indicating it was probably a flavonol.[42]

The colour reactions on paper[43] also indicated it was a flavonol. With no reagent in visible light it appeared yellow and under ultraviolet light, brown. After being exposed to ammonia vapour it turned a brighter yellow in visible light and a light brown in ultraviolet light.

Larger amounts of equisporoside were purified by column chromatography on Woelm polyamide-celite (8:2). Equisporoside was not very soluble in water so a mixture of methanol-water was used. In a typical experiment, equisporoside (788 mg.) was dissolved in methanol-water (1:1, 40 ml.) and applied to a column of polyamide-celite (10 g.) which had been packed with water. Elution with methanol-water (1:1) yielded equisporoside (608 mg., 77% recovery). Equisporoside crystallized as small yellow needles from

aqueous acetic acid. After drying at 85° under vacuum for 12 hours, it melted at 202-204°. An authentic sample of gossypitrin (obtained from Geissman), melted at 199-201°; mixed m.p. 199-201°. ν_{max} (KBr): 3400, 2900 (shoulder), 1650, 1605, 1557 and 1510 cm^{-1}; superimposable on that of gossypitrin.

Preparation of Trimethylsilyl Ether of Equisporoside for NMR Study

Equisporoside (40 mg.) was dissolved in anhydrous pyridine (3 ml.) and hexamethyl disilazane ($[(CH_3)_3Si]_2NH$, 0.5 ml) and trimethylchlorosilane (0.5 ml) was added. The solvent and excess reagents were immediately removed under high vacuum and the dry residue was extracted with carbon tetrachloride. The clear solution obtained by filtering off the salts was concentrated to a suitable volume (0.4 ml) and used directly for the NMR measurement. NMR signals: 2.34 (multiplet, 1.8 H, $H_{2'}$ and $H_{6'}$), 3.20 (doublet, 1 H, $H_{5'}$), 3.73 (singlet, with shoulder, 1 H, H_6), 5.2 (broad doublet, J = 6 cps., 1H, anomeric proton of the sugar), 6.20-6.70 (multiplet, 6H, sugar protons), 9.62-9.90(silyl ethers).

Hydrolysis of Equisporoside

Equisporoside (66.7 mg.) in methanol (20 ml) and 2N sulphuric acid (20 ml) was refluxed for 4 hours, cooled and the solution extracted with ethyl acetate. Evaporation of the combined ethylacetate extracts yielded the aglycone, equisporol, (43 mg.). It crystallized from aqueous acetic acid as yellow needles, m.p. 301-304° (dec., Kofler preheated to 290°). There was no depression of the melting point when mixed with authentic gossypetin.

Equisporol had an R_F value of 0.51 on paper chromatography using butanol-acetic acid-water (4:1:5) as eluting solvent. Dropwise addition of methanolic potassium hydroxide, to a methanol solution of equisporol

caused the originally yellow solution to change initially to blue and then to green. In acidic solution the colour was bright red. ν_{max} (KBr): 3360, 3270, 1648, 1620 (1602, shoulder), 1573, 1513 cm^{-1}; superimposable on that of authentic gossypetin; λ_{max}^{EtOH} (log ε): 384 (4.08), 341 (4.01), 276 (4.21), 263 mμ (4.22); immediately after addition of one drop of a 2% ethanolic aluminum chloride solution λ_{max} (log ε): 450 (3.97), 380 (3.96), 287 mμ (4.26); after addition of excess fused sodium acetate to both the sample and solvent cells λ_{max} (log ε): 328 (4.15), 249 mμ (4.29); 20 minutes after the addition of saturated ethanolic boric acid solution (2 ml.) to the ethanol solution of equisporol (2 ml.), dilution to 10 ml. and addition of excess anhydrous sodium acetate λ_{max} (log ε): 412 (3.92), 357 (4.03), 285 (shoulder), 274 mμ (4.30); after addition of an 0.03 M sodium ethoxide solution (2 ml.) to the ethanol solution of equisporol (2 ml.) and dilution to 10 ml. λ_{max} (log ε): about 387 (broad peak), 287 mμ (4.26); Gossypetin λ_{max} (log ε): 386 (4.15), 341 (shoulder), 278 (4.23), 262 mμ (4.26);[36] NMR (acetone-d_6): 0.93, 1.61, and 1.82 (broad singlets each integrating for 1 H, presumably due to three of the hydroxyl protons), 2.19 (doublet, J = 2 c.p.s., 1 H, H_2'), 2.27 (quartet, J = 8.5 c.p.s. and 2 c.p.s., H_6'), 3.10 (doublet, J = 8.5 c.p.s., H_5'), 3.74 (singlet, 1 H, H_6).

The aqueous layer was neutralized with barium carbonate, the bulk of the salts removed by filtration and the solution passed through a series of columns containing Amberlite IR-120 H C.P. medium porosity, strongly acidic cation exchange resin (100 ml.) which had been regenerated with 2N HCl and backwashed with water until neutral, Duolite A-4 anion exchange resin (100 ml.) which had been regenerated with 2N NaOH and backwashed with water until neutral, and Amberlite IR-120 H C.P. resin (20 ml.). Evaporation of the water at 40°C. gave a clear gum (22.09 mg.) which was identified as

glucose by paper chromatography in three different solvent systems. (See Table 4). The papers were developed by immersing them twice in silver nitrate-aqueous acetone (silver nitrate (1 g.) in acetone (100 ml.) to which just enough water was added to obtain a clear solution), once in ethanolic sodium hydroxide solution until the spots developed and finally in aqueous sodium thiosulphate solution. In each instance the papers were allowed to dry between immersions.

TABLE 4

R_F Values of Sugars

Solvent	Hours developed	galactose	glucose	unknown	sucrose	galactose + unknown	glucose + unknown
formic acid-acetic acid-water-ethyl-acetate 1:3:4:18	65	14	16.2	16.1	8.3	14.6 16.5	15.8
ethylacetate-pyridine-water 8:2:1	68	7.3	9.6	9.6	3.6	9.9 7.9	9.6
butanol-ethanol-water 3:1:1	68	6.8	8.0	7.8	4.7	8.2 7.2	8.0

Equisporol Hexamethyl Ether

Equisporol (108.2 mg.) was dissolved in anhydrous acetone (40 ml.), anhydrous potassium carbonate (1.2 g.) and dimethyl sulphate (0.5 ml) added, and the mixture refluxed for a total of 36 hours. During the reflux period two additional portions of potassium carbonate and dimethyl sulphate were

added. The dark solution was poured into water and extracted with ether, dried ($MgSO_4$) and the ether removed to give a brown oil (200 mg) which was chromatographed on alumina (Woelm, neutral, activity IV) (20 g.). Elution with chloroform yielded a pale yellow solid (36.8 mg.), m.p. 152-153°. This latter substance was washed with a small amount of methanol to give a white solid, m.p. 165-168°, which after recrystallization from methanol-chloroform melted at 170-171.5°; gossypetin hexamethyl ether (obtained from Geissman), 166-168°; mixed m.p. 166-169°. ν_{max} (KBr): 2900, 1620, 1595, 1570 (shoulder), 1510 cm^{-1}; superimposable on that of gossypetin hexamethyl ether. λ_{max}^{EtOH} (log ε): 351 (4.32), 272 (4.33), 252 mμ (4.35); Gossypetin hexamethyl ether[36] λ_{max} (log ε): 351 (4.34), 273 (4.33), 252 mμ (4.34). Calc. for $C_{21}H_{22}O_8$: 402.131. Found: 402.130.

Equisporol Hexaacetate

Equisporol (19.73 mg.) was refluxed with acetic anhydride (0.12 ml) and pyridine (2 drops) for two hours, poured into water, extracted with ether and the solvent removed. The residue gave a negative ferric chloride test. Crystallization of the crude product from acetic anhydride-methanol afforded a very poor yield (1 mg.) of the hexaacetate. During the melting point determination, this compound first sintered at 190°, again at 216° and finally melted at 226-230°. Gossypetin hexaacetate,[35] sinters at 190°, again at 210° and finally melts at 226-228°.

Equisporol Pentamethyl Ether

Equisporoside (310 mg.) and anhydrous acetone (25 ml.) were placed in a 50 ml. two-necked flask fitted with a condenser and dropping funnel. The solution was stirred for a few minutes, anhydrous potassium carbonate (6 g.) added and the apparatus flushed with nitrogen. After heating to

reflux, dimethyl sulphate (5 ml.) was added dropwise over a period of 2.5 hours. Four hours after the addition of dimethyl sulphate was begun an aliquot gave a positive (olive green) ferric chloride test. After 8 hours the test was negative. The yellow solution was filtered, evaporated to dryness and taken up in ethanol (15 ml.) and 2N sulphuric acid (24 ml.). After refluxing for 2 hours the solution was cooled, extracted with chloroform and the combined extracts washed with water. Removal of the chloroform left a yellow residue which crystallized as pale yellow needles (64.2 mg.) from ethanol-chloroform, m.p. 249-252°. This compound gave a negative ferric chloride test. λ_{max}^{EtOH} (log ε): 253 (4.14), 272 (4.18), 349 mμ (4.13); after addition of a 0.03 M sodium ethoxide solution (2 ml.) to an ethanol solution of the pentamethyl ether of equisporol (2 ml.) and dilution to 10 ml. λ_{max} (log ε): 282 (4.43), 387 mμ (3.96). Calc. for $C_{20}H_{20}O_8$: 388.116; Found: 388.117. 7-hydroxy-3,3',4',5,8-pentamethoxyflavone,[36] m.p. 250-251°. λ_{max}^{EtOH}: 351, 270, 251 mμ: after addition of sodium ethoxide λ_{max}: 368, 280 mμ.

Pentamethyl equisporol (12.91 mg.) was dissolved in acetic anhydride (0.12 ml.) and pyridine (5 drops) and allowed to stand overnight at room temperature. Water was added and the solution extracted with ether. The ether was evaporated and the product crystallized as yellow needles from ethyl acetate, m.p. 159-161°. Three further recrystallizations provided 6 mg. of a pure substance, m.p. 166-168°. Calc. for $C_{22}H_{22}O_9$: 430.126. Found: 430.128. 7-acetoxy-3,3',4',5,8-pentamethoxyflavone,[36] m.p. 164-168°.

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