INVESTIGATIONS ON STEROIDS

STUDIES ON THE TRANSFORMATIONS OF KURCHI ALKALOIDS

A

T H E S I S

Submitted to

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by

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P o o n a - 8

A C K N O W L E D G E M E N T

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C O N T E N T S

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CHAPTER I

INTRODUCTION

INTRODUCTION

One of the major developments in organic chemistry in the past decade has been the development of commercial syntheses for the steroid hormones, such as progesterone, deoxycorticosterone, hydrocortisone etc., the so-called 'miracle drugs'. Ingenious use has been made of some preformed steroids such as diosgenin, hecogenin, cholesterol, phytosterols etc., available in an abundant supply from nature as starting points for those syntheses. The intense research activity in this field has not only brought the price levels of the steroid hormones down to a rock-bottom, but also has culminated in the development of a variety of interesting hormone analogues, such as the methylated steroids, the 1:4-diene-steroids, the 9-halosteroids, the 19-nor steroids and other modified steroids, some of which have very intensified and highly specific pharmacological and physiological properties in many instances different from those of the natural hormones.

The interest in the 18-substituted steroids is of a comparatively recent origin, dating back to the discovery of the mineralocorticoid hormone, aldosterone.[1] Some recent reports[2,3] indicate a bright future for this class of steroids.

The isolation of aldosterone by Simpson and Tait and Wettstein, Reichstein and co-workers[1] from the

so-called 'amorphous' fraction of the supra-renal extracts and the elucidation of its remarkable biological properties, induced the organic chemists to develop a commercial synthesis for this compound. Consequently, several elegant methods of total synthesis of aldosterone are available to-day.[4-9]

Considering, however, the number of steps involved in these syntheses and the problem of resolution of racemates, it is highly doubtful that any of these would stand the test of time as a commercially acceptable synthesis.

The major breakthrough in the steroid industry has been the development of partial synthetic methods involving the 'tailoring' of suitable natural steroids to make the hormones. In that context the 18-substituted steroidal alkaloids occurring in nature hold out some promise as being attractive starting materials for the preparation of aldosterone and other 18-substituted steroids. Other approaches which appear even more promising, are the recent synthesis of aldosterone by Barton and Beaton[10] which utilizes a photo-catalysed nitrosyl-transfer reaction from position 11 to the position 18 in 11β-hydroxy-corticosterone and the intramolecular introduction of an oxygen-function at position 18 from a 20 hydroxyl group with the aid of lead tetra-acetate by Hensler *et al.*[11]

It is necessary to record that when this work was undertaken in October 1958 no partial synthesis of

aldosterone from a naturally-occurring steroid was known, the intramolecular oxygen or nitrosyl transfer processes appearing in print at a much later date. Furthermore, even if it is conceded that for aldosterone synthesis the 18-substituted steroidal alkaloids may appear less promising than what they did in late 1958, the variety of steroids with different substituents at positions 3, 18 and 20, theoretically derivable from these alkaloids, would have justified the undertaking of the present investigation.

The 18-Substituted Steroidal Alkaloids

Only two classes of 18-substituted steroidal alkaloids which have the desirable structural features for a potential starting material are known to occur in nature. These are (1) The 18-substituted pregnane alkaloids, and (2) Isorubijervine.

The pregnane alkaloids have been isolated mainly from "kurchi" (Genus-Holarrhena), although recently a few have been isolated from the family Apocyanaceae.[12] Isorubijervine has been isolated from Veratrum viride.[13]

The 18-Substituted Pregnane Alkaloids

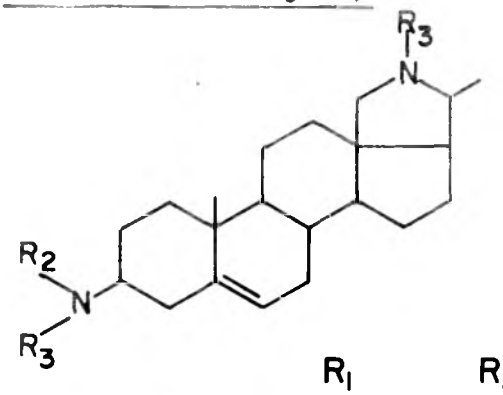
Out of the twenty-six alkaloids of this class isolated so far from the seeds and bark of the plants of the Genus Holarrhena, the Indian species, H.antidysenterica has yielded twenty-one. Two alkaloids of this class, paravallarine and methyl paravallarine, have been obtained from a species of Apocyanaceae. Structurally, the 18-substituted pregnane alkaloids can be classified into six broad groups as shown in Plate I (next page).

1. The conessine group is characterised by the presence of 3β-amino group, either unsubstituted or substituted by one or two methyl groups in a 5-pregnene nucleus and an 18-20 imino bridge nitrogen which is either unsubstituted or methylated.

18-Substituted steroidal alkaloids

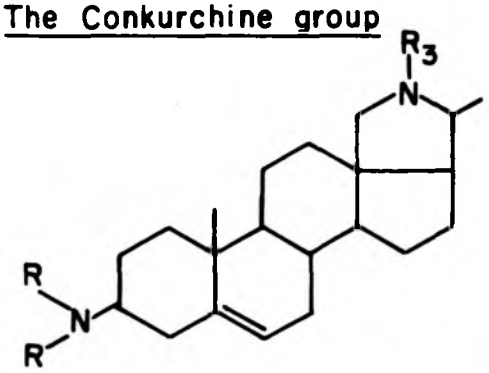
The Holarrhena alkaloids

1) The Conessine group



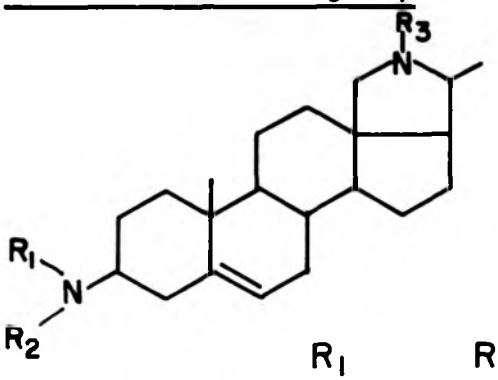
	R_1	R_2	R_3	m.p	$[\alpha]_D$
Conessine	CH_3	CH_3	CH_3	126°	−1·9° 20·6° (ethanol)
isoConessimine	CH_3	H	CH_3	92°	+30 (ethanol)
Conessimine	CH_3	CH_3	H	100°	−22·3
Conimine	CH_3	H	H	130°	−30 (ethanol)
Conamine	H	H	CH_3	130°	−19 (ethanol)
Conarrhimine	H	H	H	not pure	

2) The Conkurchine group



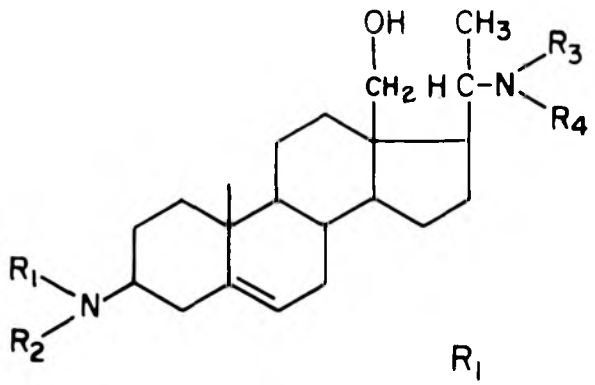
	R_1	R_2	R_3	Δ^5	m.p	$[\alpha]_D$
Conkurchine	H	H	H	present	152-153°	−51·9 (ethanol)
Conessidine	CH_3	H	H	"	123°	−63·5
Trimethylconkurchine	CH_3	CH_3	CH_3	"	125-127°	+12
Kurchamine	H	H	H	Saturated	115-117°	+16

3) The Holarrhenine group



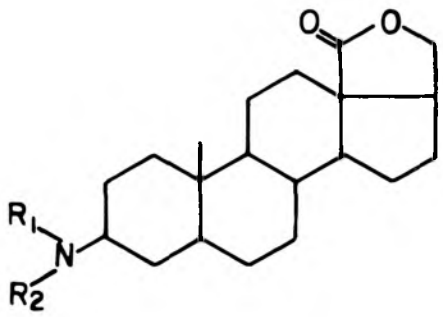
	R_1	R_2	R_3	R_4	m.p	$[\alpha]_D$
Holarrhenine	CH_3	CH_3	CH_3	H	197-198°	−7·1
Holafrin	CH_3	CH_3	H	$(CH_3)_2C{=}CHCH_2\overset{O}{\overset{\|\|}{C}}-$	116-117°	−19·1
Holarrhetine	CH_3	CH_3	CH_3	"	74-75°	−14·9

4) The Hollarrhimine group



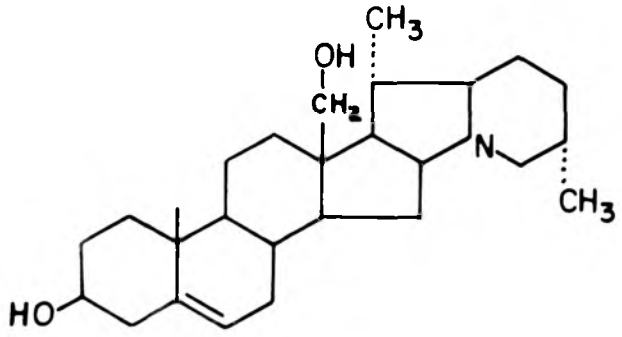
	R_1	R_2	R_3	R_4	Config at 3	m.p	$[\alpha]_D$
Holarrhimine	H	H	H	H	β	183°	-14·2
3N-Methyl holarrhimine	CH_3	H	H	H	β	above 360°	-28(Meth)
20N-Methyl holarrhimine	H	H	CH_3	H	β	163-164°	-19
Tetramethyl holarrhimine	CH_3	CH_3	CH_3	CH_3	β	227-228°	-33
Holarrhidine	H	H	H	H	α	181-182°	-23

Paravallaris alkaloids



	R_1	R_2	m.p	$[\alpha]_D$
Paravallarine	CH_3	H	181°	-52°
Methyl paravallarine	CH_3	CH_3	140°	-37·7°

The Veratrine alkaloid



Isorubijervine	m.p	$[\alpha]_D$
	237°	+ 6·5 (alcohol)

2. The alkaloids of the conkurchine group have the same basic structural features as those of the conessine group, excepting for the presence of an extra double bond between the positions 17 and 20. In kurchamine the 5,6-double bond is absent.

3. The bases of the holarrhenine group are structurally similar to the conessine alkaloids excepting for the presence of a free or esterified hydroxyl group at the 12β position.

4. In the alkaloids of the holarrhimine group, the nitrogen bridge across the positions 18 and 20 is absent. These bases have 18-hydroxy 3β-or α-, 20α-diamino-5-pregnene as the basic skeleton where both the amino groups may be unsubstituted or either partially or completely substituted by methyl groups.

5. The paravallarines have most of the basic features of the holarrhimines excepting for the positions 18 and 20 which are connected by a lactone-bridge.

6. Isorubijervine contains the 18-hydroxy-5-pregnene nucleus with two fused heterocyclic rings attached to ring D.

The Kurchi Alkaloids and Their Interrelationships

The ditertiary base, conessine, was isolated more than hundred years ago by Haines.[14] The first systematic investigations, however, on the alkaloids of Holarrhena antidysenterica were undertaken by Siddiqui and co-workers only thirty years ago. They isolated several new alkaloids

such as iso-conessimine, conessimine, holarrhimine, holarrhine, conimine, conamine and conarrhimine from the seeds and bark of Indian 'kurchi'.[15-18] They were also able to demonstrate some interesting interrelationships among these alkaloids.[18] All the bases of the conarrhimine type were converted to the ditertiary base, conessine, on refluxing with formaldehyde and formic acid. Conessine, on the other hand, could be progressively demethylated to the secondary-tertiary base, iso-conessimine, and the di-secondary base, conimine. They also recorded an interesting observation that the completely un-substituted base, conarrhimine, was converted to holarrhimine by refluxing with moist ethyl acetate.[17] Now that the chemistry of the kurchi alkaloids has been firmly established, it is difficult to visualise a plausible mechanism for such an unusual transformation. It may be mentioned in this connection that the purity of connarrhimine isolated by Siddiqui and Siddiqui[17] was not rigorously established.

The bases of the conkurchine group were isolated by Bertho,[19] and later by Tschesche and co-workers,[22-24] and correlated with the conessine group by hydrogenation of the easily reducible 17-20 double bond.

The holarrhenine group of bases have been isolated so far only from the African variety of kurchi.[25-28] Oxidation of the 12β-hydroxyl group followed by the

hydrogenolysis of ethylene dithioketal of the resulting 12-oxo compound converts this class of compounds to the conessine group.[26]

Alkaloids of the holarrhimine group have been isolated by Siddiqui and Pillay,[15] Tschesche and co-workers, and Sorm and co-workers.[29-39] Labler *et al.* were able to convert dihydrotetramethyl holarrhimine to dihydroconessine by refluxing with thionyl chloride which resulted in an intramolecular displacement of the 18-hydroxyl group by the 20-dimethylamino group and demethylation.

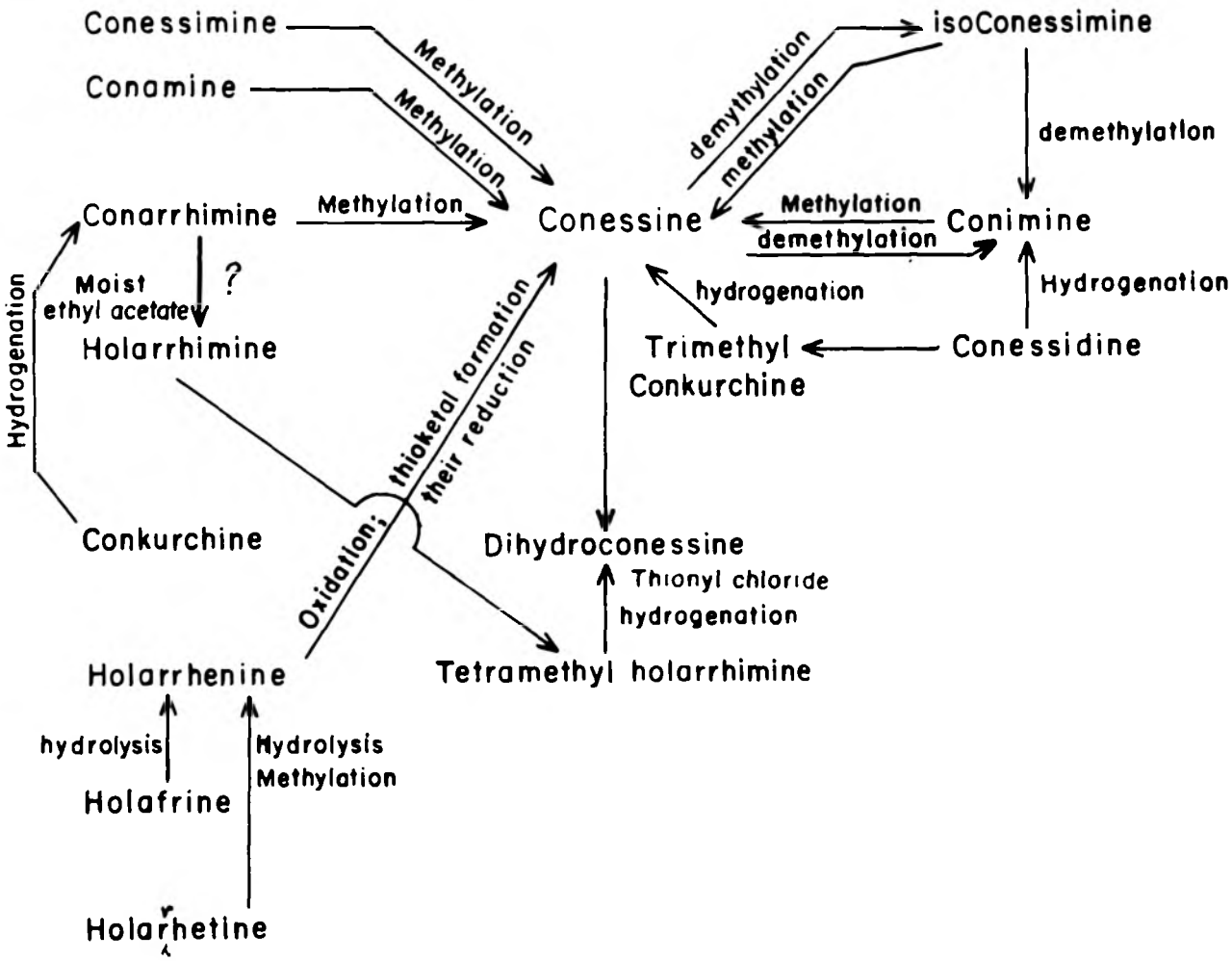
The interrelationships of these alkaloids are summarised in Fig.I.

From these relationships it is clear that if the constitution of any one of the alkaloids could be established, the structures of other alkaloids could also be deduced by a few simple transformations. This is precisely what followed immediately after the elucidation of structure of conessine by Haworth and co-workers.[35-45]

The shrub kurchi, *Holarrhena antidysenterica* grows abundantly in India especially in the State of Bombay, the Malabar Region and the Himalayan Belt. The extract of kurchi has been used for the treatment of dysentery from the ancient times and the bark is available commercially @ £ 1/- per 40 kg. The chloroform soluble alkaloidal content of the bark varies from 1.9 to 2.2 percent. The three alkaloids conessine, *iso*-conessimine

Figure I

Interrelationships among the Kurchi Alkaloids



and conessimine make up approximately half of the total alkaloid mixture. The above considerations led to the choice of these three alkaloids from kurchi as starting materials for the synthesis of 18-substituted steroids.

The Chemistry of Conessine

Conessine, a ditertiary base, $C_{24}H_{40}N_2$, is the most abundant alkaloid from kurchi occurring to the extent of 0.4 to 0.7 percent of the dry weight of the bark. Pharmacological studies of pure conessine is a more recent development. The reported anti-amoebic action of conessine *in vivo* could not be demonstrated *in vitro*.[46] Meissner and Hesse[47] have shown that it inhibits the growth of tubercle bacilli *in vitro*.

Systematic work on the constitution of conessine was started in the early thirties by Siddiqui and co-workers[18] and culminated in the eventual establishment of its structure by Haworth and co-workers.[35-45]

The preliminary work of Siddiqui and co-workers[18] led to the conclusion that conessine contained the following structural features:-

1. A double bond which is rather difficult to reduce;
2. A tertiary dimethylamino group;
3. A tertiary methylimino group.

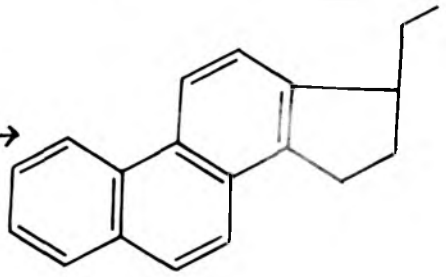
On the basis of this, the structure was developed to $C_{21}H_{31}N^{a}Me_2$ - $N^{b}Me$ (N^{a} representing the dimethylamino and N^{b} representing the methylimino groups).

The physical data especially the lack of an ultraviolet absorption peak above 200 mμ, suggested that neither of the amino groups was adjacent to the double bond or in other words, a vinylamine type of structure was absent.

Siddiqui obtained on dry distillation of the dihydro-iodide of conessine a hydrocarbon, $C_{21}H_{30}$, conessene.[48] Haworth and co-workers[35] obtained 3'-ethyl-1, 2-cyclopentanophenanthrene by selenium dehydrogenation of conessene.

$$C_{24}H_{40}N_2 \cdot 2HI \xrightarrow{\Delta} C_{21}H_{30} \xrightarrow[\Delta]{Se}$$

Conessene



A steroidal basic skeleton for conessine was, therefore, strongly indicated.

After exhaustive methylation and Hofmann degradation, conessine yielded four products:-

(a) Apoconessine, $C_{21}H_{29}$ $N^{b}Me_2$(ii) with three double bonds;

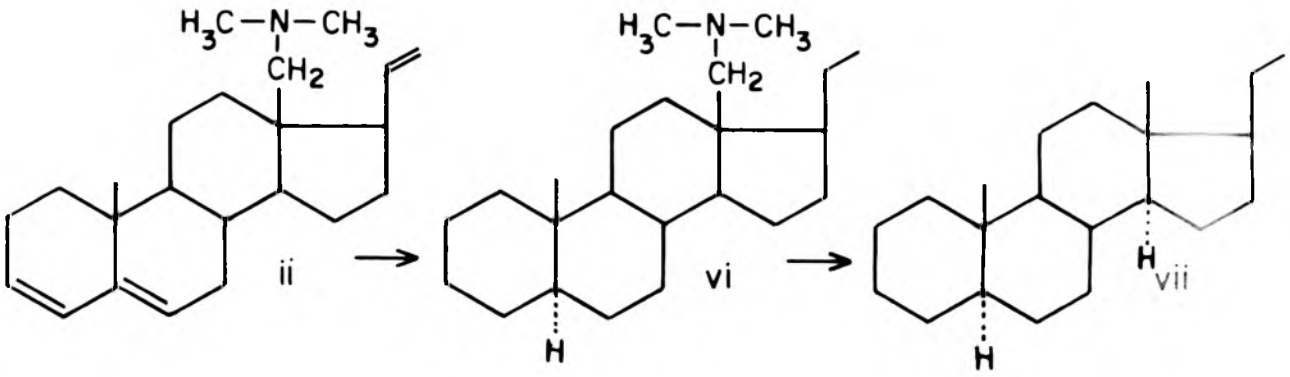
(b) a methiodide, $C_{21}H_{30}$ $N^{b}Me2I$(iii) with two double bonds;

(c) conessimethine, $C_{21}H_{30}$ $N^{a}Me_2$-$N^{b}Me_2$(iv) with two double bonds; and

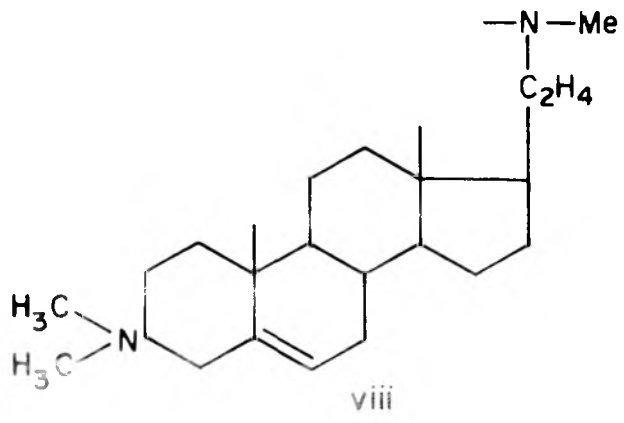
(d) heteroconessine, $C_{21}H_{30}N^{a}Me_2$-$N^{b}Me$(v) with one double bond.

Chemical studies on these four products led to the complete elucidation of the structure of conessine.

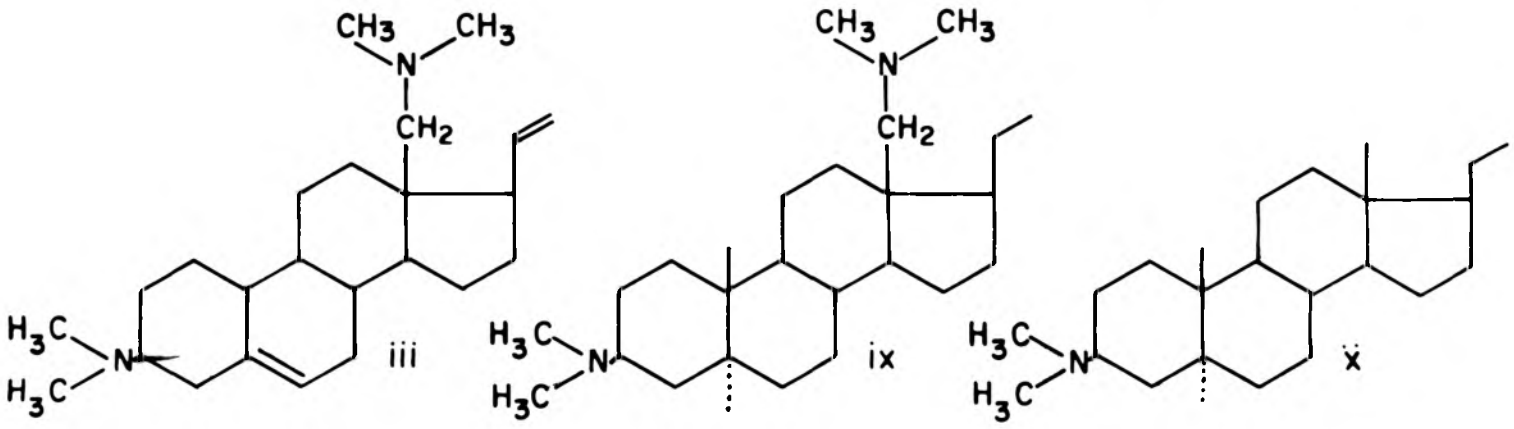
(a) Apoconessine (ii) was converted by Emde degradation to a $\Delta^{3,5,20(21)}$ pregnatriene (vi) which was hydrogenated to the known 5α-pregnane (vii).



(b) Since apoconessine (ii) and the methiodide (iii) ii) retained the N^b nitrogen only and since it was found that the double bond produced by the elimination of N^a nitrogen was in conjugation with another double bond, a likely partial structure for conessine could be formulated as in (viii).

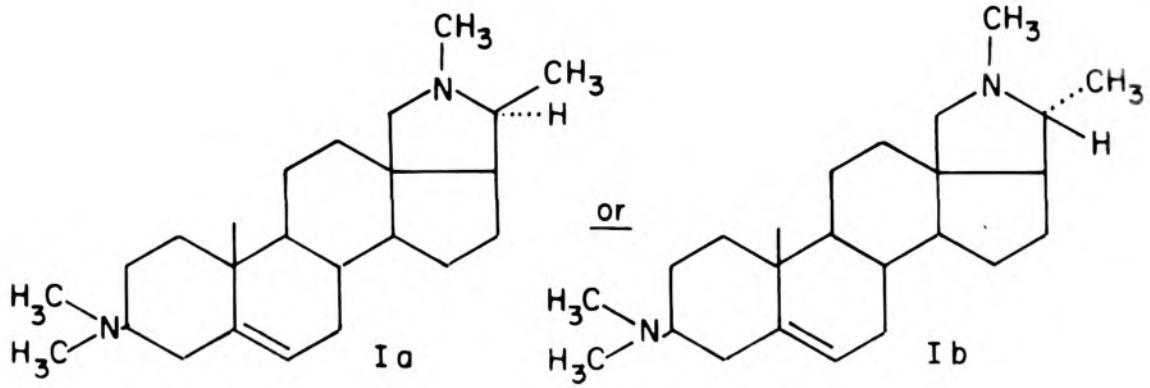


(c) Conessimethine (iii) was hydrogenated to the tetrahydroderivative (ix) which was transformed to the known 3β-N-dimethyl-5α-pregnane (x). The assignment of 3β configuration for -N^aMe_2 group was therefore justified.



(d) Heteroconessine (v) on Kuhn-Roth degradation gave the same number of C-methyl groups as conessine. Furthermore, the nitrogen from apoconessine could not be removed by Hofmann degradation.

From these data, the following two isomeric structures (Ia or Ib) are admissible for conessine.



The final proof for Ia as the structure for conessine had been supplied by Labler *et al.*[48] by synthesis of dihydroconessine from dihydrotetramethyl holarrhimine.

The syntheses of dihydroconessine by Corey and co-workers[49] and by Jeger[50] and co-workers by photo-catalysed reactions and the recent syntheses of conessine itself by Johnson and co-workers[51] and by Barton and Morgan[52] using a photochemical reaction of azides, leave no doubt about the structure of conessine (Ia).

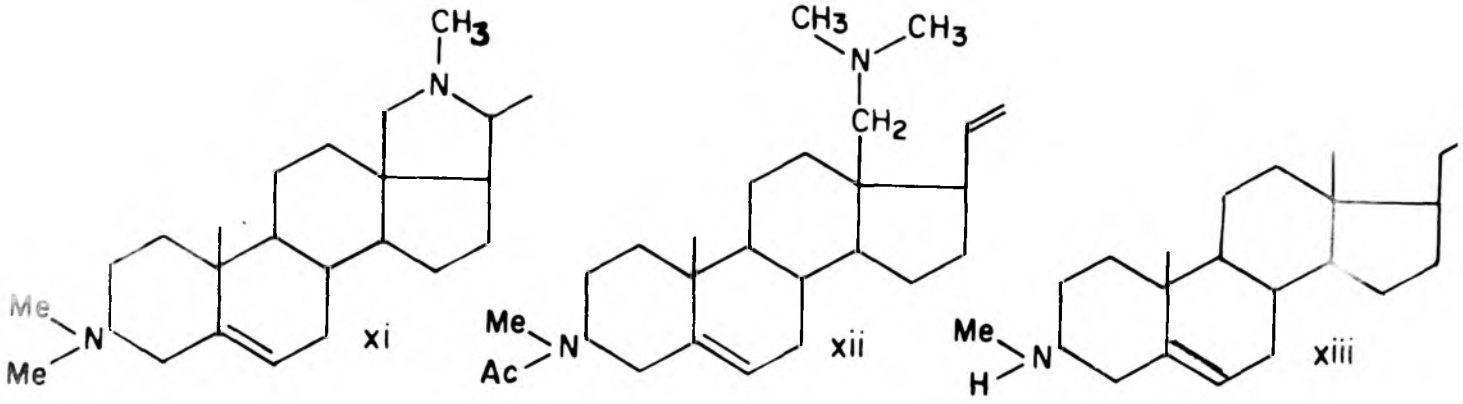
Chemistry of Isoconessimine

Iso-conessimine, a secondary-tertiary base, having the molecular formula, $C_{23}H_{38}N_2$, was isolated from the bark and seeds of kurchi in 1934 by Siddiqui.[16] It was also prepared by cyanogen bromide demethylation of conessine by Siddiqui and Siddiqui.[18] The base had one active hydrogen and two N-methyl groups in the molecule.

The formulation of isoconessimine as NH.Me $C_{21}H_{31}$ - NMe was proposed by Siddiqui on the following argument - when conessine was treated with one mole of cyanogen bromide, one of the two nitrogens reacted exclusively with this reagent to yield cyano-isoconessimine which could be converted to isoconessimine by saponification and decarboxylation. The reactivity of the exo-cyclic N-dimethyl group N^a was expected to be greater to cyanogen bromide than that of the tertiary ring nitrogen N^b. This

led to the conclusion that in isoconessimine the secondary amino group is exocyclic. However, no rigorous proof was advanced for this proposition.

In the course of their studies on the structure of conessine, Haworth and co-workers[36] also established the structure of isoconessimine as (II) by the Hofmann degradation of N-acetyl isoconessimine to N-acetyl isoconessimine methine (xi) and hydrolysis of the degradation product to the free base (xii). After Emde degradation and selective hydrogenation (xii) yielded the known 3β-methylamino-5-pregnene (xiii). This indicated that the secondary nitrogen in isoconessimine is at position 3β.



Chemistry of Conessimine

Conessimine, a tertiary-secondary base having the molecular formula, $C_{23}H_{38}N_2$, isomeric with isoconessimine, was isolated by Siddiqui and Pillay[15] from the kurchi bark. The base had one acylable active hydrogen and two N-CH_3 groups.

The structural formula, $N.Me_2 - C_{21}H_{31}-NH$, was proposed by Siddiqui[26] in 1934, since the other possibility, $NH.Me - C_{21}H_{31}N\ Me_2$, had been assigned to isoconessimine. Since the structure of conessine and isoconessimine were conclusively established by Haworth and co-workers, the only logical structure admissible for conessimine was (III).

In the present investigation, it was considered desirable to establish a proof for the structure (III) by the synthesis of conessimine from conessine.

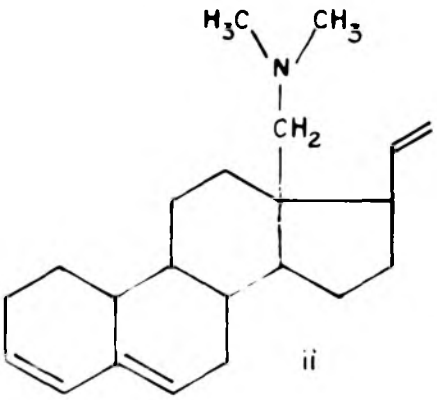
When this work was undertaken excepting for McNiven's[33] solitary note on the transformation of conessine to 18-hydroxy-5α-pregnane, no literature data were available on the transformation of the Holarrhena alkaloids to 18-substituted neutral products. However, during the course of the present investigation, many publications have come out from different laboratories on the transformations of kurchi alkaloids to steroidal intermediates.

Haworth and co-workers[41] were the first to convert conessine into 18-amino-5α-pregnane. Conessine on Hofmann degradation yielded apoconessine (ii) which was hydrogenated to hexahydroapoconessine (xiv). On treatment with cyanogenbromide (xiv) gave the N-cyano derivative (xv) which was hydrolysed to N-desmethyl-hexahydroapoconessine (xvi). The secondary amino group

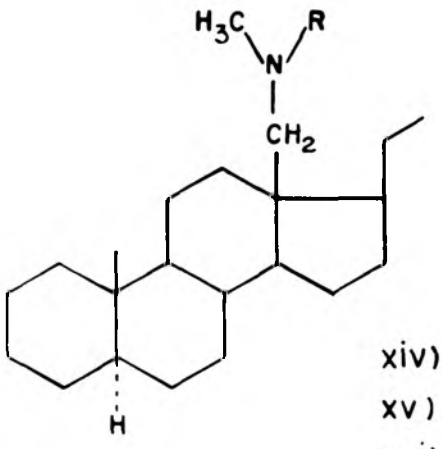
in (xvi) was demethylated by treatment of the corresponding benzoyl derivative with phosphorous pentachloride and hydrolysed by acid to (xvii).

McNiven[53] converted 18-amino-5α-pregnane (xvii) by Sommelet's reaction into 18-oxo-5α-pregnane (xviii) which on reduction with sodium borohydride yielded 18-hydroxy-5α-pregnane (xix).

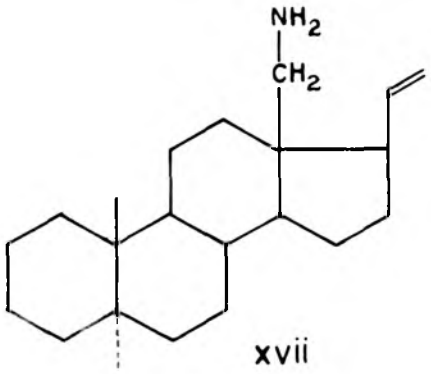
Transformations of Conessine by Haworth and co-workers and McNiven



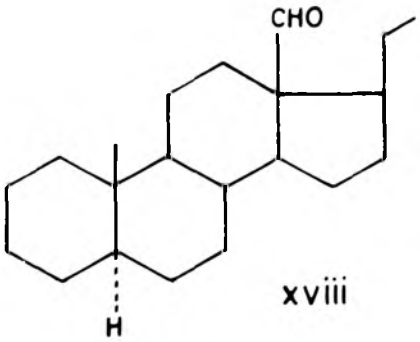
ii



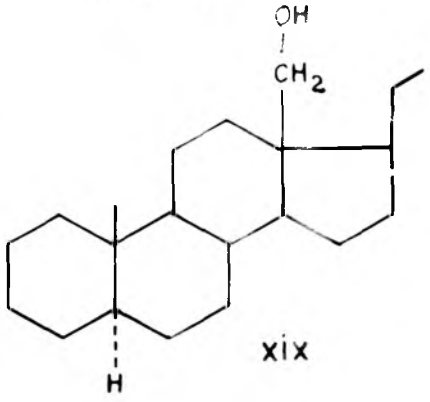
xiv) R = CH_3
xv) R = CN
xvi) R = H



xvii



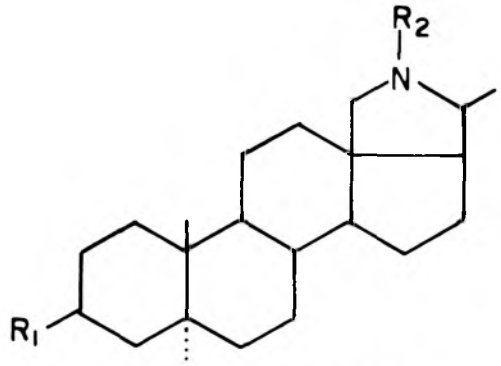
xviii



xix

Cerny and Sorm[54] in 1959 reported the synthesis of 3 β-hydroxy-18-benzoylamino-20-oxo-5 α-pregnane from dihydroconessine.

On treatment with cyanogenbromide dihydroconessine (xx) was converted to a N-cyano-derivative (xxi) which was saponified by hydrochloric acid to yield dihydroisoconessimine (xxii). By Ruschig's method of N-chlorination, dehydrochlorination and hydrolysis (xxii) gave the keto-compound (xxiii) which was reduced by tri (tert-butoxy) aluminohydride to conanine-3β-ol (xxiv). Cyanogenbromide treatment of (xxiv) gave the N-cyano derivative (xxv) which on hydrolysis yielded 3β-hydroxy-18,20-imino-5α-pregnane (xxvi). The pyrroline derivative (xxvii) was obtained by Ruschig's procedure of N-chlorination and dehydrochlorination on (xxvi). Benzoylation of (xxvii) with benzoyl chloride afforded 3β-hydroxy-18-benzoyl amino



(xx) $R_1 = NMe_2$, $R_2 =$ Me

(xxi) $R_1 = NMeCN$, $R_2 =$ Me

(xxii) $R_1 = NHMe$, $R_2 =$ Me

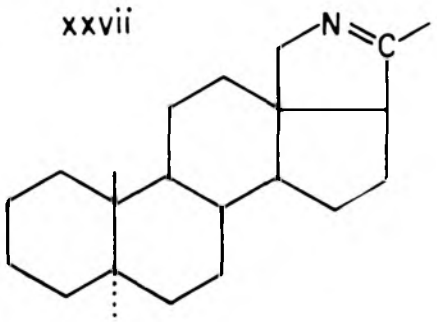
(xxiii) $R_1 = O$, $R_2 =$ Me

(xxiv) $R_1 = OH$, $R_2 =$ Me

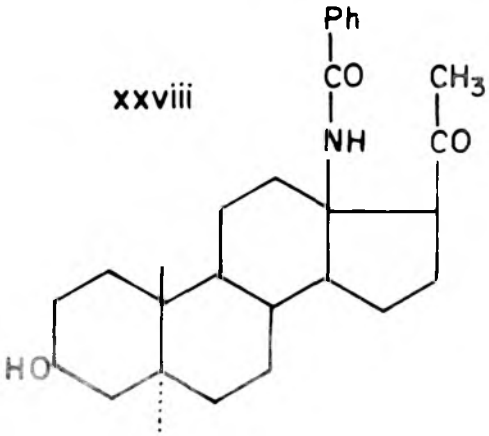
(xxv) $R_1 = OH$, $R_2 =$ CN

(xxvi) $R_1 = OH$, $R_2 =$ H

xxvii



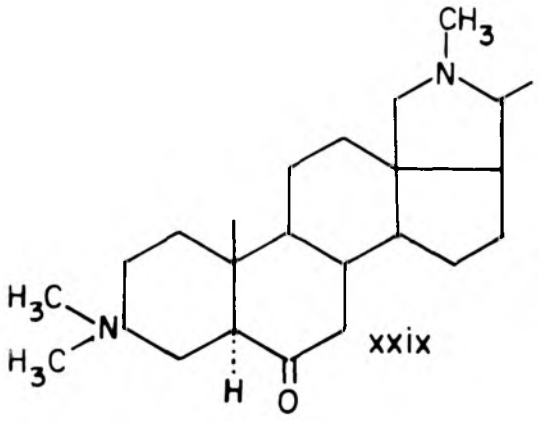
xxviii



Pappo[35] in 1959 worked out a synthesis of 18-hydroxyprogesterone from conessine involving a number of steps:

Conessine on treatment with sodium-borohydride and aluminium chloride gave a 6-boron complex which was oxidised by chromic acid to 3β N-dimethyl-6-oxo-18,20-N methyl-5α-pregnane (xxix). The dimethiodide of (xxix) under Hofmann degradation with potassium-t-butoxide afforded 18-dimethylamino-3:5-cyclo-6-oxo-20-pregnene (xxx). The metho-p-toluene sulphonate of (xxx) was hydroxylated to the 20-epimeric diols (xxxi). The nitrogen-free 18-20 oxido product (xxxii) was obtained by refluxing (xxxi) with base. Ether (xxxii) was converted via the 21-tosylate to the 21-dimethylamino derivative (xxxiii). The 6-keto group was reduced by lithium aluminium hydride to a mixture of epimeric 6-alcohols (xxxiv). Treatment with formic acid regenerated the Δ^5-3-ol-system (xxxv) and Oppenauer oxidation of (xxxv) afforded the 21-dimethylamino-18,20-oxido-3-oxo-4-pregnene (xxxvi). The corresponding N-oxide, when refluxed in t-butyl benzene resulted in the formation of the vinyl ether (xxxvii), which was hydrolysed by dilute acids to 18-hydroxy-progesterone (xxxviii) occurring as a mixture of two readily interconvertible 20-epimeric 18,20-hemiketals.

Synthesis of 18-hydroxyprogesterone from Conessine by Pappo.



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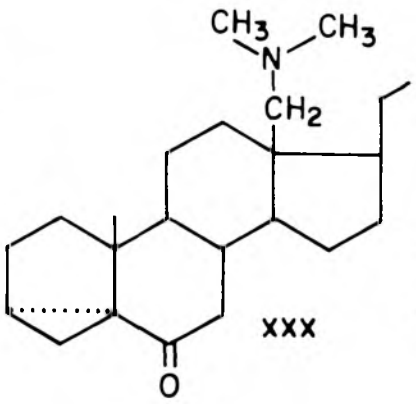

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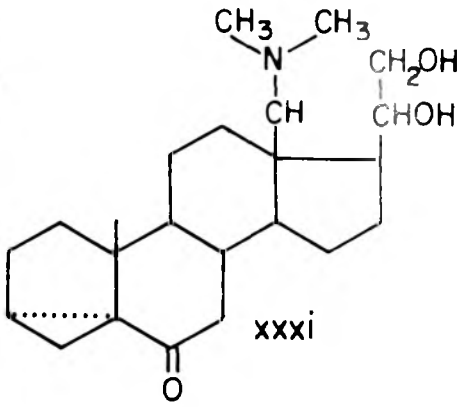

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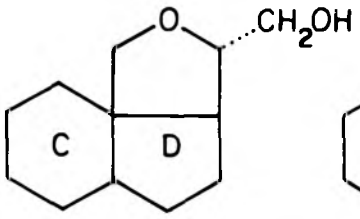
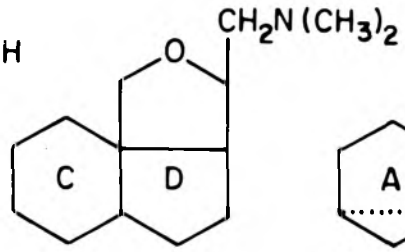
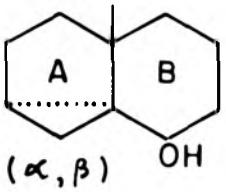


xxxii



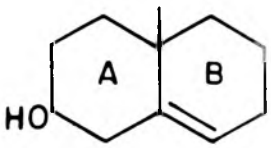
xxxiii

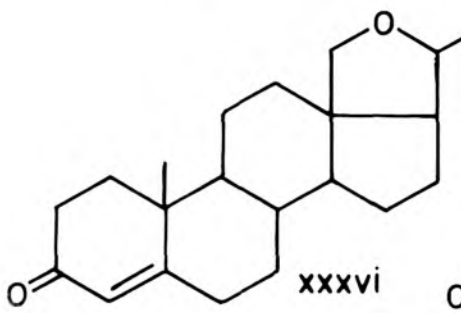
xxxiv



(α, β)

xxxv





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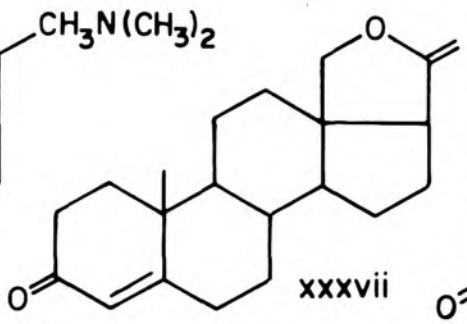
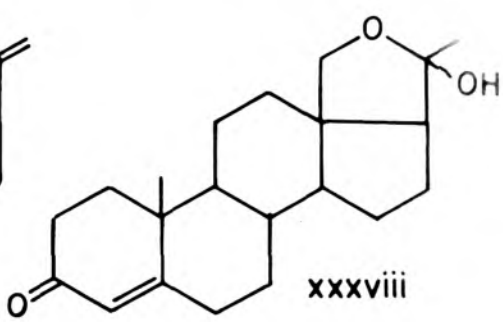


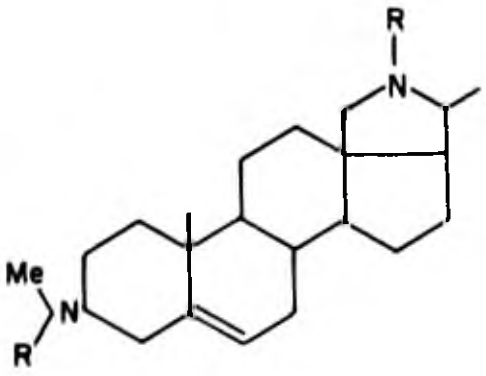
xxxvii



xxxviii

In the same year Buzzetti et al[56] developed a short and elegant method for the synthesis of 18-hydroxy progesterene from conessine in only 6-steps.

Conessine on treatment with cyanogen bromide gave N-N dicyanoconimine (xxxix) which on hydrolysis with alcoholic alkali yielded conimine (xl). The action of two mols. of N-chloro succinimide on conimine resulted in the dichloro derivative (xli) which when refluxed with sodium ethoxide and then with sulphuric acid afforded $\Delta^{4,20N}$ -imino-3-keto-pregna-diene (xlii). The action of nitrous acid converted (xlii) to 18-hydroxy progesterene (xxxviii)

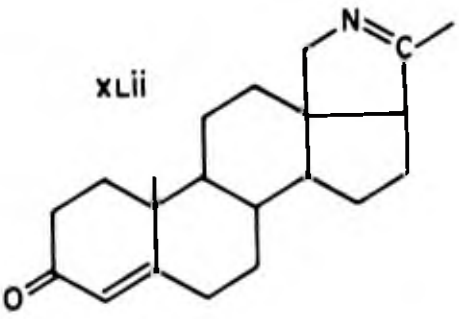


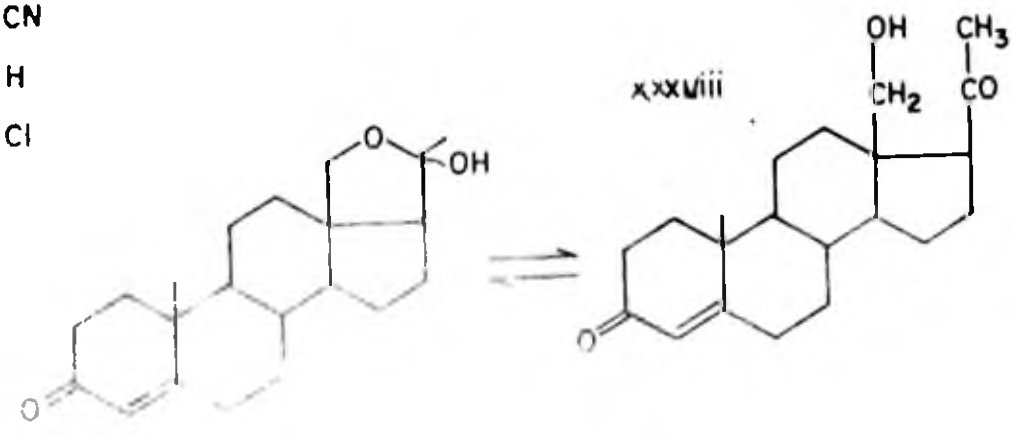
(I) R = Me

(xxxix) R = CN

(xL) R = H

(xLi) R = Cl





Aim of the Present Investigation

The purpose of the present investigation was 1) To convert three of the kurchi alkaloids conessine, isoconessimine and conessimine into partially or completely deaminated steroidal intermediates with different substituents at positions 3, 18 and 20. Apart from their potential utility as convenient starting materials for the synthesis of aldosterone, it was not considered unreasonable to expect that these intermediates would constitute a class of steroids with some interesting physico-chemical and pharmocological properties. That this expectation is not unjustified, has been demonstrated by the reports on aldosterone-blocking action of 18-hydroxy progesterone[2] and 18,21-dihydroxyprogesterone and the antifungal activity of 3-oxo-cora-1,4-diene[3] and 2) To examine the bark of kurchi to determine, if the plant accumulates any of the neutral hypothetical precursors of the alkaloids in appreciable quantities.

The Present Investigation

The present investigation thus comprises of -

(A) Examination of the neutral non-saponifiable steroidal components of kurchi bark.

(B) Development of a convenient procedure for the isolation of the alkaloids, conessine, iso-conessimine and conessimine from kurchi bark.

(C) Transformations of conessine.

(D) Transformations of isoconessimine.

(E) Transformations of conessimine.

C H A P T E R II

METHODS AND MATERIALS

II. METHODS AND MATERIALS

1. Kurchi Bark

Kurchi bark was procured from Messrs Jadavjee Lallobhai & Co. in Bombay, India. The authenticity of the material was established by a comparison with a sample obtained through the courtesy of the Forest Department of Bombay State, India.

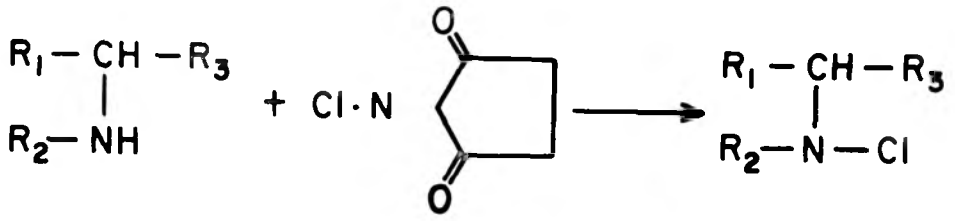
2. Isolation of Alkaloids

The final scheme for the isolation of the three alkaloids, conessine, isoconessimine and conessimine consisted in a combination of the essential features of the method of Siddiqui and Pillay[15] with those of Tschesche and Petersen.[22] Details of the procedure are discussed under the heading "Isolation of Alkaloids".

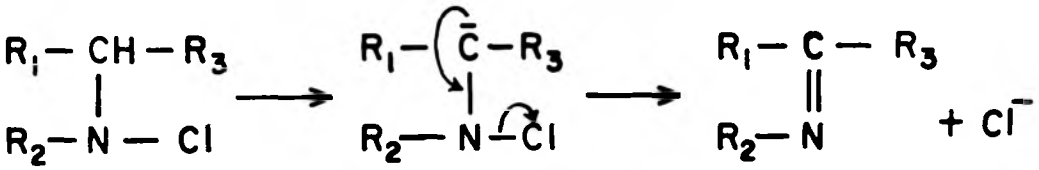
3. Removal of Nitrogen

The general procedure employed in the removal of partially substituted nitrogen atoms from the alkaloids was the Ruschig 's procedure of N-chlorination, dehydrochlorination and acid hydrolysis.[57]

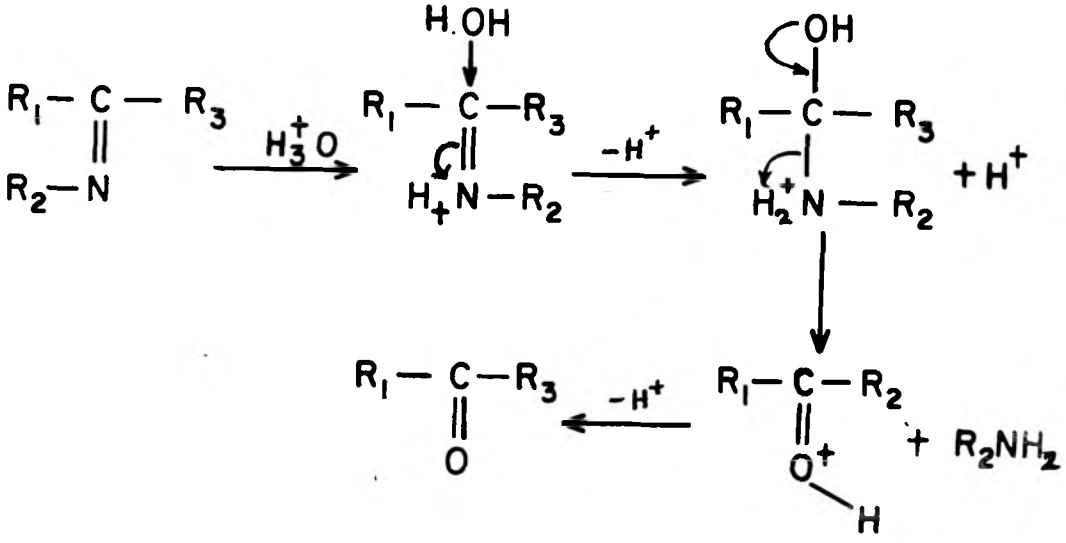
In the initial step, a chlorine radical from N-chlorosuccinimide is presumed to displace a hydrogen radical from a primary or a secondary amino-nitrogen to form a chloramine.



On treatment with a base such as an alkoxide ion, the chloramine is dehydrochlorinated to a Schiff's base.



In the final step, the Schiff's base is hydrolysed with acid to form the carbonyl compound.



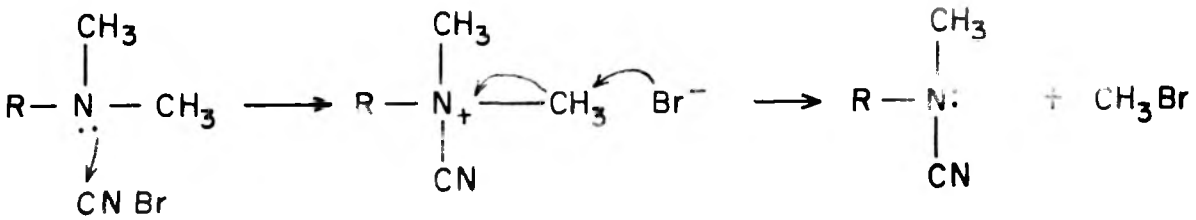
N-chlorosuccinimide was prepared according to the procedure of J.Tschernijac.[58]

For completely substituted tertiary amines the von Braun cyanogen bromide procedure[59] of demethylation was used to convert it to secondary bases.

On the basis of X-ray diffraction studies and Raman spectra, cyanogen bromide has the structure -

$$Br - C \equiv N \quad \text{rather than} \quad Br - \overset{+}{N} = \overset{-}{C}$$

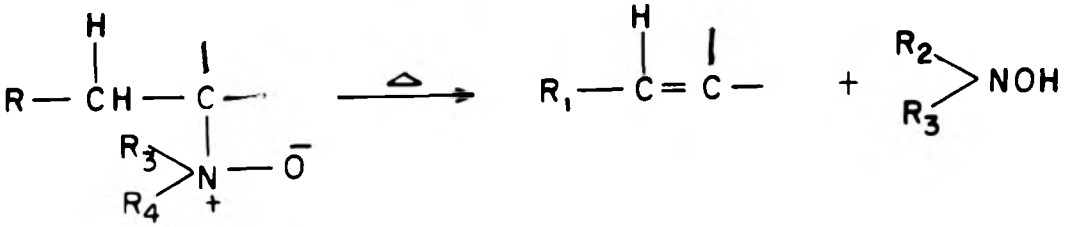
The initial reaction of cyanogen bromide on a basic nitrogen involves the nucleophilic displacement of the bromine in cyanogen bromide by the nitrogen of the base with the formation of the ionic addition product in which the nitrogen becomes quaternized. In the final step another nucleophilic displacement of the N-cyano-tertiary-amine by the bromide ion takes place.[60]



Cyanogenbromide was prepared according to the method described in Organic Syntheses.[61]

The five-membered 18,20- cyclic Schiff's bases proved to be stable to acid hydrolysis. These were treated with nitrous acid to form the corresponding 18-hydroxy-20-ketones.[56]

Another method of deamination of tertiary base has been employed for conessine. This consisted in the pyrolytic cleavage of tertiary N-oxides.[62]



Alumina for Chromatography

For the chromatography of alkaloids, basic alumina was prepared from commercial hydrated alumina (100-250 mesh) from Indian Aluminium Co., Bihar (India). The alumina was thoroughly washed with distilled water until the pH of the effluent was just below 8.6. After drying in air the alumina was heated at 400-450° for 6 hours according to the method of Djerassi.[63] To prepare Grade II

alumina, the dry alumina was treated with the requisite amount of water (3 ml./100 g.) according to the procedure of Lederer and Lederer.[64]

For the chromatography of neutral steroids, neutral alumina prepared according to the procedure of Djerassi[63] was used.

Buffer Solutions

The buffer solutions for the countercurrent extraction of the alkaloids were prepared according to McIlwain.[65]

Liebermann-Burchard Colour Reaction[66]

To the steroidal material in ca. 0.5 ml. of chloroform was added one or two drops of the test reagent prepared freshly by chilling 5 ml. of acetic anhydride in a 10 ml. flask and adding 0.5 ml. of concentrated sulphuric acid. The formation of a transient purple colour changing to blue, green, pink or purple indicated the presence of steroids or triterpenes.

2:4-Dinitrophenylhydrazine Reagent[67]

The reagent was prepared by heating on a steam bath a mixture of 0.5 g. of 2:4-dinitrophenylhydrazine, 8-10 ml. of 85% ethanol and 1 ml. of concentrated hydrochloric acid until a clear solution was obtained.

Ultraviolet and Infrared Spectra

The ultraviolet absorption spectra were taken in ethanol solutions with a Beckman Quartz Spectrophotometer Model DU.

The infrared spectra were recorded in nujol on a Grubb-Parsons Double Beam Spectrophotometer or a Perkin-Elmer Infracord with a sodium chloride prism unless otherwise mentioned.

Optical Rotation

Optical rotations were recorded in chloroform, unless otherwise stated, using a 1 dm. tube. The concentrations are reported in parenthesis.

Melting Point

The melting points recorded are uncorrected.

Van Slyke Amino Nitrogen

Van Slyke primary amino nitrogen determinations were carried out according to the method described by Van Slyke.[97]

N.M.R. Spectra

The n.m.r. spectra were determined in a Model H.R.-60 Spectrometer of the Varian Associates in $CDCl_3$ solution with benzene as a reference compound.

C H A P T E R III

EXAMINATION OF NEUTRAL FRACTIONS FOR STEROID CONTENTS

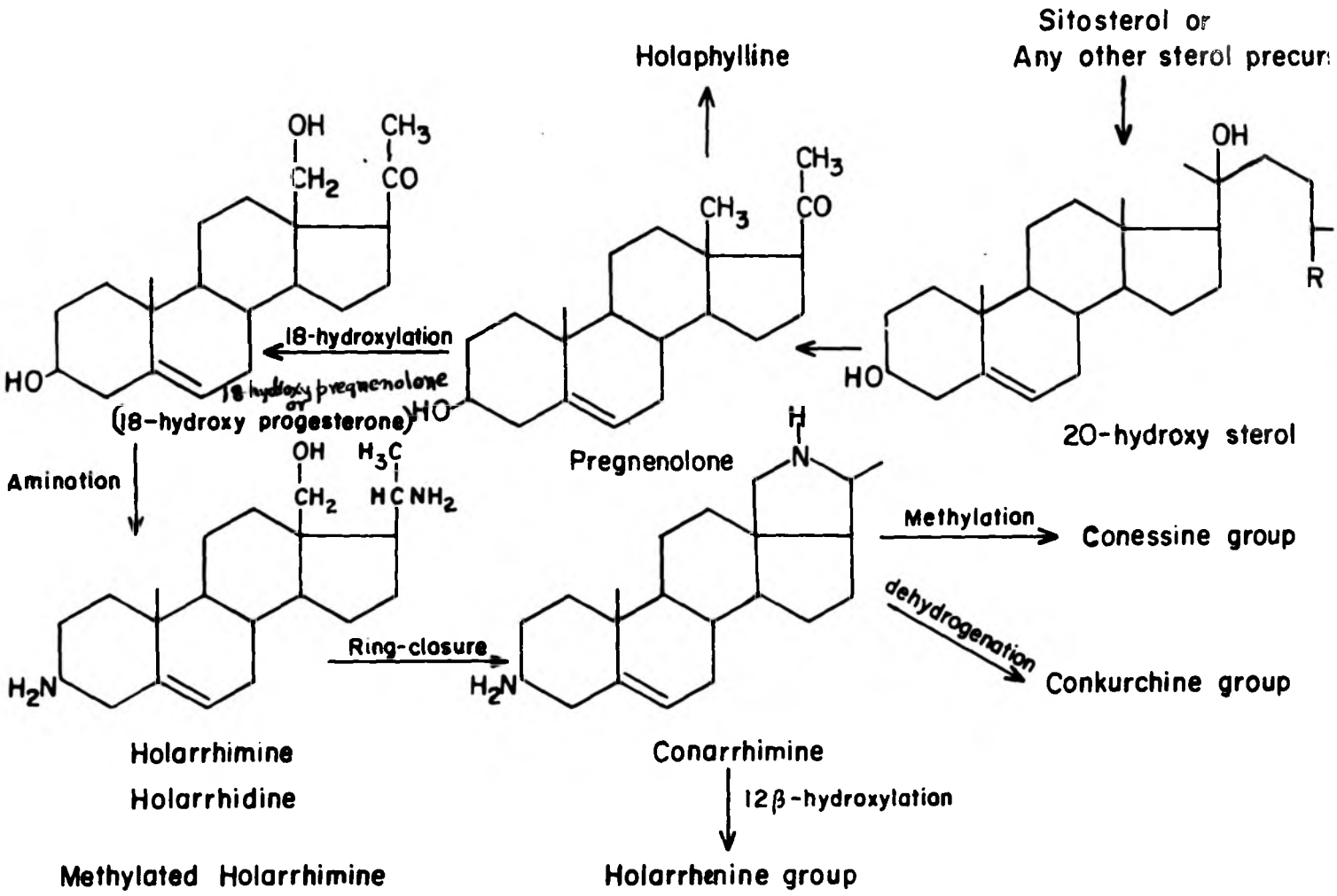
III. Examination of Neutral Fraction for Steroid Content

DISCUSSION

All the steroidal alkaloids isolated so far from Holarrhena antidysenterica have the twenty-one carbon pregnane type of steroid nucleus. From a biogenetic point of view, as far as it is known, the synthesis of sterols in plants and animals appear to follow broadly similar pathways.[68]

It has been established that in the adrenal gland in the animal, the origin of the C-21 steroids is by an oxidative cleavage of the isoprenoid side-chain of cholesterol initiated by a hydroxylation at C-20.[69]

Extending further the concept of biogenetic parallelism in plants and animal for the formation of the C-21 steroids, the following tentative scheme for the biogenesis of the kurchi alkaloids may be formulated.



The question arises, since the plant accumulates the pregnane alkaloids in large quantities in the bark, would it also store any of the neutral precursors, such as 20-hydroxy steroids, pregnenolone or 18-hydroxy progesterone in appreciable amounts?

A systematic examination of the neutral components of kurchi bark was therefore indicated.

The powdered bark was exhaustively percolated with petroleum ether, ether, ethanol, methanolic ammonia and 10% aqueous hydrochloric acid. The extracts were examined for compounds responding to the Liebermann Burchard reaction for sterols and triterpenes. As a specific colour test, this reaction is not entirely satisfactory. The twenty-one carbon Δ^4 or Δ^5 pregnenes do not respond to the reaction in minute concentrations. Moreover, the colour change produced by the pregnenes in acetic anhydride and sulphuric acid is more or less pink or light green in colour and reminiscent of the triterpenes. Saturated steroids do not respond to this test. The test is not entirely satisfactory with glycosides and the higher fatty acid esters of sterols and terpenes. However, in the absence of a more convenient method, the Liebermann colour reaction was employed to follow the separation and any change in colour to green, blue or pink was considered to be a positive reaction.

Practically all the lipid material from the bark with some amount of glycosides and chlorophyll was recovered in the petroleum ether extract. By a solvent-solvent distribution in hexane and 90% ethanol,

the viscous oily material obtained on evaporation of the petroleum ether extract, was separated into non-polar and polar fractions. Almost all the Liebermann positive compounds were concentrated in the non-polar fractions mainly as fatty acid esters, the bulk of which could be precipitated with alcohol. The free non-saponifiable steroidal and triterpenoid alcohols were obtained by saponification and separated into two crystalline components by twenty-five transfer Craig distributions between hexane and 90% alcohol followed by column chromatography over alumina. These compounds were identified as β-sitosterol and lupeol.

The Craig fractions of intermediate polarity from the petroleum ether extracts were subjected to a ten-transfer distribution between hexane, ethylacetate, alcohol and water (7:3:5:5). The third fraction from the non-polar end, showing a positive Liebermann reaction was crystallised out of acetone to give another neutral Liebermann-positive material, compound C (m.p.170^{o}). The mother-liquors from compound C were chromatographed over alumina to yield lupeol and a fourth substance, compound D (m.p.122-25^{o}). The analysis of D indicated that it was impure. In the I.R. spectra, it showed strong hydroxyl bands. The quantities of compounds 'C' and 'D' were too small to warrant any further studies on their constitution.

A literature search revealed that the only available data on the neutral non-saponifiable components of kurchi are due to the work of Siddiqui and Pillay,[70] who reported the isolation of lupeol from Indian variety of kurchi and β-sitosterol from the African variety.

A cursory examination of various fractions obtained from kurchi bark at this stage indicated that in all probability the plant does not accumulate in the free form any of the postulated steroidal intermediates in appreciable quantities in the bark. Further studies in that direction were not considered economically promising and therefore abandoned.

It may be mentioned here that it was not possible to exclude the possibility that some complex form of the hypothetical intermediates appear in the alcoholic extract. A clear cut separation of the neutral components from this extract proved to be rather difficult due to the presence of chlorophyll and alkaloids which were present as tannates and salt of other plant acids. Even separation of the alkaloids from these complexes proved to be time-consuming and incomplete.

EXPERIMENTAL

Twelve kg. of the powdered bark were percolated successively with 5 gallon-quantities of the following solvents - petroleum ether, ether, ethanol, methanol containing 5% ammonium hydroxide and aqueous hydrochloric acid (10%).

The petroleum ether and ether extracts were evaporated to dryness after a mild acid washing to remove the alkaloids. Since the ether extract did not contain much material, the residue from these two extracts was pooled (240 g.).

The greenish brown oily residue was subjected to a nine-transfer Craig distribution between hexane and 90% aqueous ethanol in separating funnels, using 1 l. of each phase in each separating funnel. The solvent layers from two funnels from each end were evaporated separately. Small aliquots from each fraction were subjected to a Liebermann reaction of steroids and triterpenes. The results of the distribution are given in Table 1.

T A B L E I

Results of a nine-transfer distribution of neutral petroleum ether extractives from kurchi bark between hexane and 90% aqueous ethanol.

Weight of the material ... 240 g.

Volume of each phase ... 1000 ml.

No. of transfers ... 9

Fr.No.	Solvent-layer	Weight (g)	Liebermann reaction
1a	Hexane	28.27	+
1b	Alcohol	2.60	+
2a	Hexane	46.09	+
2b	Alcohol	6.03	+
3	Hexane + alcohol	39.06	-
4	"	27.90	-
5	"	20.16	-
6	"	13.98	-
7	"	11.49	-
8	"	13.76	-
9a	Petroleum ether	1.28	-
9b	Alcohol	9.43	-
10a	Petroleum ether	0.88	-
10b	Alcohol	15.13	-
	Recovery -	236.06	

Fractions 3, 4 and 5 were pooled (91.18 g.) and redistributed in hexane and 90% aqueous ethanol in another ten-transfer Craig distribution using 500 ml. of each phase. The results are presented in Table II.

T A B L E II

Results of a Ten-transfer distribution of Fractions 3, 4 and 5 from Table I between hexane and 90% aqueous ethanol.

Weight of the material ... 91.1 g.
Volume of each phase ... 500 ml.
No. of transfers ... 10

Fr.No.	Solvent layer	Weight (g)	Liebermann reaction
1a	Hexane	16.11	+
1b	Alcohol	0.61	+
2a	Hexane	13.36	+
2b	Alcohol	3.40	+
3	Hexane + alcohol	16.51	±
4	"	16.62	-
5	"	9.34	-
6	"	6.85	-
7	"	3.58	-
8	"	3.88	-
9	"	1.88	-
10a	Hexane	0.40	-
10b	Alcohol	1.05	-
11a	Hexane	0.20	-
11b	Alcohol	0.72	-
	Recovery -	90.40	

The non polar components from the first and second Craig distributions giving a positive Liebermann reaction (fractions 1a and 2a in each case) were pooled (102.83 g.).

An aliquot of 51 g. was taken in 400 ml. of ether and poured slowly into 1600 ml. of refluxing ethanol. After refluxing for 15 minutes the mixture was allowed to settle overnight. A creamy white precipitate, A, separated out. After decantation, the filtrate B, was evaporated under reduced pressure to three-fourths of its volume. After standing overnight at 0° another crop of precipitate A was obtained. The precipitates A were combined (21 g.). It was soluble in ether and acetone and insoluble in alcohol. The filtrate B on evaporation yielded 31 g. of an oily material. Both the precipitate A and the filtrate B gave a positive Liebermann reaction.

Saponification of the Precipitate A

An aliquot of 14 g. of the precipitate was dissolved in minimum volume of ether and added in small portions to boiling 95% ethanol containing 20% potassium hydroxide. After refluxing for 3 hours, the cooled mass was carefully extracted with ether, washed free from alkali and alcohol by repeated washing with water, dried over anhydrous sodium sulphate and evaporated under reduced

pressure to a gum (11 g.). The gum was subjected to a 25-transfer Craig-distribution between hexane and 90% aqueous ethanol using 200 ml. of each phase. The results are presented in Table III.

T A B L E III

Results of a 25-transfer distribution between hexane - 90% aqueous alcohol of precipitate A after saponification.

Weight of the material ... 11 g.
Volume of each phase ... 200 ml.
No. of transfers ... 25

Fr.No.	Weight (mg)	Liebermann reaction	Fr.No.	Weight (mg)	Liebermann reaction
0	465	-	13	791	+
1	470	-	14	641	+
2	240	-	15	486	+
3	145	-	16	344	+
4	198	±	17	270	+
5	316	+	18	215	+
6	409	+	19	210	+
7	611	+	20	187	+
8	798	+	21	147	+
9	946	+	22	127	+
10	1136	+	23	181	+
11	1001	+	24	88	+
12	970	+	25	95	+

Recovery - 10.78 g.

Fractions 5 onwards gave strong Liebermann test. Fractions 6 to 9 representing the ascending side of a peak were pooled (2.76 g.) and chromatographed over 30 times its weight of alumina (neutral Gr.II), and developed successively with benzene containing 10%, 25%, 50% chloroform, chloroform, chloroform-methanol (1:1) and methanol. The results are given in Table IV.

T A B L E IV

Chromatography of fractions 5-10 from Table III.

Weight of the material ... 2.76 g.
Weight of alumina ... 90 g.
Each fraction collected ... 25 ml.

Fr.No.	Eluent	Volume (ml)	Weight (mg)
1-2	5% chloroform-benzene	50	78
3-4	"	"	95
5-6	10% chloroform-benzene	"	19
7-8	"	"	11
9-10	"	"	9
11-12	"	"	11
13-14	"	"	123
15-16	"	"	150
17-18	25% chloroform-benzene	"	131
19-20	"	"	139
21-22	50% chloroform-benzene	"	186
23-24	"	"	234
25-26	Chloroform	"	303
27-28	"	"	401
29-30	50% methanol-chloroform	"	386
31-32	"	"	232
33-34	Methanol	"	45
35-36	"	"	20
		Recovery -	2.67 g.

Fractions 29-34 were pooled (663 mg.) and recrystallized from methanol after filtration through a 2 g. alumina column (610 mg).

Physical constants

Melting point - 138, 139.5°

Mixed m.p. with authentic sample of β-sitosterol - 138, 140°

Optical rotation

$[\alpha]_D$ * - 37° (C = 0.02)

Analysis	C.	H.
$C_{29}H_{50}O$ requires	83.99;	12.15 %
Found:	83.35;	11.96 %

The identity of the compound with β-sitosterol was established by the physical constants, mixed melting point and comparative I.R. spectrum.

The acetate of the compound was prepared in the usual manner (m.p. 128-29°; Analysis - C, 81.2; H, 11.3%. $C_{31}H_{52}O_2$ requires C, 81.52; H, 11.48%), and was found to be identical with β-sitosteryl acetate.

Fractions 17 to 30 (1.78 g) were pooled and recrystallized from methanol. The crystalline material (1.3 g.) showed a range in melting point (195-203°). On repeated recrystallisation, a small amount of lupeol (48 mg; m.p. 213°) was obtained. More of lupeol was obtained from the Craig fractions from the saponified filtrate B.

Saponification of the filtrate B

The alcohol-soluble fraction B (31 g.) was saponified by refluxing with 1.5 l. of 25% potassium hydroxide in 95% ethanol for 3 hours. The ethereal extract from the mixture after treatment in the manner described before (page 35) yielded 30.9 g. of a gummy material which exhibited a tendency of crystallization on prolonged storage. The product was subjected to a 25-transfer Craig distribution between hexane and 90% aqueous alcohol. The results are presented in Table V.

T A B L E V

Results of a 25-transfer Craig distribution between Hexane and 90% aqueous ethanol fraction B after saponification.

Weight of the material ... 30.9 g.
Volume of each phase ... 200 ml.
No. of transfers ... 25

Fr.No.	Weight(g)	Fr.No.	Weight (g)
0	0.378	13	1.946
1	1.102	14	1.212
2	0.777	15	0.903
3	0.620	16	0.371
4	0.784	17	0.371
5	1.377	18	0.374
6	1.754	19	0.308
7	2.465	20	0.240
8	3.259	21	0.196
9	3.788	22	0.137
10	3.697	23	00063
11	3.168	24	0.034
12	2.736	25	0.013
		Recovery -	31.196 g.

Fractions 8, 9 and 10 were pooled (10.7 g.) and repeatedly crystallized from methanol as aggregates of needles.

Physical constants

Melting point 215°

Optical rotation $[\alpha]_D$ + 27° (C = 0.019)

Analysis	C.	H.
$C_{30}H_{50}O$ requires	84.90;	11.8 %
Found:	84.47;	11.63%

The acetate of the product was prepared in the usual manner (m.p. 213-14°) (Analysis - C, 81.68; H, 10.53. $C_{33}H_{52}O_2$ requires C, 82.5; H, 10.8%).

The product was identified as lupeol through its physical properties such as m.p., rotation, analysis and the O-acetyl derivative.

Other fractions from the Craig appeared to be mixtures of sitosterol and lupeol and yielded no other neutral steroids.

Fractions 6, 7 and 8 from Table I and 6, 7, 8 and 9 from Table II were combined and subjected to a 10-transfer Craig-distribution between a solvent system consisting of hexane-ethyl acetate (7:3) and ethanol - water (1:1). The results of the distribution are given in Table VI.

T A B L E VI

Results of a ten-transfer distribution of fractions 6, 7 and 8 from Table I and 6, 7, 8 and 9 from Table II between hexane-ethylacetate and aqueous ethanol.

Weight of the material ... 55.4 g.

Volume of each phase ... 500 ml.

No. of transfers ... 10

Fr.No.	Solvent layer	Weight (g)	Liebermann reaction
0A	Hexane ethyl-acetate	20.0	+
0B	Ethanol	0.22	+
1A	Hexane-ethylacetate	9.50	+
1B	Ethanol	2.30	+
2	Hexane-ethylacetate + Ethanol	2.47	+
3	"	2.26	±
4	"	1.75	-
5	"	1.11	-
6	"	1.02	-
7	"	1.03	-
8	"	0.82	-
9A	Hexane-ethylacetate	0.07	-
9B	Ethanol	1.00	-
10A	Hexane-ethylacetate	0.70	-
10B	Ethanol	6.18	-
		Recovery- 50.43	

Fraction 2 showed signs of crystallisation. It was, therefore, taken in 6 ml. of hot acetone. On cooling, the solution deposited 630 mg. of crystalline material (m.p. 152-163°). On repeated crystallisation from acetone, this material was resolved into a lower melting component (100 mg; m.p. 166-68°), which appeared first and a higher melting component (73 mg; m.p. 184-198°). On repeated crystallisation, the lower melting component, yielded pure compound 'C' with m.p. 170-171°; $[\alpha]_D$ = + 33°; Analysis - C, 84.3; H, 11.25%. Calcd. for $C_{30}H_{50}O$. C, 84.90; H, 11.8%.

The higher melting point component was identified as lupeol.

The mother liquors from compound C and lupeol on evaporation yielded 1.7 g. of material which was chromatographed over 50 g. of alumina (neutral; Grade II) and eluted successively with 200 ml. of each of benzene, 5% chloroform, benzene- 10% chloroform, benzene - 25% chloroform, benzene - 50% chloroform and chloroform. The first fraction eluted with benzene was a gum followed by a crystalline fraction (750 mg., m.p. 177-197°) which was spread over the tail end of benzene and the front end of benzene-chloroform eluates. Another crystalline fraction (192 mg) was recovered in benzene - 10% chloroform eluate.

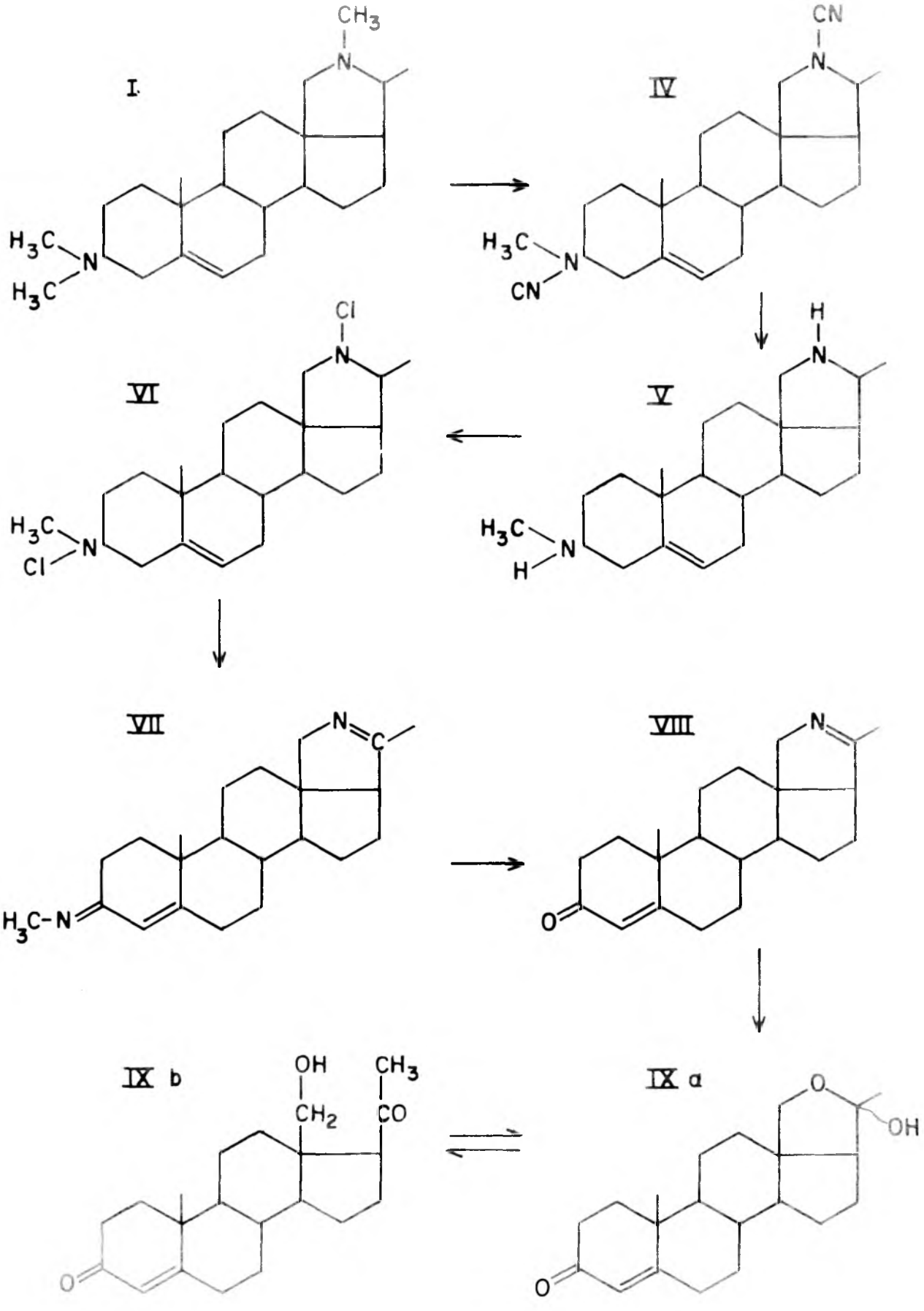
On repeated crystallization, this material yielded a colourless crystalline compound D (22.2 mg.) with m.p. 122-124°; $[\alpha]_D$ - 32°; Analysis - C, 76.50; H, 11.10% and I.R. spectra ν/λ max. 3260 cm^{-1}, 1052 cm^{-1}.

C H A P T E R V

TRANSFORMATIONS OF CONESSINE

Plate 2

Transformations of Conessine



V. Transformations of Conessine

DISCUSSION

A. 18-Hydroxy Progesterone from Conessine

When this work was in progress, Jeger and co-workers[56] reported the conversion of conessine into 18-hydroxyprogesterone by a six-step procedure. Pappo[55] also accomplished the same conversion by employing a series of reactions involving 15 steps. The experimental details of Jeger's procedure have not been published as yet. Since the current investigation was moving in parallel lines, it was decided to investigate in detail the experimental conditions for this very attractive synthesis of 18-hydroxyprogesterone.

Siddiqui and Siddiqui[18] reported that the action of cyanogen bromide on conessine at room temperature in ether leads almost exclusively to the mono N-cyano-isoconessimine which could be converted to dicyano-conimine by a further prolonged treatment for seven days with cyanogen-bromide.

The stoichiometry of the reaction-sequence can be represented as follows:

$$2CNB_r + 3\ C_{24}H_{40}N_2 \longrightarrow 2\ C_{23}H_{37}N_2\text{-}CN + C_{24}H_{40}N_2\ (2\ CH_3Br)$$

$$2\ C_{23}H_{37}N_2CN + 2CNB_r \longrightarrow C_{22}H_{34}\text{-}(2CN) + C_{23}H_{37}N_2\text{-}CH(CH_3Br)$$

From the above equation, it is apparent that the

limit of the yield of dicyanoconimine can never exceed 35 percent, since a third of conessine and half of the monocyano isoconessimine are utilized to trap the methyl-bromide liberated. To achieve better yields of dicyanoconimine some ways had to be devised to cause a rapid removal of the methyl bromide from the reaction mixture, before it had a chance to react with conessine. The use of a higher temperature for running the reaction was chosen for two reasons:

(i) It would allow the volatile methyl bromide to escape from the reaction mixture before it has a chance to react with conessine;

(ii) It would enhance the rate of demethylation particularly at the relatively inert bridge nitrogen.

On the other hand, the temperature should not be too high for the volatile cyanogen bromide to escape.

The reaction was, therefore, run in refluxing benzene and the yield of dicyanoconimine was advanced to 70%. It was not possible to prevent the formation of quarternary bromides entirely.

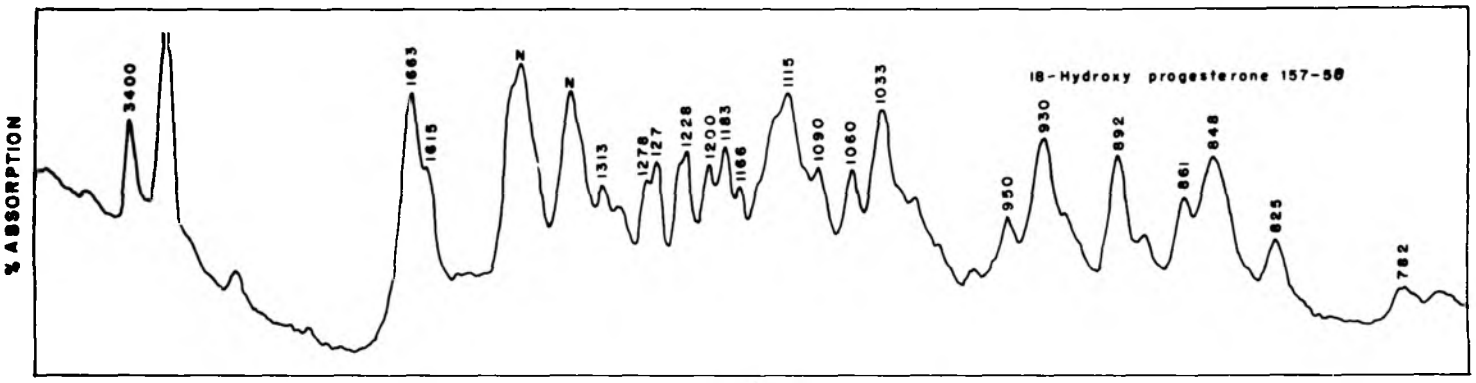
On alkaline hydrolysis of dicyanoconimine (IV), the disecondary base conimine (V), was obtained in about 66% yield, the overall yield from conessine being 46-50%. It was not possible to achieve the 100% yield reported by Buzzette *et al*.[56] under the experimental conditions employed.

Conimine was subjected to deamination by Ruschig's procedure. The crystalline N-chloro compound (VI) was isolated and characterised. By refluxing with sodium ethoxide, it was dehydrochlorinated to compound (VII), which could not be obtained in a crystalline form and was subjected as such to acid hydrolysis. The acid hydrolysis product (VIII) was purified by extraction with perchloric acid and re-extraction of the base after basification in chloroform rendered it readily crystallizable.

The overall yield of the Schiff's base (VIII) was of the order of 75 percent based on conimine (V) and 35 percent on conessine.

On nitrous acid treatment, the Schiff's base yielded both the forms of 18-hydroxyprogesterone (IX) in 70-80 percent yield, the overall yield from conessine being 24-28 percent.

The identity of the compound was established by a comparison of its properties with those of 18-hydroxyprogesterone prepared from holarrhimine.[73]


% ABSORPTION
3400
1663
1615
1313
1278
1227
1228
1200
1183
1166
1115
1090
1060
1033
950
930
892
861
848
825
782
18-Hydroxy progesterone 157-58

B. Microbiological Transformation of the Schiff's base(VIII)

DISCUSSION

As a part of the steroid programme undertaken in this laboratory, the conversion of some of the steroidal intermediates, obtained from the kurchi alkaloids to aldosterone was under investigation. In this connection, the possibility of introduction of an 11-oxygen function with the aid of micro-organisms was considered. A strain of Aspergillus niger, which was used in the microbiological transformation of terpenes in this laboratory,[75] was found to give reasonable yields (70-85%) of 11α-hydroxy progesterone from progesterone in shake cultures as well as in deep tank fermentation. 18-Hydroxyprogesterone was converted to 11α-18-dihydroxyprogesterone in about 20% yield by this mould.[72]

The Schiff's base (VIII) was considered to be one of the prospective raw materials for the microbiological transformation. It had the Δ^4-3-keto structure necessary to obtain reasonable yields of the hydroxylated compound.[76] And from the anticipated product, the 11α-hydroxy derivative of the Schiff's base, some theoretically feasible routes to aldosterone could be visualized.

The fermentation was carried out in shake cultures essentially according to the procedure of

Peterson *et al.*[76] The metabolites from this mould after fermentation were separated by extraction of both the culture filtrates and the acetonised mycelium with methylene chloride. The bases were removed from the solvent layer, by extraction with dilute perchloric acid in the aqueous phase and recovered again in chloroform after basification.

The unreacted Schiff's base, (VIII), was separated from the more polar transformation products by a solvent-solvent distribution between hexane and 70% aqueous ethanol. From the hexane phase the unreacted Schiff's base was obtained in 72% yield. The polar basic transformation products, obtained from the ethanol phase (ca. 17%) could not be obtained in a crystalline form. The u.v. and I.R. spectra of this material after purification revealed that the Δ^4-3-keto system of the starting material had completely disappeared. However, the I.R. absorption spectra indicated that a hydroxyl band (3500 cm^{-1}) has been introduced and the internal Schiff's base structure (1648 cm^{-1}) was unaffected.

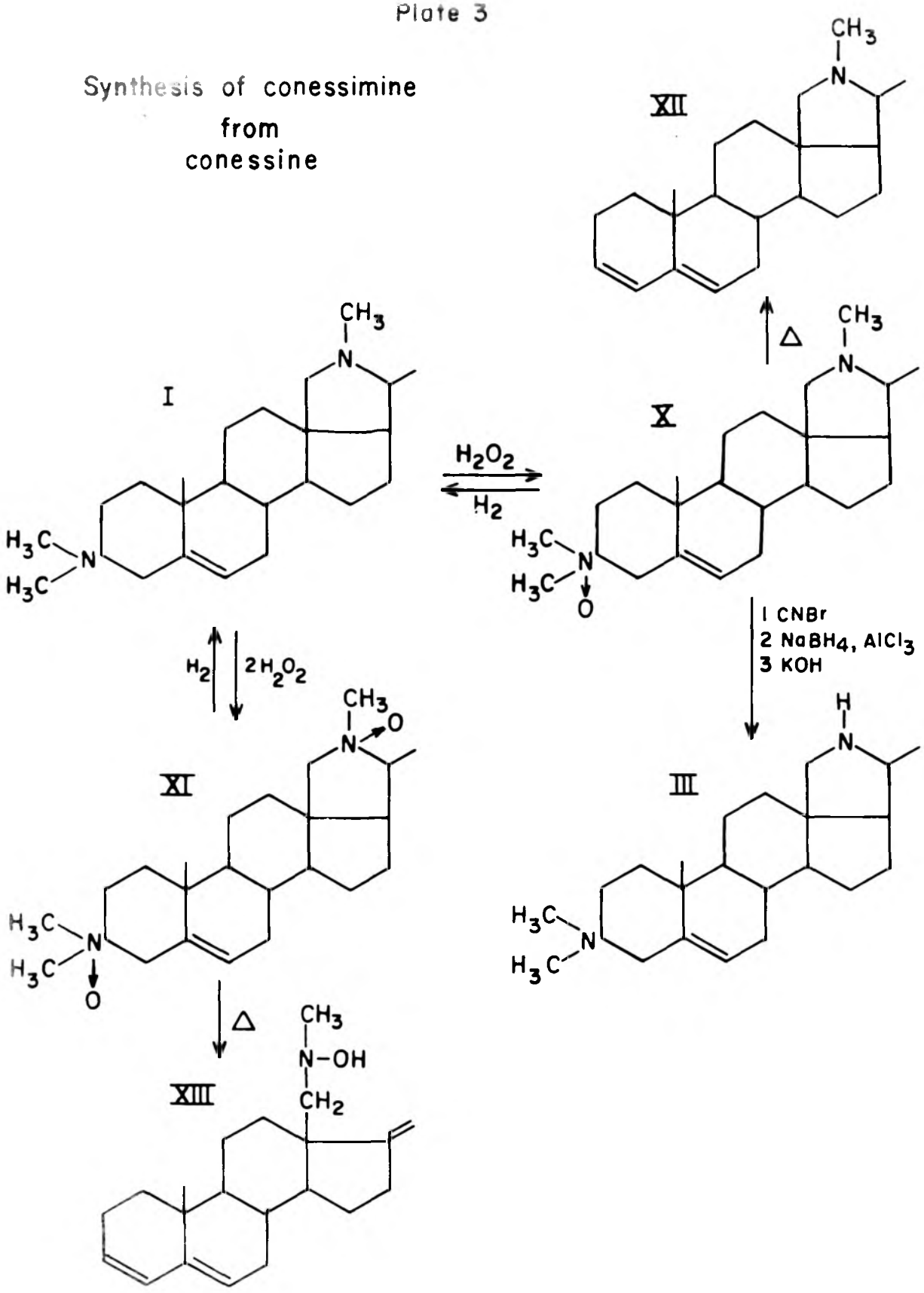
Since the yields of the microbiological transformation products were not encouraging, further experiments were not carried out.

[illegible]

The structure of conessimine was established

Plate 3

Synthesis of conessimine
from
conessine



are secondary-tertiary bases and both yield conessine on methylation with a mixture of formaldehyde-formic acid. Since it was conclusively established that iso-conessimine had the secondary amino group at position 3β and the 18-20 bridge nitrogen fully substituted (II), the other alternative structure (III) with a 3β-N - dimethyl group and an unsubstituted imino 18-20 bridge nitrogen had to be assigned to conessimine.

The primary point of attack of cyanogen bromide is on the 3β tertiary nitrogen of conessine leading predominantly to the 3β N-cyano derivative at the first stage. The bridge nitrogen is comparatively inert and reacts comparably slowly.[18] If, however, the nucleo-pliticity of the 3β tertiary nitrogen could be reduced by any means, an electrophilic attack exclusively on the bridge nitrogen might be rendered feasible.

It was in this connection that the 3-mono N-oxide of conessine proved to be a suitable starting material. Moreover, no difficulty was anticipated in the reduction of 3β-mono N-oxide at any stage with sodium borohydride and aluminium chloride.

The mono N-oxide of conessine was, therefore, subjected to the action of cyanogen-bromide under the conditions established for the preparation of conimine

fron conessine. The N-cyano N-oxide of conessimine was sparingly soluble in benzene (ca. 1 g. in 300 ml.) and could be recovered by working up the filtrate from the cyanogen bromide reaction free from other quarternary bromides. The gummy product was, however, unstable in nature and hygroscopic. It could not be obtained in a crystalline form. The material was reduced by sodium borohydride and aluminium chloride and the product without purification was saponified with methanolic potassium hydroxide.

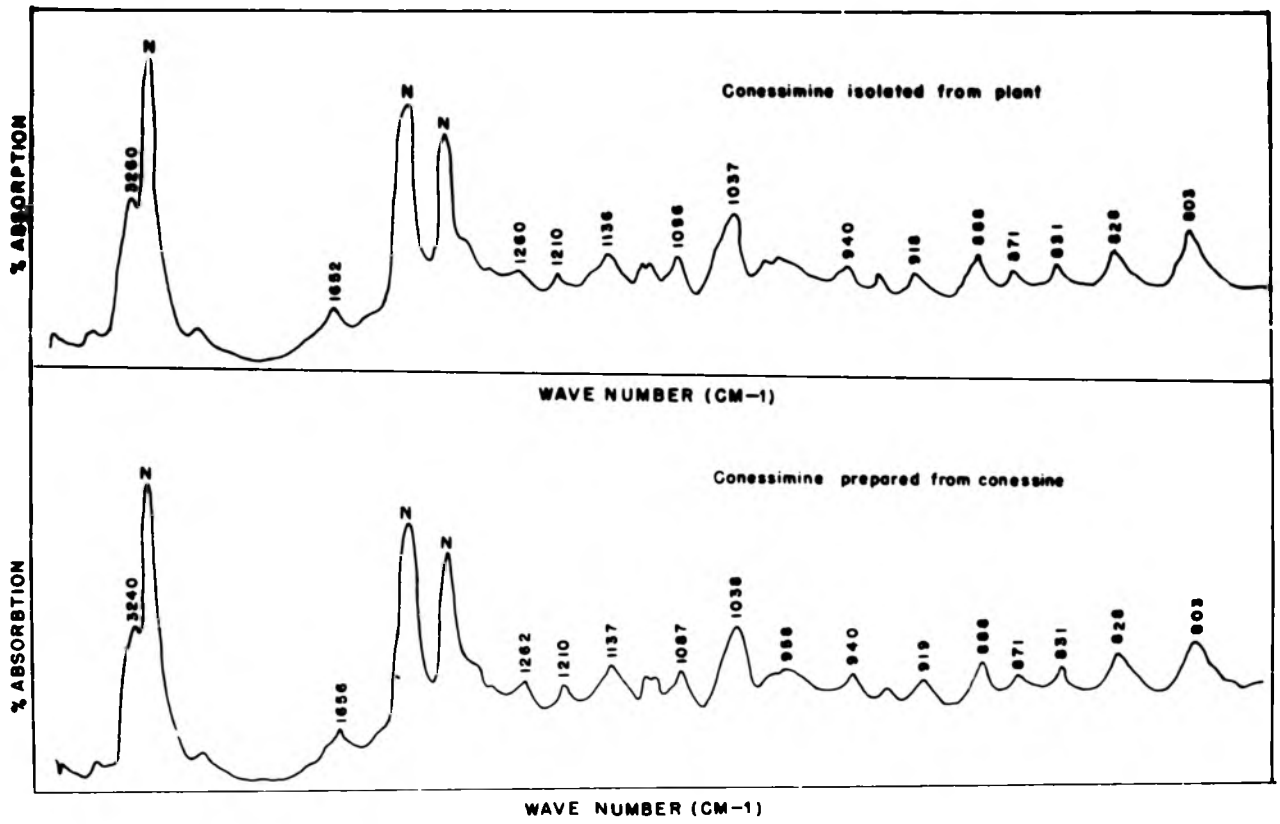
The final reaction product on column chromatography yielded conessimine (III) in low yields. The identity of this product was established by mixed melting point with an authentic sample of conessimine and through comparative I.R. spectra.

EXPERIMENTAL

Demethylation of Conessine to Conimine

(i) Action of cyanogen bromide on conessine

Ten grams of conessine (I) (0.0238 mole) in 100 ml.


% ABSORPTION
Conessimine isolated from plant
3260
1652
1260
1210
1136
1036
1037
940
918
888
871
831
828
803
WAVE NUMBER (CM-1)
% ABSORBTION
Conessimine prepared from conessine
3240
1656
1262
1210
1137
1037
1038
940
919
888
871
831
828
803
WAVE NUMBER (CM-1)

of benzene were added slowly during a period of 10 minutes to a solution of 7 g. of cyanogen bromide (0.066 mole) in 75 ml. of benzene in a 250 ml. two-necked flask provided with a cold water condenser, a separatory funnel and a magnetic stirrer. The mixture was then allowed to warm up to room temperature and finally heated to 80-85° under efficient refluxing for 7-8 hours with continuous stirring. The stirring was continued for 24 hours at room temperature. The benzene layer was decanted off from the residue which separated out during refluxing and evaporated in vacuo to yield a gummy product. This product was taken up in chloroform and the chloroform solution extracted with dilute acetic acid to remove any basic material present. After washing the chloroform extract free from acid with water, it was dried over anhydrous sodium sulphate and evaporated in vacuo to a neutral semicrystalline product (7 g.). The product was crystallized from acetone.

Physical constants

Melting point 168°

Optical rotation $[\alpha]_D$ = + 63° (C ± 0.019).

Analysis		C.	H.	N.	$N\text{-}CH_3$
$C_{24}H_{34}N_2$	requires	76.25	9.05	14.66	3.93 %
	Found:	76.02	8.83	14.19	3.80 %

Infrared absorption spectrum

$1/\lambda$ max. 2215 cm^{-1} for CN.

The physical data of the product were consistent with the structure (IV).

Hydrolysis of dicyanoconimine to conimine

An aliquot of 5 g. of dicyanoconimine (IV) was refluxed with 20% of alcoholic potassium hydroxide (100 ml.) on a steam bath for 18 hours, when crystalline potassium carbonate separated out and the colour of the solution became reddish brown. After removal of most of the alcohol under reduced pressure, the residue was diluted with 100 ml. water and the colourless precipitate recovered in chloroform. The chloroform layer was extracted three times with dilute acetic acid to separate the base in aqueous phase from neutral colouring matters in the solvent phase. From the combined aqueous phase the base was liberated by the addition of 5N sodium hydroxide and recovered in chloroform. The chloroform extract was washed free from alkali, dried over anhydrous sodium sulphate and evaporated under reduced pressure to a gummy product (3.1 g.). It was crystallized from ethyl acetate.

Physical constants

Melting point 130-131°

Optical rotation $[\alpha]_D$ 28.5° (C=0.017, ethanol).

Analysis

		C.	H.	N.	$N\text{-}CH_3$	Active H.
$C_{22}H_{36}N_2$	requires	80.43	11.03	8.50	4.60	0.60 %
	Found:	80.12	10.91	8.44	4.36	0.57 %

Infrared absorption spectra

ν/λ max. 3260 cm^{-1} for -NH.

The identity of the product as conimine (V) was established by preparing derivatives such as conimine hydrochloride and conessine according to the method of Siddiqui.[18]

N-Chlorination of conimine (V)

To 200 mg. of conimine (V) (0.61 mole) in 75 ml. of methylene chloride was added 164 mg. (1.22 mole) of N-chlorosuccinimide in small lots with stirring. After 15-20 minutes of stirring, the methylene chloride extract was washed repeatedly with water to remove succinimide and any unreacted N-chloro-succinimide. This extract gave a positive test with starch-iodide paper characteristic of N-chloro compounds. After drying the solution over anhydrous sodium sulphate, it was carefully evaporated *in vacuo* at 30-35° bath temperature on account of the unstable nature of the compound, when a crystalline material separated out (174 mg.). It was crystallised from ethyl-acetate.

Physical constants

Melting point 115° (decomposed)

Optical rotation $[\alpha]_D$ = + 29° (C = 0.021).

Analysis

	C.	H.	N.	Cl.
$C_{22}H_{34}N_2Cl_2$ requires	66.48	8.62	7.05	17.90 %
Found:	66.20	8.50	6.82	17.58 %

Infrared absorption spectra

$1/\lambda$ max. 735 cm^{-1} for N-Cl.

The physico-chemical behaviour and the infrared spectra of the product were consistent with the structure (VI).

Dehydrochlorination of N,N-dichloroconimine (VI)

To 200 mg. of the dichloro compound (VI) was added 50 ml. of freshly prepared sodium ethoxide (1 g. of sodium/ 50 ml. ethanol) and the mixture was refluxed for 45 minutes when it lost its property to respond to the starch-iodide test. At the end ca. 100 ml. of water was added until the appearance of a white precipitate which was extracted with chloroform and the chloroform extract worked up in the usual manner to yield 184 mg. of a gummy product. Attempts to crystallize the ketimine did not succeed. The u.v. absorption spectrum showed a peak at 242 mμ (ϵ=7200) and I.R. spectrum showed absorption bands at 1678, 1613 cm^{-1} (α,β-unsaturated ketimine) 1650 cm^{-1} (C=N) and 838 cm^{-1} (trisubstituted double bond).

The physical data of the gum were consistent with the structure (VII).

Hydrolysis of ketimine (VII)

An aliquot of 180 mg. of the ketimine (VII) was refluxed with 50 ml. of 2N sulphuric acid for 8 hours. The mixture was made alkaline by 5 N sodium hydroxide and extracted with chloroform. The chloroform extract was extracted three times with 1% perchloric acid and the perchloric extract made alkaline with 5N sodium-hydroxide

to precipitate the base which was recovered by chloroform extraction. After washing, drying over anhydrous sodium sulphate and evaporation the chloroform extract yielded a colourless gum (142 mg.) which was crystallised from petroleum ether and acetone, as clusters of needles. It could also be crystallised from ethylacetate and methanol.

As an alternative to recrystallization a single sublimation of the gummy material at 135-140° and 0.01 mm. pressure yielded the pure product.

Physical constants

Melting point 180-81°

Optical rotation $[\alpha]_D = +84^\circ$ (C = 0.018).

Analysis	C.	H.	N.
$C_{21}H_{29}ON$ requires	80.98	9.39	4.50 %
Found:	80.78	9.21	4.35 %

Infrared absorption spectrum

ν_{max} 1647 cm^{-1} for N=C and a doublet at 1667-1619 cm^{-1} for α,β-unsaturated ketone.

Ultraviolet absorption spectrum

λ max. 240 mμ (ϵ max = 15,900).

Estimation of nitrogen by VanSlyke

By employing the usual nitrite-acetic acid procedure of VanSlyke for amino-nitrogen determination, it was found that the compound gave off 4.23% of nitrogen (theory - 4.50%).

The physico-chemical data of the product were consistent with the structure (VIII).

Nitrous acid reaction with ompound (VIII)

To 500 mg. of the ompound (VIII) in 50 ml. of 25% acetic acid were added 500 mg. of sodium nitrite in 5 ml. of water with stirring when a precipitate separated out. It was extracted with chloroform and the chloroform extract was washed with dilute acetic acid to remove unreacted base, if any. The solvent layer was then washed free from acid, dried over anhydrous sodium sulphate and evaporated under reduced pressure to a gum (430 mg.) which was crystallised from methanol after filtration through a small column of alumina (yield 210 mg, m.p. 156-157°). On chromatography of the mother-liquors on 50 times its weight of alumina, another crystalline form of 18-hydroxyprogesterone was obtained (yield 202 mg, m.p. 182-183°). On repeated crystallization from aqueous acetone the high melting form yielded the lower melting form of 18-hydroxyprogesterone.

Physical constants

Melting point Form I: 156-57°; Form II: 182-83°

Mixed m.p. of form I with 18-hydroxyprogesterone prepared from holarrhimine[72] 156-58°.

Optical rotation $[\alpha]_D$ = + 159° (C = 0.019) for both the forms I and II

Analysis

		C.	H.
$C_{21}H_{30}O_3$	requires	76.32	9.15 %
	Found:	76.21	9.01 %

Infrared absorption spectra

ν_{λ} max. 3400 cm^{-1} (OH), 1666-1615 cm^{-1} (α,β- unsaturated ketone) with shoulder at 1704 cm^{-1} (sometimes absent).

Ultraviolet absorption spectrum

λ max. 242 mμ ϵ = 14,300

The physical data of this compound were consistent with the structure IX (a or b).

EXPERIMENTAL

Fermentation

The Stock culture of A.niger (NCIM 612) was maintained on potato-dextrose agar. The spores on the agar slants, grown for 4-5 days at 28° were used for inoculation of 100 ml. of sterile Czapeck-Dox medium containing 0.5% corn-steep liquor in 500 ml. Erlenmeyer flasks. The flasks (20 in number) were incubated on a rotatory shaker at 28-29° for 24 hours. An aliquot of 10 mg. of the Schiff's base (VIII) in ethanol was added to each flask under aseptic conditions. The fermentation was allowed to continue for 24 hours, after which the flasks were steamed for 15 minutes.

Recovery of Fermentation Products

The contents of all the flasks were pooled together and filtered. The mycelium was acetonised, extracted repeatedly with methylene chloride and discarded. The filtrate, pooled with the acetone extract from the mycelium, was extracted three times with methylene chloride and the methylene chloride extracts pooled.

The combined solvent extracts were extracted with 1% perchloric acid. The perchloric acid extracts were basified and extracted with chloroform. The final chloroform extract, after drying over anhydrous sodium sulphate, yielded 183 mg. of a gummy material on evaporation.

The gummy product was then distributed in a 4-transfer modified Craig distribution (loc.cit) between hexane and 70% aqueous ethanol. The combined hexane layers yielded 144 mg. of a gummy product which was crystallised from a mixture of petroleum ether and acetone (m.p. 179-181°), and was identified as the unreacted Schiff's base (VIII).

The alcoholic layer, after removal of most of the alcohol under reduced pressure, was extracted with chloroform. The chloroform extract on drying and evaporation yielded a colourless treacle (34 mg.). Attempts to crystallize the product, after repeated chromatography, were not successful. The purified product showed a band in the hydroxyl region of the I.R. spectrum at 3500 cm^{-1} and the N = C band at 1648 cm^{-1}. However, the U.V. and I.R. absorption spectra indicated the absence of α,β-unsaturated carbonyl absorption in the compound.

The N-oxides of Conessine

Preparation of Conessine-mono-N-oxide

To 1 g. of conessine in 10 ml. of ethanol in a two-necked 100 ml. flask, provided with a magnetic stirrer, outlet tube and a dropping funnel, was added 0.33 ml. of 30% hydrogen peroxide keeping the temperature of the mixture 8-10°. The mixture was allowed to warm to room temperature and kept for 7-8 hours stirring after which most of ethanol was evaporated *in vacuo* keeping the bath temperature below 60°. A white crystalline solid separated out in the flask (1.1 g.). It was crystallized from methanol, after keeping for 4-5 days in cold under anhydrous conditions.

Analytically pure sample was prepared by passing 500 mg. of N-oxide over an alumina column (1x30; neutral, Grade II) and eluting successively with 250 ml. each of ethylacetate, acetone and methanol. On evaporation of the solvents 350 mg. of a crystalline product was obtained from the ethyl acetate and acetone eluates.

Physical constants

Melting point 142-143°

Optical rotation $[\alpha]_D$ = + 24° (ethanol C=0.02).

Analysis

	C.	H.	N.
$C_{24}H_{40}N_2O$ requires	77.42	10.75	7.53 %
Found:	77.18	10.49	7.41 %

The compound was extremely hygroscopic in nature, soluble in chloroform, ethanol, less so in benzene (1 g./ 300 ml) and insoluble in ether.

It showed a tendency for decomposition at temperatures above 70°. The structure of conessine mono-N-oxide may be written as (X).

Reduction of Conessine-mono-N-oxide (X) by Sodium borohydride and aluminium chloride

Conessine-mono-N-oxide (X) was reduced to conessine according to the method of Brown and Subba Rao.[78]

Sodium-borohydride (56 mg.) was placed in a 100 ml. two-necked flask, provided with a condenser, a separating funnel and magnetic stirrer. To the flask was added 1.g. conessine-mono-N-oxide dissolved in 10-15 ml. of diglyme and the temperature of the flask was kept 5-6°. To the well-stirred solution was added 0.75 ml. of ether solution containing 76 mg. of aluminium chloride dropwise, when slight turbidity appeared. After stirring for half an hour at 5-10° and for 2 hours at room temperature, the reaction mixture was poured in a beaker containing 100 g. of crushed ice and 5 ml. of hydrochloric acid. After keeping for 15 minutes, the acidic solution was made strongly alkaline by 5N sodium hydroxide to precipitate the base which was extracted with chloroform and the chloroform extract worked up in the usual manner to yield

Thermal decomposition of conessine-di-N-oxide (XI)

The N-oxide was decomposed in the same manner as for mono-N-oxide to a brown gum from which no crystalline material could be obtained. The u.v. absorption spectrum showed a peak at 234 mμ with a shoulder at 241 mμ (ϵ =22000) indicating the presence of a 3:5-diene system (XIII).

D. Synthesis of Conessimine from Conessine

Action of cyanogen bromide on conessine-mono-N-oxide

To 1.58 g. of the conessine 3β-mono-N-oxide (X) dissolved in 400 ml. of dry benzene in a 1 litre two necked-flask, provided with a cold water condenser, a dropping funnel and a magnetic stirrer, was added 0.5 g. of cyanogen bromide in 25 ml. of benzene with stirring, over a period of five minutes. The solution was stirred for 6 hours at 80-85° and then at room temperature for 24 hours when semi-crystalline mass separated out. The benzene layer was decanted off and the residue washed two times with benzene and washings added to the original solution. On evaporation of the solvent in vacuo a semicrystalline product was obtained (1.1 g.). The substance was hygroscopic and difficult to purify. The crude N-cyano N-oxido conessimine was subjected to reduction by sodium borohydride and aluminium chloride.

Reduction of N-cyano, conessimine 3 N-oxide

The above product (1.1 g.) was reduced by sodium borohydride and aluminium chloride by the method of Brown and Subba Rao[78] as described before for reduction of the N-oxides of conessine. The gummy material (0.72 g.) was subjected to hydrolysis without purification.

Hydrolysis of N-cyano-conessimine

The crude N-cyano conessimine obtained after reduction was refluxed with 20% of alcoholic potassium hydroxide (25 ml.) for 18 hours. After decanting the alcoholic solution from the precipitated potassium carbonate, it was evaporated in vacuo. The residue was suspended in 50 ml. water and extracted with chloroform and the chloroform layer was extracted three times with dilute acetic acid. From the aqueous phase, the base was liberated by addition of 5N sodium-hydroxide and recovered again in chloroform. After washing and drying, the chloroform extract was evaporated in vacuo to yield a gum. It was subjected to chromatography over 30 g. of basic grade II alumina. The order of elution was as follows: benzene, benzene-ethyl- acetate, ethylacetate, ethylacetate-chloroform.

The benzene-ethyl acetate and ethyl acetate eluates were combined and evaporated to a gum (68 mg.).

On crystallisation from ethyl acetate 42 mg. of conessimine (III) was obtained (m.p. 97-98°).

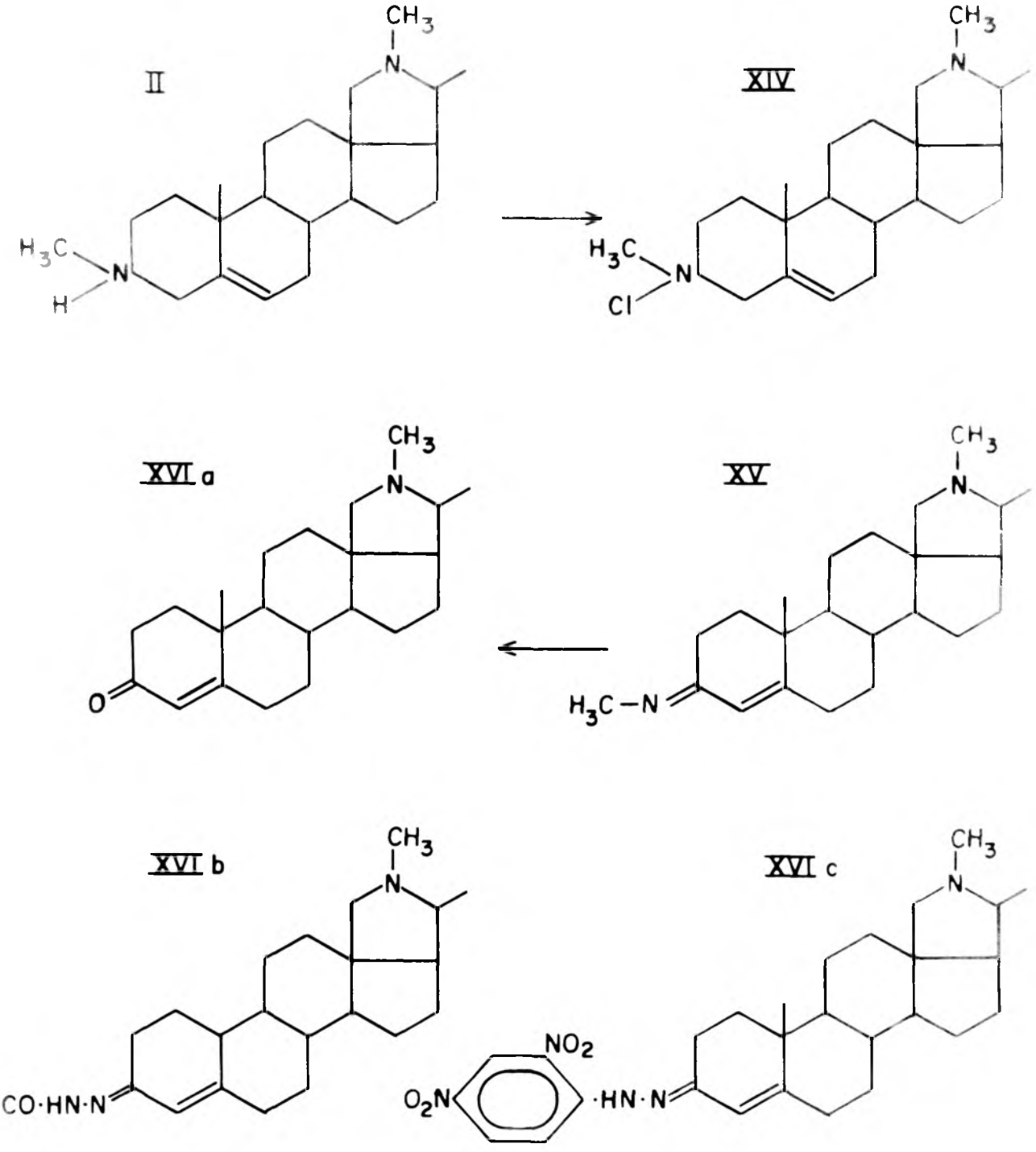
The identity of the product was established thrcugh its mixed m.p. with conessimine and a comparison of the I.R. spectrum and other physico-chemical properties.

C H A P T E R VI

TRANSFORMATIONS OF ISO-CONESSIMINE

Plate 4

Transformations of isoconessimine



VI. Transformation of Iso-Conessimine

DISCUSSION

Iso-conessimine occurs in the kurchi bark to the extent of 0.06 - 0.2% and is a minor alkaloid as such. However, conessine can be converted to iso-conessimine in reasonable yields by partial demethylation by the cyanogen bromide procedure.[18]

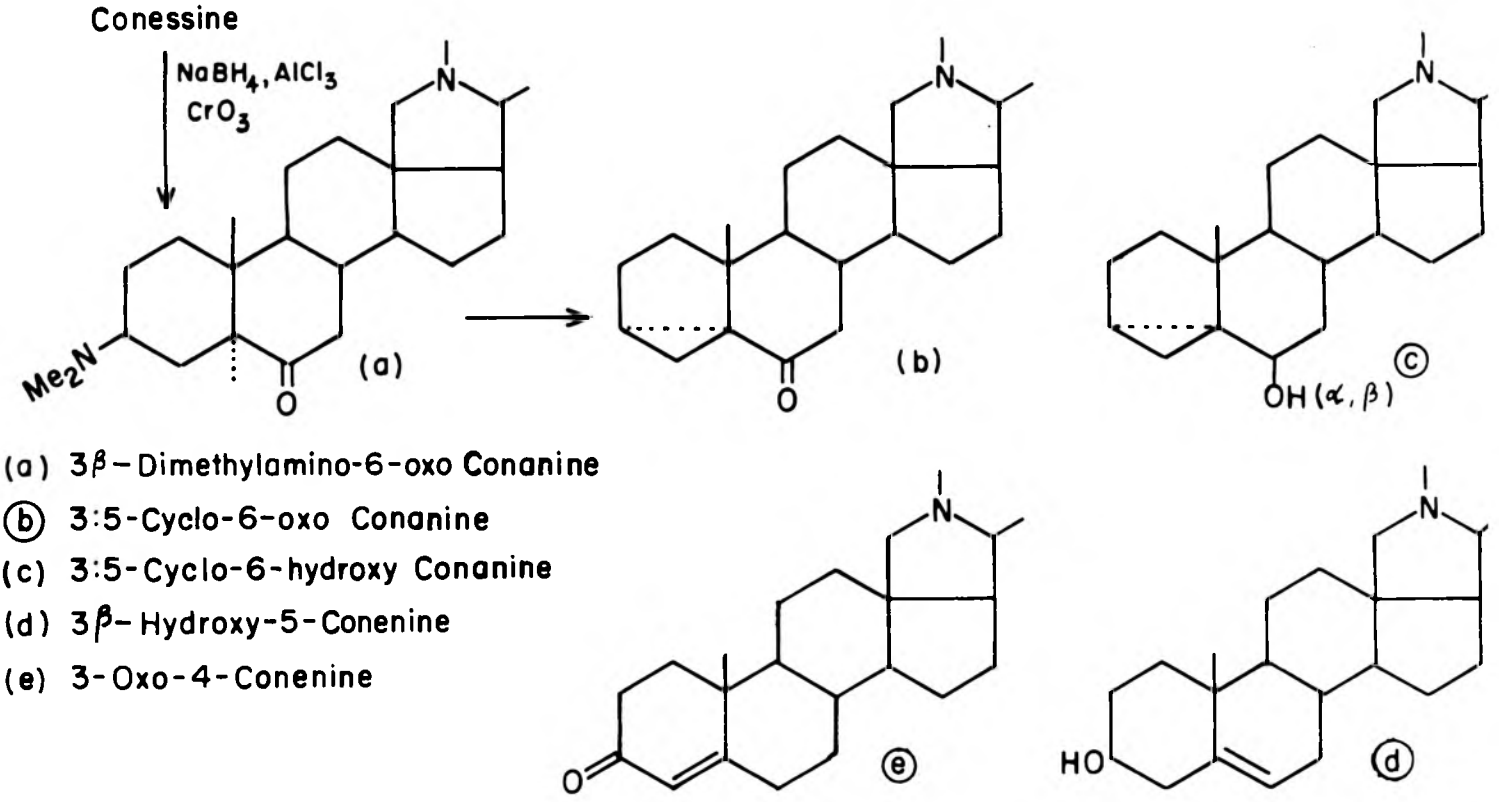
From the structure of iso-conessimine it is apparent that the 3β-amino group in this compound is partially substituted and as such is expected to be a convenient starting material for the synthesis of 3- oxygenated steroids containing the 18-20 bridge nitrogen.

Iso-conessimine (II) on treatment with one mole of N-chlorosuccinimide formed the 3N-chloroderivative in near quantitative yields. The product crystallised without difficulty from ethylacetate and was characterised by its elemental analysis, physical properties and I.R. spectrum which indicated a distinctive absorption band at 735 cm^{-1} for N-Cl. The product liberated iodine in the test starch-iodide paper. As compared to N^a-N^b-dichloroconimine and N^b-chloro-conessimine, this product appeared to be less stable to storage.

On refluxing with sodium ethoxide in alcohol,

the N-chlorocompound lost its property of responding to the starch iodide test, indicating the removal of the N-chloro group. The crude gummy product from this reaction could not be isolated in the crystalline form, but was characterised by its absorption spectra (λ max. 240 mμ, ν_{max} 1678, 1613 cm^{-1} α,β- unsaturated C=N), 1650 cm^{-1} (C=N) and 838 cm^{-1} (trisubstituted double bond). The crude ketimine was subjected to acid hydrolysis with 2N sulphuric acid. The product isolated on basifying the reaction mixture failed to crystallise. It was, however, characterised as XVIa by its u.v. and I.R. spectra (λ max. 241, ϵ 14000); ν_{max} 1676, 1614 cm^{-1} (α,β -unsaturated ketone). The product yielded a micro-crystalline semi-carbazone (XVIb) and a crystalline 2:4-dinitrophenyl hydrazone (XVIc). The physico-chemical behaviour and absorption spectra of these two derivatives were consistant with the structure (XVIa) for the original product.

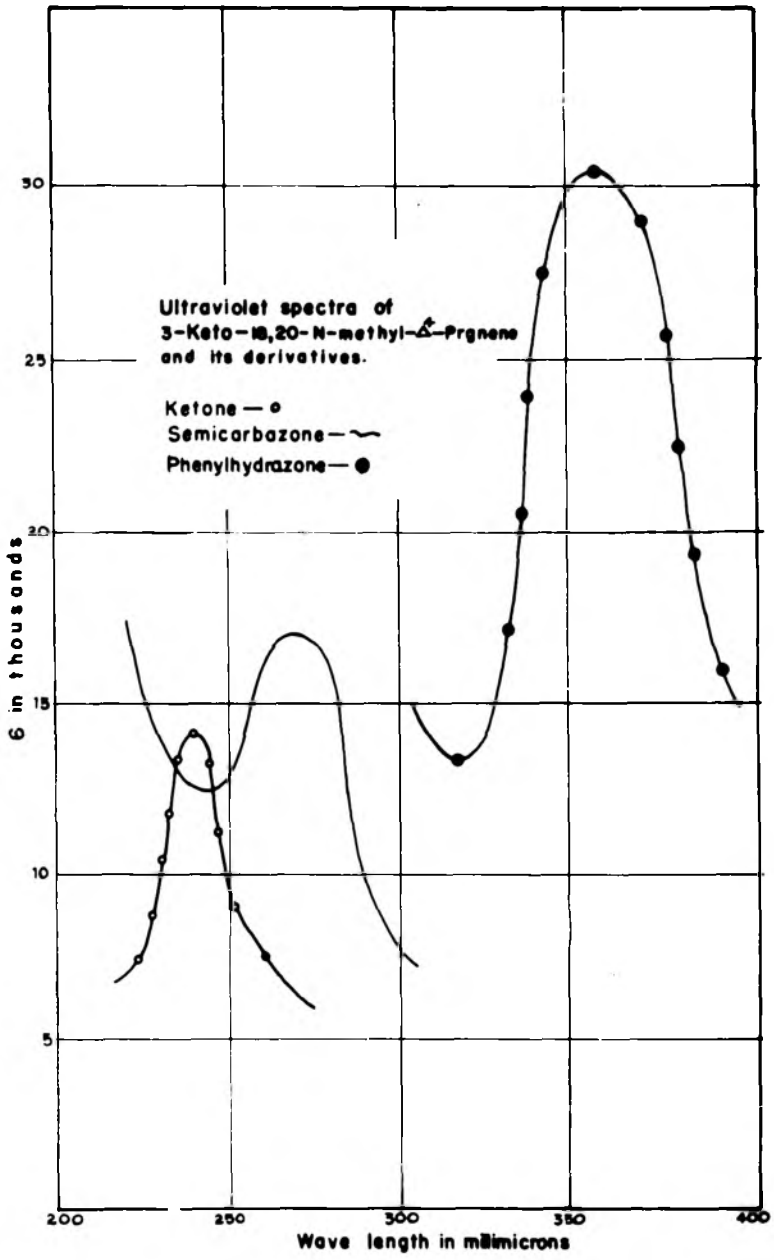
Pappo[79] has synthesized the compound (XVIa) by the following route:



(a) 3β-Dimethylamino-6-oxo Conanine
(b) 3:5-Cyclo-6-oxo Conanine
(c) 3:5-Cyclo-6-hydroxy Conanine
(d) 3β-Hydroxy-5-Conenine
(e) 3-Oxo-4-Conenine

Pappo was unable to crystallize the compound. Johnson,[51] who has used this compound as the starting material for the synthesis of conessine was, however, able to crystallise it from methanol.

It is interesting to record that Bavan and Pappo[3] reported the compound $\Delta^{1,4}$-3-oxo-conanine, a derivative of 3-oxo-4-conenine exhibits strong antifungal properties.


Ultraviolet spectra of
3-Keto-18,20-N-methyl-Δ⁴-Prgnene
and its derivatives.
Ketone — ○
Semicarbazone — ~
Phenylhydrazone — ●
ε in thousands
30
25
20
15
10
5
200
250
300
350
400
Wave length in millimicrons

EXPERIMENTAL

N-Chlorination of Iso-Conessimine

To 250 mg. (0.73 mole) of isoconessimine (II) in 50 ml. of dry methylene chloride were added in small lots, 100 mg. (0.74 mole) of N-chlorosuccinimide with stirring. After 15-20 minutes of stirring the methylene chloride extract was washed with water to remove succinimide and any unreacted N-chlorosuccinimide. The extract gave a positive test with starch-iodide paper characteristic of N-chloro-compounds. After drying the extract over anhydrous sodium sulphate, it was carefully evaporated *in vacuo* at 30-35° bath temperature (on account of the unstable nature of the compound) when a crystalline material (258 mg.) separated out. It was crystallised from ethylacetate.

Physical constants

Melting point 109° (decomposed).

Optical rotation $[\alpha]_D$ = + 12.6° (C = 0.023).

Analysis

		C.	H.	N.	Cl.
$C_{23}H_{37}N_2Cl$	requires	73.40	9.84	7.45	9.30 %
	Found:	73.15	9.65	7.30	9.20 %

Infrared absorption spectrum

$1/\lambda$ max. 735 cm^{-1} for Cl.

The physical data of the product were consistent with the structure (XIV).

Dehydrochlorination of N-chloro-Iso-Conessimine

To 200 mg. of the N-chloro-compound (XIV) were added freshly prepared 50 ml. of sodium-ethoxide (1 g. of sodium/50 ml. ethanol) and the mixture refluxed for 30 minutes when it lost its property to respond to starch-iodide test, indicating the completion of dehydro-chlorination. At the end, after removal of most of the alcohol, water (50 ml.) was added until the appearance of a white precipitate which was extracted with chloroform. The chloroform extract was washed thoroughly with water, and dried over anhydrous sodium sulphate. On evaporation of chloroform under reduced pressure, a gummy product was obtained (174 mg.). Attempts to crystallize the material from common organic solvents did not succeed. The infrared spectrum of the crude product showed absorption bands at 1678, 1613 cm^{-1} (α,β-unsaturated ketimine), 1650 cm^{-1} (N=C) and 838 cm^{-1} (trisubstituted double bond). The ultraviolet absorption spectrum showed a peak at 240 mμ (ϵ =7600).

The physical data were consistent with the structure (XV).

Hydrolysis of Ketimine (XV)

The crude ketimine (XV) (175 mg.) was refluxed for two hours after the addition of 25 ml. of 2N sulphuric acid. The mixture was then made alkaline by 5N sodium hydroxide and the precipitated base extracted with

chloroform. The chloroform extract was worked up in the usual manner yielding a gummy product (151 mg.). Attempts to crystallize the product from various solvents and by a chromatographic procedure did not succeed. However, from ethylacetate a white amorphous precipitate was obtained. The u.v. absorption spectrum exhibited a peak at 241 mμ (ϵ=14000). The I.R. spectra exhibited a doublet at 1676, 1614 cm^{-1} (for α,β- unsaturated ketone), and band at 1650 cm^{-1} being absent. The physical data were consistent with the structure (XVIa) 3-oxo-18,20-N-methyl-4-pregnene.

Semicarbazone of (XVIa)

An aliquot of 80 mg. of (XVIa) in 10 ml. of 80% alcohol, 100 mg. of semicarbazide hydrochloride and 50 mg. of sodium acetate were heated on water bath for one hour. Most of the alcohol was then removed by evaporation in vacuo. The aqueous phase was carefully basified with 5N sodium hydroxide in the cold and the white precipitate obtained was extracted with chloroform. The chloroform extract was worked up in the usual manner to yield a gummy product (63 mg.). Attempts to crystallize the product from methanol or ethanol did not succeed. When chromatographed over 50 times its weight of alumina one fraction of white micro-crystalline material (18 mg.) was obtained. The compound exhibited the u.v. absorption characteristics of semi-carbazone of an α,β-unsaturated carbonyl compound (λ max. 271 mμ) (ϵ=17000). The data are consistent with the structure (XVIb).

Preparation of 2:4-Dinitrophenyl hydrazone

To 5 ml. of 2,4-dinitrophenyl hydrazine reagent 100 mg. of the crude ketone (XVIa) was added. The mixture was heated for 4-5 minutes, concentrated to one half of its volume and left in cold overnight. Orange coloured crystals separated out. After three crystallizations from aqueous alcohol 15 mg. of the pure crystalline product was obtained, m.p. 115°.

The u.v. spectra (page 89a) indicated a strong absorption, characteristic of hydrazones of $\alpha\beta$-unsaturated carbonyls λ max. 360 mμ (ϵ=30150).

Analysis

	C.	H.	N.	
$C_{28}H_{38}N_5O_4$ requires	66.30	7.48	13.78	%
Found:	66.10	7.42	13.51	%.

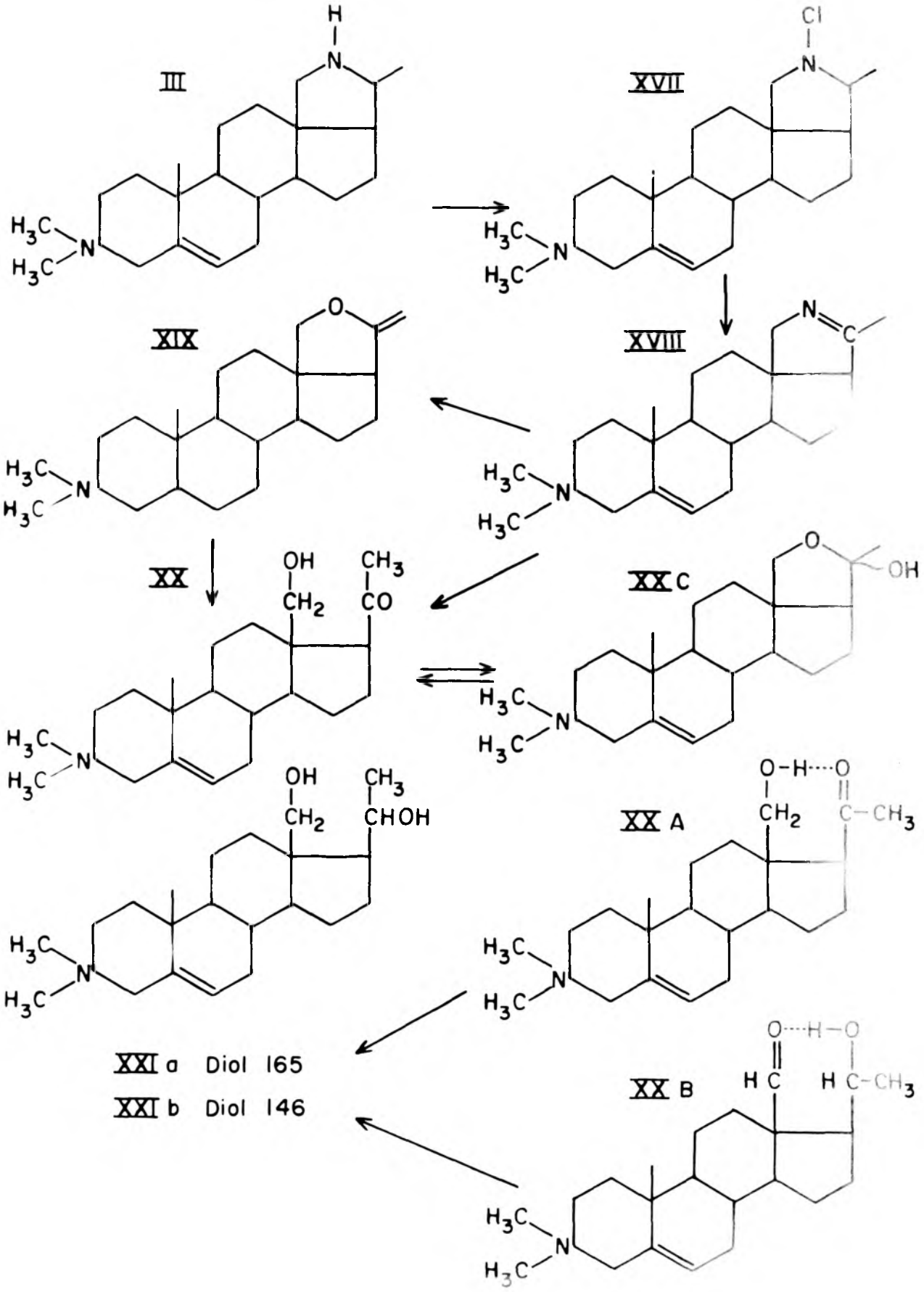
The data were consistent with the Structure XVIc for the 2:4-dinitrophenyl hydrazone.

C H A P T E R VII

TRANSFORMATIONS OF CONESSIMINE

Plate 5

Transformations of Conessimine



VII. Transformations of Conessimine

DISCUSSION

Conessimine, $C_{23}H_{38}N_2$, (III), occurs in Kurchi bark to the extent of 0.1 to 0.2 percent. No work has been reported in literature so far on the transformation of this alkaloid to steroidal intermediates.

The 18-20- unsubstituted imino N^b-nitrogen bridge in conessimine provides a convenient point of attack for the rupture of the heterocyclic pyrrolidinering eventually leading to 18,20-disubstituted steroids. As a primary step, the Ruschig procedure appeared to be attractive.

On treatment with N-chlorosuccinimide, conessimine yielded the expected N-chlorocompound in near-quantitative yields in a crystalline form. Response to the starch-iodide test and the I.R. band at 735 cm^{-1} strongly indicated the structure (XVII) for the product. It was more stable to storage at room temperature than the corresponding N-chloro derivative of isoconessimine.

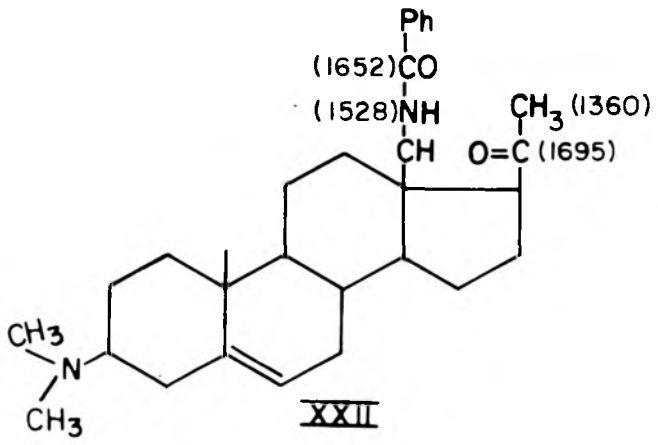
Dehydrohalogenation of N-chloro-conessimine with sodium ethoxide proceeded smoothly without much discoloration and was complete in 10 to 15 minutes at the reflux temperature of ethanol. The internal Schiff's base of 18 amino-3β-N-dimethyl-20-oxo-5-pregnene (XVIII) was obtained in the crystalline form by working up the reaction mixture in yields exceeding 80 percent based on conessimine.

Characterisation of Compound XVIII

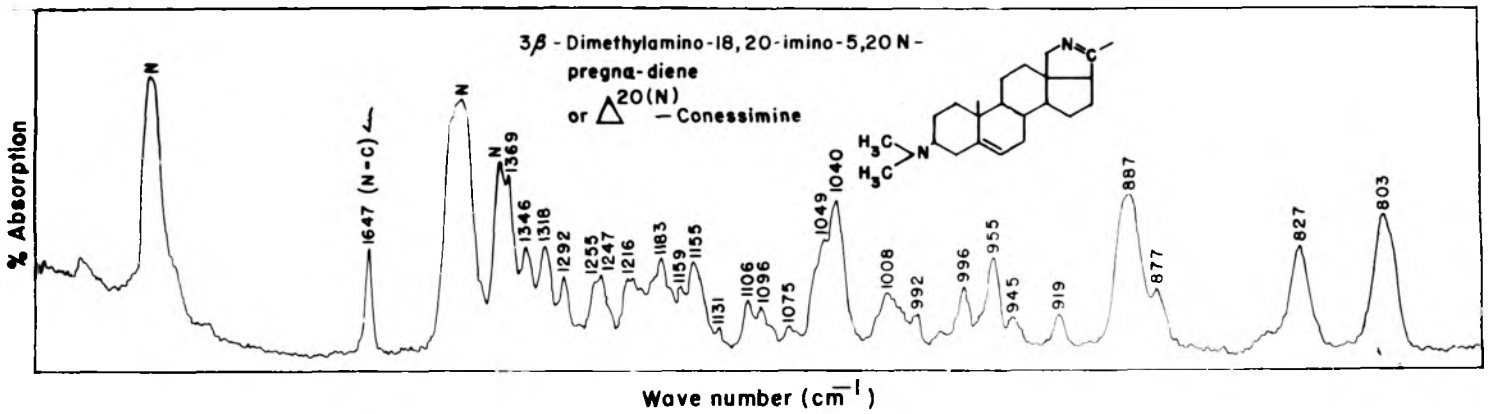
The I.R. spectra (ν_{max} 1648 cm^{-1} for N=C and 848cm^{-1} for the trisubstituted double bond) were consistent with Structure (XVIII) for the Schiff's base. Van Slyke determination indicated the existence of a potential primary amino group in the molecule. Optical rotation, analytical data and the finding of two N-methyls indicated that the 3β N-dimethyl-5-pregnene structure was not affected by these transformations.

Treatment with dilute acid followed by basification gave back the compound unchanged. The conclusion was, therefore, drawn that the Schiff's base was intramolecular in nature and in a 5 or 6 membered ring.

The pyrroline ring could be effectively opened, however, on benzoylation to yield the crude 18-benzamido 3β-N-dimethyl-20-oxo-5-pregnene (XXII) which exhibited the anticipated I.R. absorption characteristics of the functional groups in the structure (XXII) (wave numbers in parenthesis).

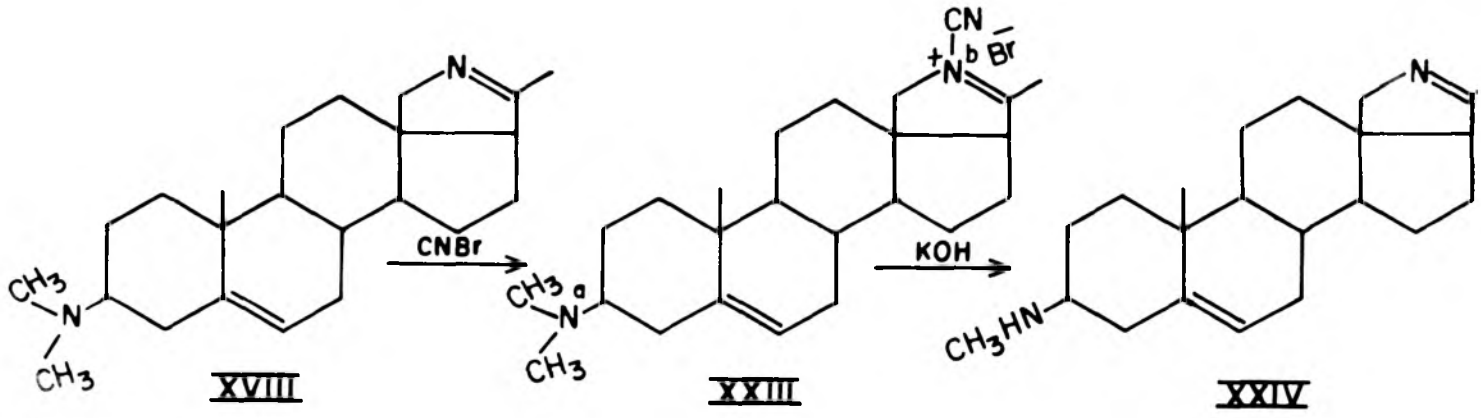


XXII

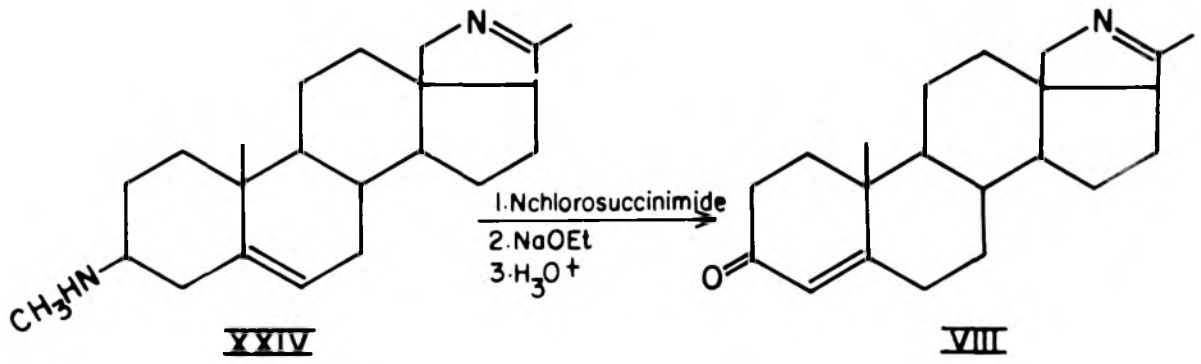

3β - Dimethylamino-18, 20-imino-5,20 N-
pregna-diene
or Δ20(N) — Conessimine
% Absorption
Wave number (cm⁻¹)
1647 (N-C)
1369
1346
1318
1292
1255
1247
1216
1183
1159
1155
1131
1106
1096
1075
1049
1040
1008
992
996
955
945
919
887
877
827
803

In this connection it may be mentioned that Sorm and co-workers reported that the Schiff's base of 3β-hydroxy-18-amino-20-oxo-5α-pregnane normally resistant to acid hydrolysis, also yielded the 18-benzamido-20-oxo-derivative on benzoylation.

The conclusive evidence for Structure (XVIII) for the Schiff's base was obtained by correlating it with 18,20- Schiff's base of 18-amino-3,20-dioxo-4-pregnene (VIII) obtained from conimine (V) prepared by the procedure of Jeger et al.[56] To accomplish this, the von Braun cyanogen bromide procedure for demethylation at the 3β-nitrogen was employed on the Schiff's base. It was found that both the nitrogens in the molecule reacted with cyanogen bromide in the initial step and that the di-N-cyano intermediate, (XXIII), was difficult to purify. It was subjected directly to alkaline hydrolysis and decarboxylation with the anticipation that along with the N^a-cyano-group at 3β-position, the N^b-cyano group on the pyrroline ring would be eliminated through this reaction, leaving the partially demethylated Schiff's base (XXIV). Unfortunately, the reaction product could be isolated only as a gummy material.



The finding of approximately one N-methyl and the I.R. bands at 3260 cm^{-1} (NH) and 1648 cm^{-1} (N=C) in the reaction product supported Structure (XXIV). It was then subjected to the Ruschig procedure of N-chlorination, dehydrochlorination and hydrolysis. No attempts were made to isolate the intermediates in this sequence of reactions. The final product was, however, obtained in a crystalline form after purification through the perchloric acid extraction step described in connection with the transformations of conessine(page 59) and proved to be identical with Compound (VIII) obtained from conimine.



XXIV VIII

These transformations gave additional evidence for the structure of Compound (XVIII) and established a route to 18-hydroxyprogesterone from conessimine. Why this rigorous procedure for establishing the structure of Compound (XVIII) became necessary would be apparent when some unusual behaviour of the subsequent transformation products would be discussed.

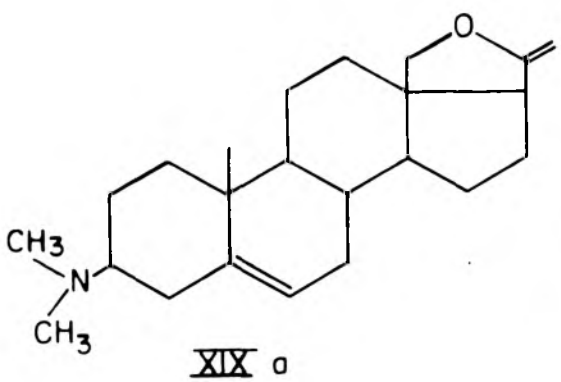
Action of Nitrous Acid on the 18,20-Schiff's base of 18-amino-3β-N-dimethyl-20-oxo-5-pregnene(XVIII)

In course of the transformations of conessine it was evident that the Schiff's base yielded predominantly 18-hydroxyprogesterone (IX) on treatment with nitrous acid. It was also mentioned that Compound (IX) existed in two forms, one melting at 156-57° and the other at 182°-83°, both having almost the same optical rotation and superimposible I.R. spectra. A difference in the polarity of these forms made them separable by column chromatography over alumina. Since 18-hydroxyprogesterone exists predominantly in the 20 - 18 hemiketal form, it was presumed that these two forms were epimeric at position 20.

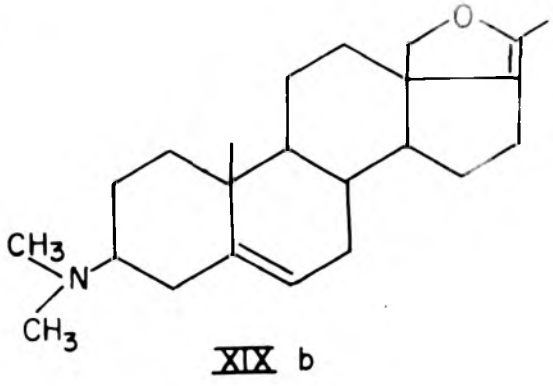
From the analogy of the behaviour of Compound(VIII) towards nitrous acid, Compound (XVIII) was anticipated to yield the corresponding 18-hydroxy-20-oxo-compound(XX) as the major product. Furthermore, the possibility that like 18-hydroxyprogesterone, Compound (XX) would also exist in two forms had to be considered. When the actual experiment was performed, however, three crystalline products were isolated from the nitrous acid reaction mixture. Compound (XIX), m.p.180° which was isolated directly by crystallisation from ethyl acetate and two products (XXA) and (XXB) were presumed to be two forms of Compound (XX) melting at 74° and 89° respectively by column chromatography of the mother liquors from Compound (XIX).

The Structure of Compound(XIX)

Compound (XIX) analysed for $C_{23}H_{35}NO$ and contained no active hydrogen. The I.R. spectra(page 95a) indicated the absence of hydroxyl or carbonyl absorption in the normal frequency ranges. However, a strong band at 1589 cm^{-1} indicated that the oxygen function was probably associated with a vinyl ether system. Out of the two probable structures (XIXa) and (XIXb),



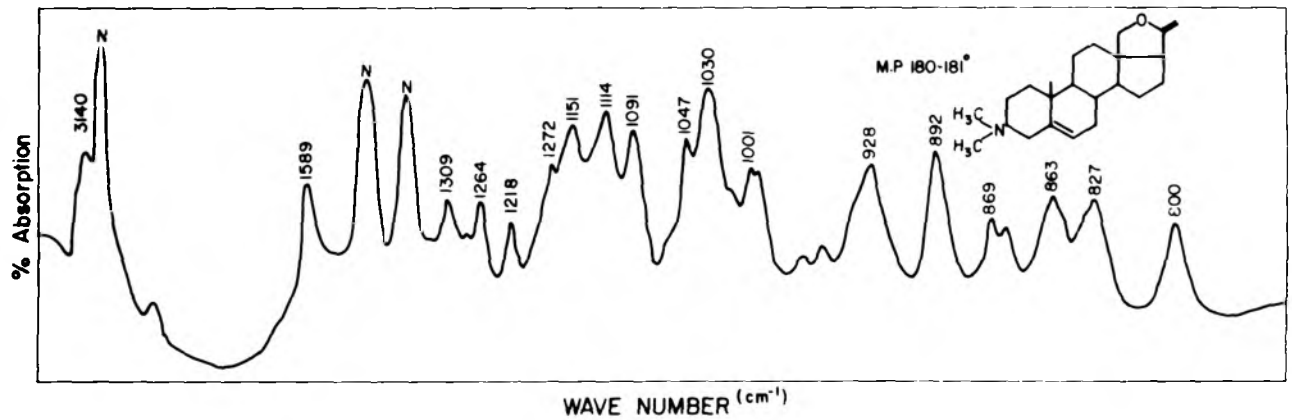
XIX a

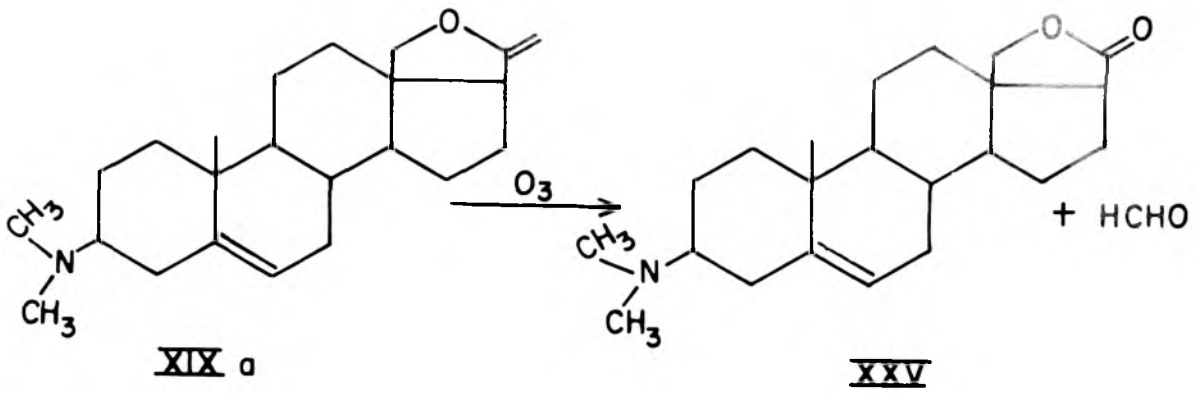


XIX b

(XIXa) was favoured because of an I.R. band of medium intensity at 892 cm^{-1}. That this choice was justified was borne out by chemical studies.

On ozonolysis, Compound (XIX) yielded in a substantial yield formaldehyde, characterised through its dimedone derivative, as the only volatile product. The non-volatile part of the ozonolysis mixture was obtained as a gum which exhibited the expected I.R. absorption at 1770 cm^{-1} of a five membered ring lactone as in (XXV).


% Absorption
WAVE NUMBER (cm⁻¹)
3140
1589
1309
1264
1218
1272
1151
1114
1091
1047
1030
1001
928
892
869
863
827
800
M.P 180-181°
H3C
H3C
N
O



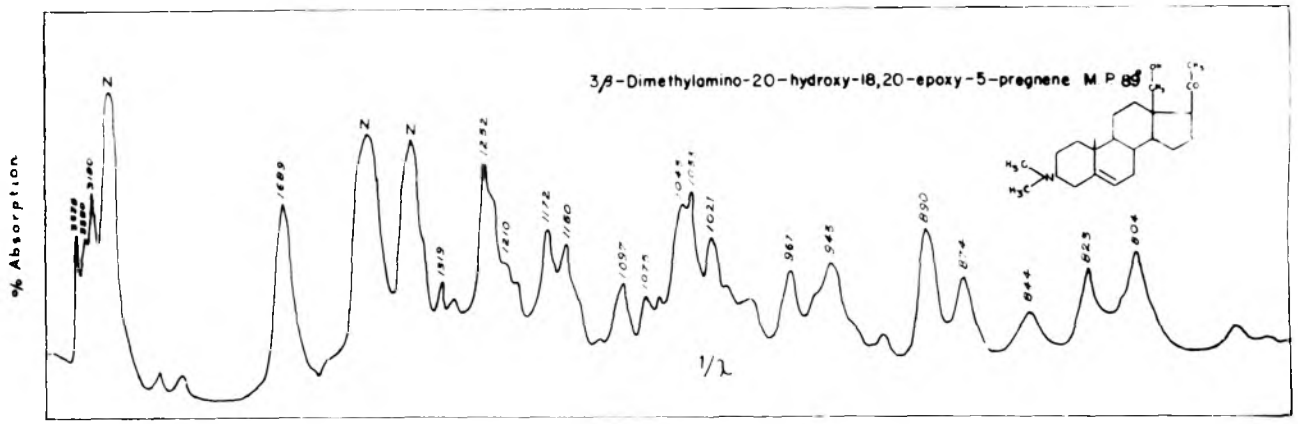
XIX a XXV

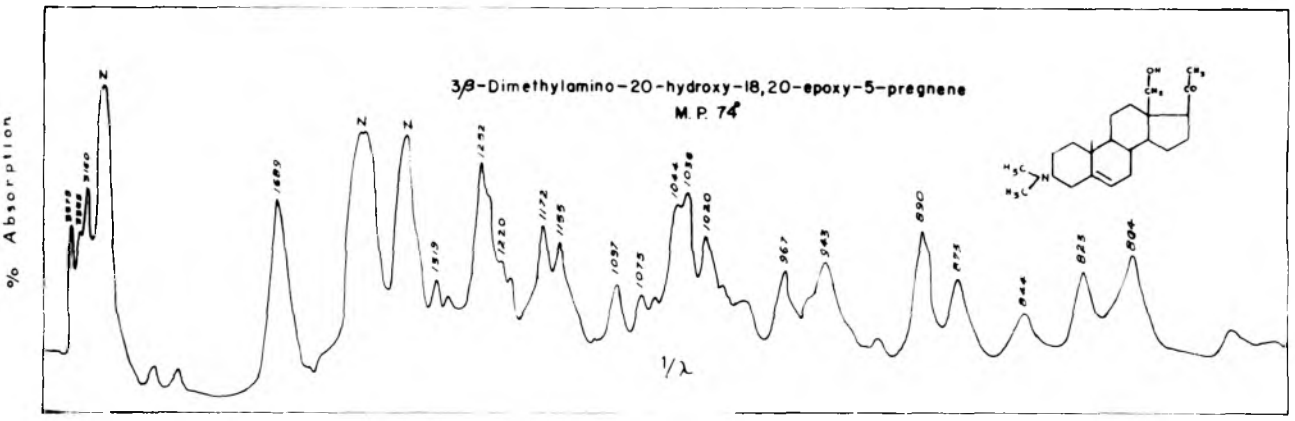
Compound (XIX) was converted to a mixture of both the forms of Compound (XX) by acid-catalysed hydration.

The yield of Compound (XIX) in the mixture varied with the acid concentration in the nitrous acid reaction mixture. When the nitrous acid reaction was carried out in 25 percent aqueous acetic acid, the yield was 33 percent. On the other hand, with 50% aqueous acetic acid it went upto 45 percent.

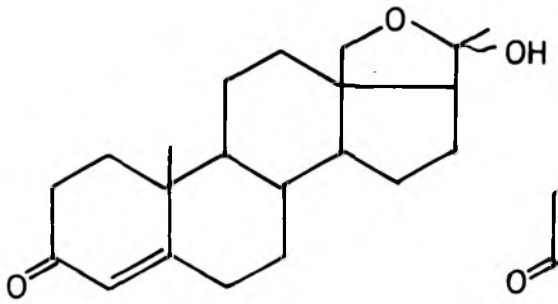
The Structures of Compound (XX) (Form A & B)

Both the forms of Compound (XX) analysed for $C_{23}H_{37}NO_2$ and two N-methyls. There was no significant difference in the optical rotation of these two forms. The solid state I.R. spectra in ∫ nujol of both these forms were identical and exhibited bands at 3575 and 3200 cm^{-1} regions for hydroxyl and at 1689 cm^{-1} for carbonyl grouping.

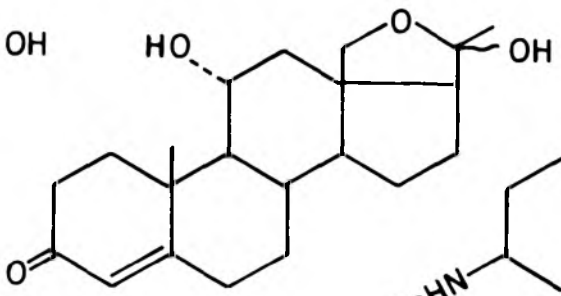

% Absorption
3β-Dimethylamino-20-hydroxy-18,20-epoxy-5-pregnene M.P. 89°
1/λ


% Absorption
3β-Dimethylamino-20-hydroxy-18,20-epoxy-5-pregnene
M.P. 74°
1/λ

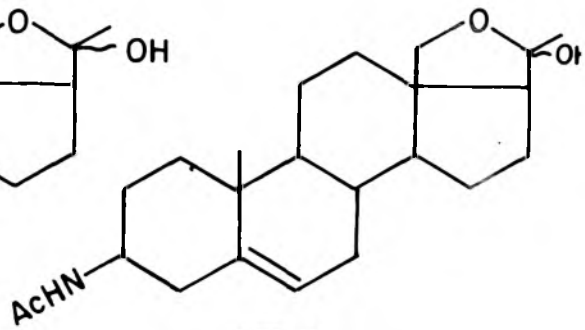
However, a comparison of the I.R. spectra of Compounds, (XXA) and (XXB) with those of three other 18-hydroxy-20-oxo-compounds (IX, XXVI and XXVII) available in this laboratory[71] revealed some unexpected differences.



IX



XXVI

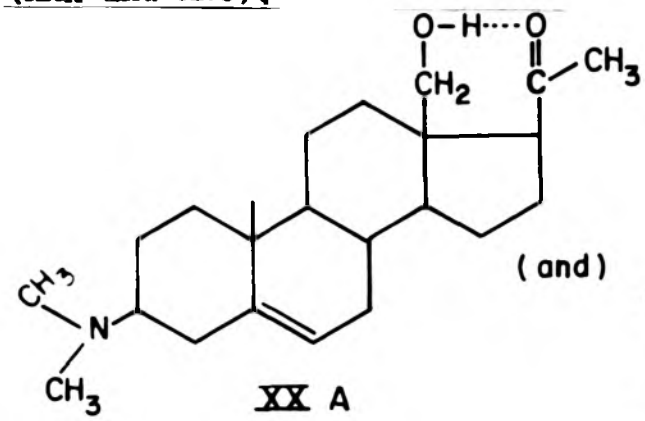


XXVII

The 20-carbonyl bands in the case of 18-hydroxy progesterone (IX), 11α-18-dihydroxyprogesterone(XXVI), and 18-hydroxy-3β-acetamido-20-oxo-5-pregnene (XXVII) do not usually show up in the I.R. spectra excepting for 18-hydroxyprogesterone where it is only occasionally observed as a shoulder. In contrast, Compound (XX) exhibited a sharp carbonyl band at 1689 cm^{-1} and two bands in the hydroxyl region at 3575 and 3100 cm^{-1}. The shift of the carbonyl absorption frequency to 1689 cm^{-1} from the normal range of 1705 to 1710 cm^{-1} along with the appearance of the 3200 cm^{-1} band for the

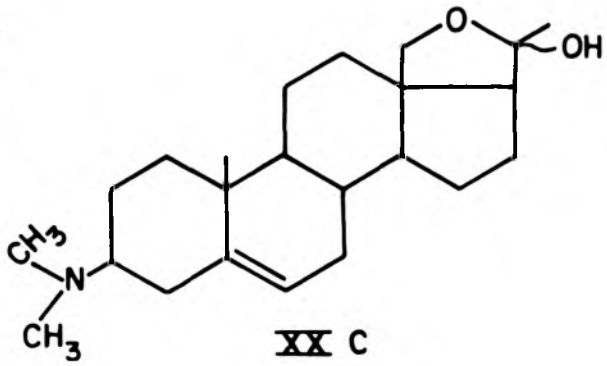
hydroxyl indicated an intramolecular hydrogen bonding as in the structure(XXA). At the same time, the bands at 3575 cm^{-1} and 1130 cm^{-1} represented the tertiary hydroxyl group in Structure (XXC).

The tentative conclusion, therefore, suggested itself that the crystalline structure of (XXA) or of (XXB) was built up by an aggregation of both the forms, (XXA and XXC),



XX A

(and)



XX C

and that in (XXA) and (XXB) the (XXC) component was epimeric at position 20. The alternative explanation that the existence of two forms, (XXA) and (XXB) is just another example of polymorphism, could also be entertained. However, these simple interpretations of the I.R. spectra ran into some unexpected difficulties when some unusual behaviour of the lithium aluminium hydride reduction products from (XXA) and (XXB) came to light.

Lithium Aluminium Hydride Reduction of Compounds XXA and XXB

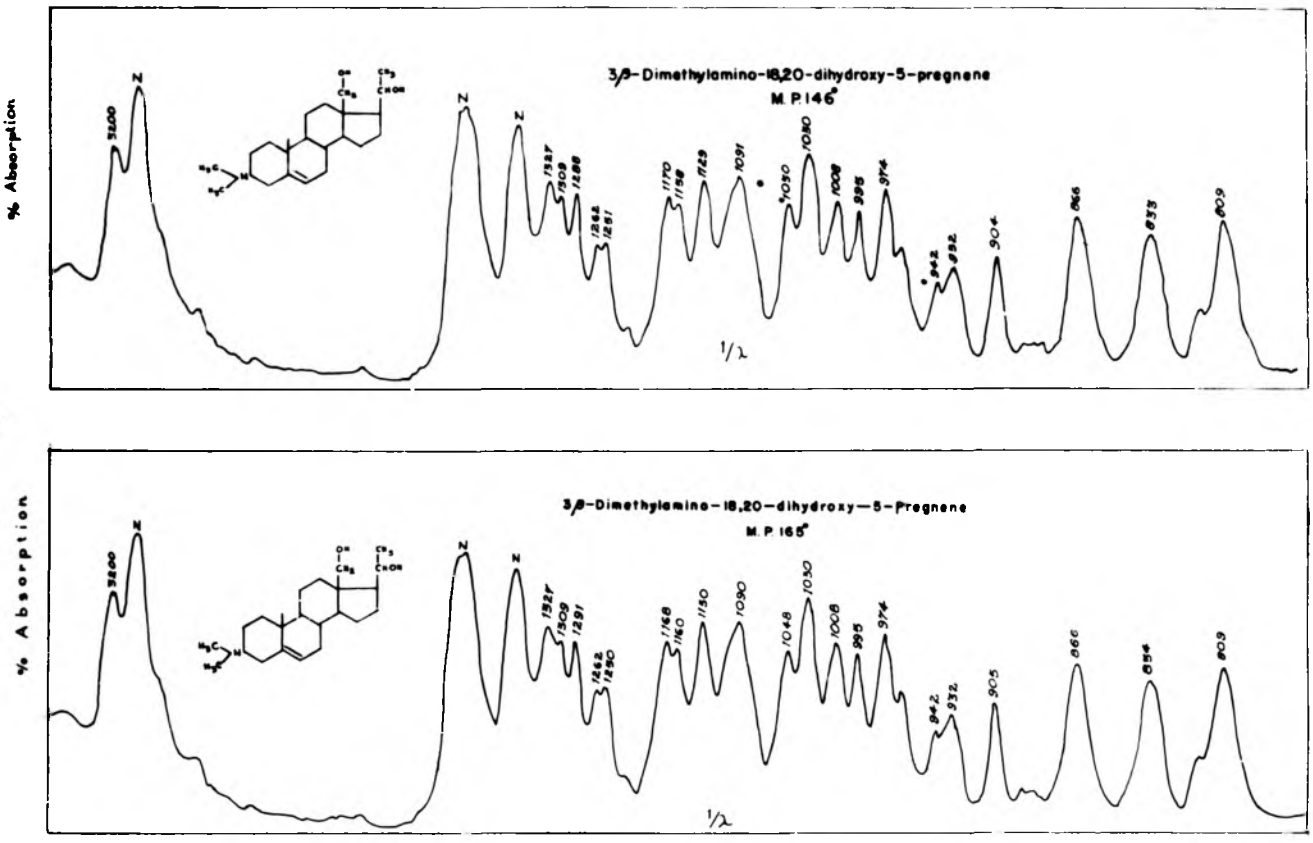
On reduction with lithium aluminium hydride Compound (XXA) was converted quantitatively to a crystalline

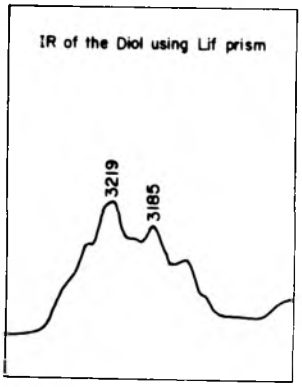
Compound (XXIa), $C_{23}H_{39}NO_2$, melting at 165°. The solid state I.R. spectra in nujol (page 99a) indicated a complete disappearance of the carbonyl absorption at 1689 cm^{-1} and the enhancement of a strongly bonded hydroxyl absorption at 3200 cm^{-1}. With lithium fluoride prism, the latter band was split into two peaks, one appearing at 3219 cm^{-1} and the other at 3185 cm^{-1} (page 99a). The physical data, I.R.spectra, solubility etc. were consistent with Structure (XXI) for the compound. This compound will be referred to as Diol 165 in the subsequent discussion.

Compound (XXB) was also quantitatively reduced by lithium aluminium hydride to another diol, (XXIb), $C_{23}H_{39}NO_2$, melting at 146°, referred to as Diol 146 hereafter.

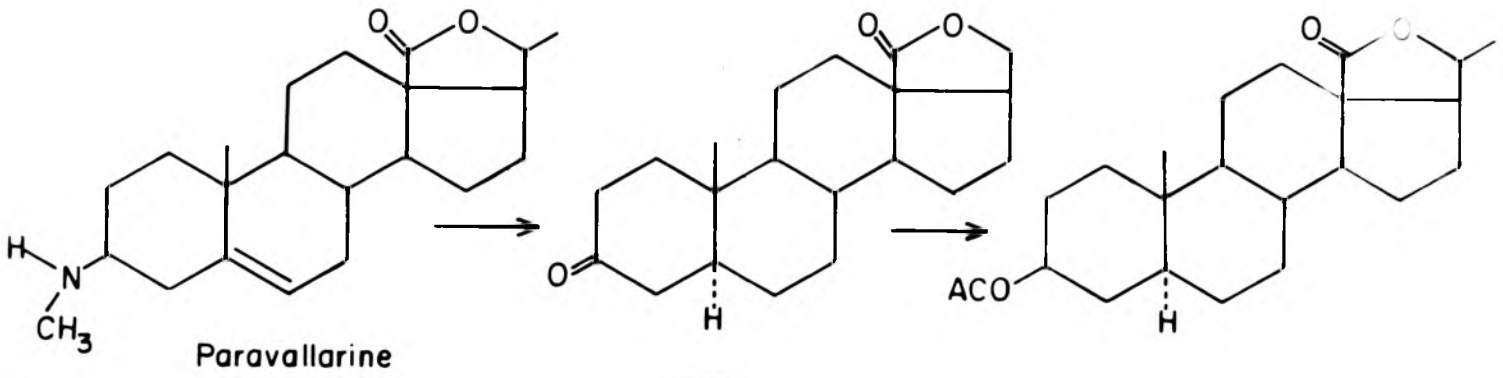
There was no significant difference in the optical rotation of these two diols (-63° and -62°). Their solid state I.R. spectra were almost superimposible (page 99a). Superficially, excepting for their differences in melting point, crystalline behaviour and solubility (Diol 165 being more soluble in ethyl acetate than Diol 146) they appeared to be identical.

The question, therefore, arose once again whether these forms of the Diol (XXI) are identical compounds showing polymorphism or they are different in some minor structural details.


% Absorption
3β-Dimethylamino-18,20-dihydroxy-5-pregnene
M.P. 146°
3200
1327
1309
1288
1264
1251
1170
1158
1123
1091
1050
1030
1008
995
974
942
932
904
866
833
809
1/λ
% Absorption
3β-Dimethylamino-18,20-dihydroxy-5-Pregnene
M.P. 165°
3200
1327
1309
1291
1268
1250
1168
1160
1130
1090
1048
1030
1008
995
974
942
932
905
866
854
809
1/λ

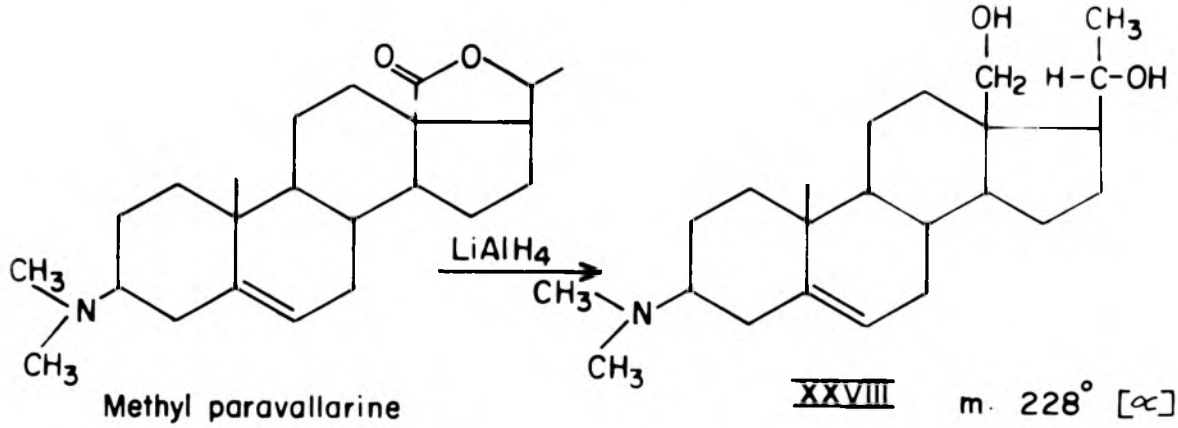

IR of the Diol using Lif prism
3219
3185

In the I.R. spectra taken with lithium fluoride prism (page 99a) some differences in the relative intensities of the two peaks at 3219 and 3185 cm^{-1} were noticeable



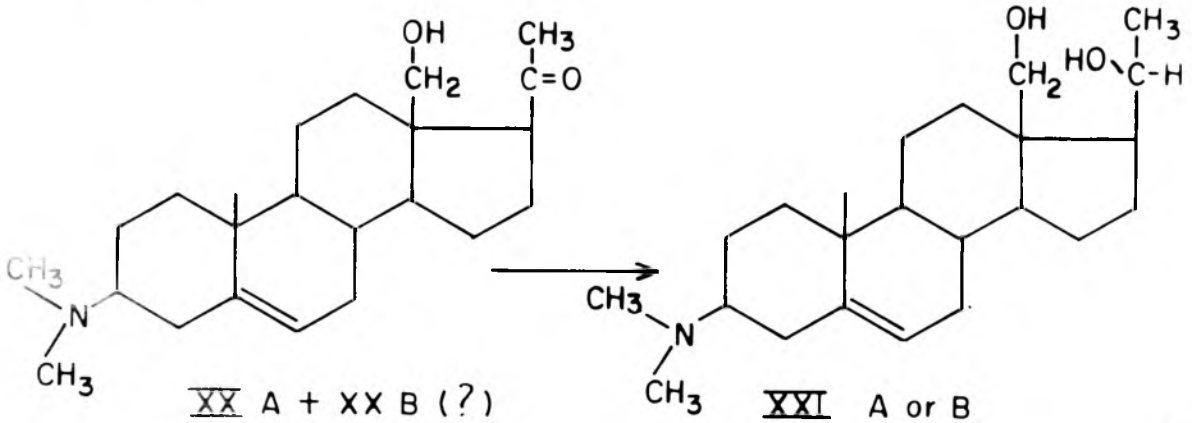
Paravallarine

XXIX



Methyl paravallarine

XXVIII m. 228° $[\alpha]_D$ -30°



XX A + XX B (?)

XXI A or B

XXI A Diol 165, m. 165°
XXI B Diol 146, m. 146°
$[\alpha]_D = -63°$

Since it was evident that the diol from methyl paravallarine was 18,20α dihydroxy 3β-N-dimethyl-5-pregnene, the 20-epimeric 18,20β -dihydroxy-3β-N-dimethyl-5-pregnene structure (XXI) had to be considered for the Diols 165 and 146. This assignment of structure was in keeping with the observed behaviour of lithium aluminium hydride on steroidal 20-ketones which are converted mainly to the 20β-hydroxy compounds by this reagent.[74]

Since physical data for several 18,20 diols were available it was possible to calculate the molecular rotation difference between the 20α and 20β diols in this series, Table IX (Page 102).

From the table it was apparent that both the diols 165 and 146 had the expected optical rotation of an 18,20β diol.

Another criterion on which a judgement of configuration at position 20 could have been possible was by the application of the so-called 'Sarett's rule'.[79] From their studies on the molecular rotational difference between several pairs of 20-acetates and the corresponding 20-hydroxy steroids, Sarett *et al.* found that acetylation changes the rotation more effectively towards the positive side in the case of 20β-hydroxy steroids. From Table IX, it could be calculated that M_D (diacetate - Diol 165) had a magnitude of + 270°. Unfortunately, Men did not report

TABLE IX

Comparison of Physical Properties of some 18-20 Diols.

Sl. No.	Compound	Configuration 3	Configuration 20	OH band.	M.P.	$[\alpha]_D$	Acetate MP	Acetate $[\alpha]_D$	Ref.
1.	Diol from paravallarine	NHMe	S		241-42	-47°	134°		
2.	Diol from methyl-paravallarine	NMe_2	S		228	-30°	114°		12
3.	Diol 165	NMe_2	R	3200	165	-63°	105.12°	+14	Present work
4.	Diol 146	NMe_2	R	3200	146	-62.3°	105.14°	+14	
5.	3β,18,20S-trihydroxy-5-pregnene	OH	S	3366	220-25	-14°	154°	-4	71
6.	3β,18-20 R-trihydroxy-5-pregnene	OH	R		241-43	-52°	131°	-13	81
7.	3β,18,20S-trihydroxy-5-α pregnane	OH	S		223-25	+35°	105-6°	+23	82, 71

M_D (5-6) = 126°

M_D (2-3) = 119°

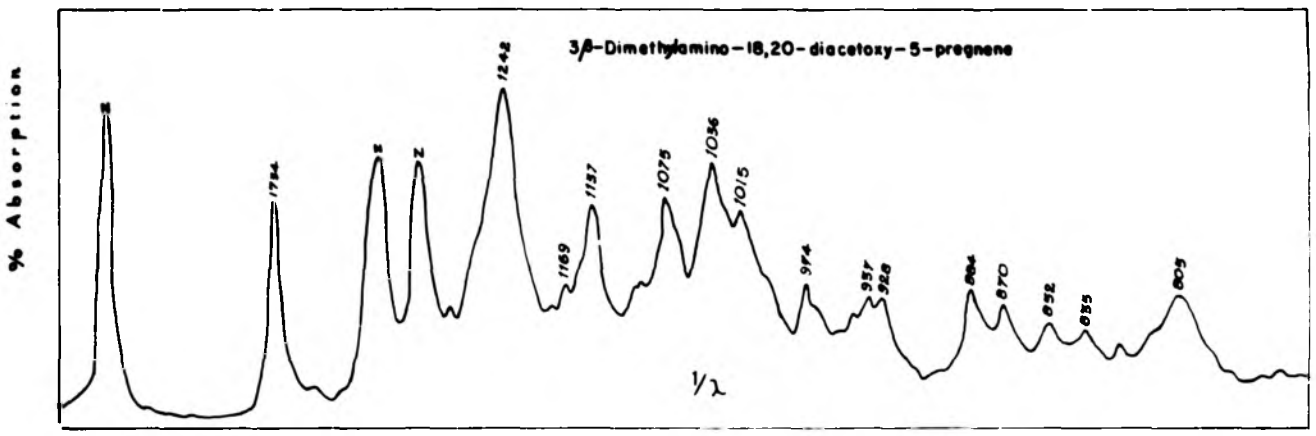
the rotation of his diacetate and hence no direct comparison could be made with the 20α series. It should also be apparent from Table (IX) that 'Sarett's rule' is not quite satisfactory as far as the sign of molecular rotation difference is concerned, that between some 20 acetates and the corresponding 20α hydroxy compounds being positive, although of a considerably reduced magnitude.

The diacetates obtained by acetylation of both the Diols 165 and 146 appeared to be identical in every respect including melting point, solubility and crystalline form. This led to the rather premature conclusion that Diol 165 and Diol 146 were just two crystalline forms of 18,20β dihydroxy 3β N-dimethyl-5-pregnene (XXI).

Some disturbing experimental observations were made which could not be accommodated with the idea of polymorphism.

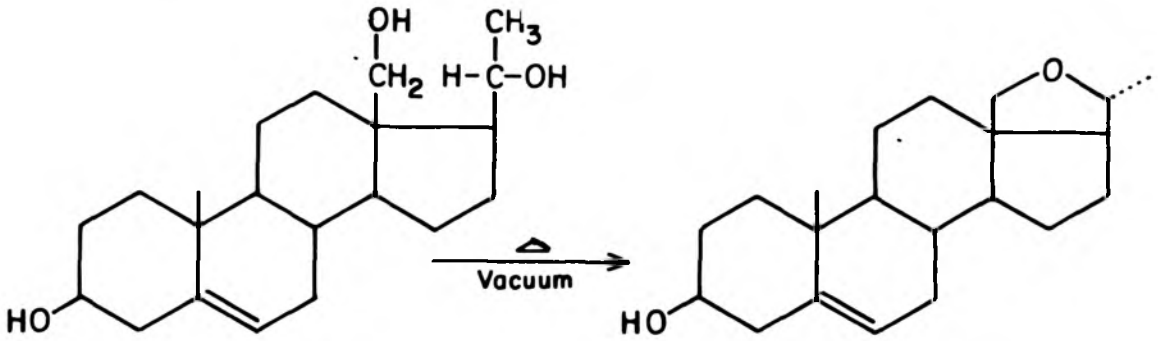
In the first place neither one of these diols could be converted into the other by repeated crystallisation from ethyl acetate. Unlike the higher melting form of 18-hydroxyprogesterone which was converted into the lower melting form by repeated crystallisation the Diol 165 retained its melting point and its distinctive crystalline form even when it was crystallised from its saturated solution after seeding with Diol 146.

In the second place, some striking differences wereobserved in their behaviour in pyrolysis.


3β-Dimethylamino-18,20-diacetoxy-5-pregnene
% Absorption
1734
1244
1169
1137
1075
1036
1015
974
957
928
884
870
852
835
809
1/λ

Pyrolysis of Diol 165

When preparing a sample of Diol 165 for analysis it was observed that the compound underwent slight discolouration with some loss in weight when the vacuum drying was performed at 110°. The dried product melted at a considerably lower temperature with a wide range (109-132°). The possibility had, therefore, to be considered that an elimination of water was taking place under the conditions of drying. This is not an unique behaviour in the 18,20-diol system, as it has been demonstrated by Bhattacharyya et al.[71] that 3β,18,20α-trihydroxy-5-pregnene (XXXI) prepared from holarrhimine yields 3β-hydroxy-18,20β oxido-5-pregnene (XXXII) with the elimination of water by heating under vacuum.

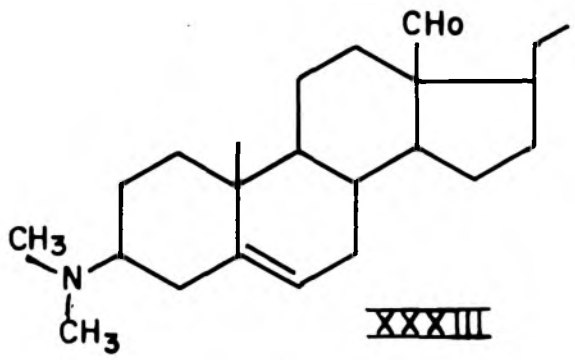


XXXI XXXII

The logical procedure was, therefore, to subject Diol 165 to sublimation under the vacuum of the aspirator pump at 130-140°. A crystalline sublimate was obtained melting at 90° which showed I.R. absorption characteristics

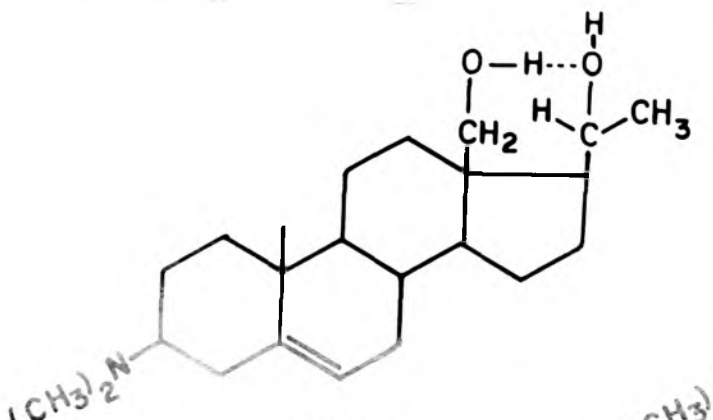
of an aldehyde (λ max. 1710 cm^{-1}, 2660 cm^{-1} (page 105a) rather unexpectedly instead of those of the expected ether. It is also apparent from the spectrum that the hydroxyl functions completely disappeared from the molecule.

The amount of sublimate obtained was, unfortunately, too small for a complete physico-chemical study on its structure. But taking all the structural features of the Diol 165 into account, Structure (XXXIII) for the sublimate suggests itself as a logical possibility.

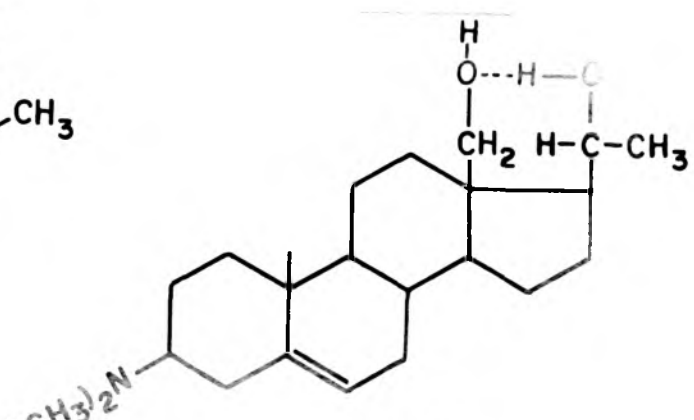


XXXIII

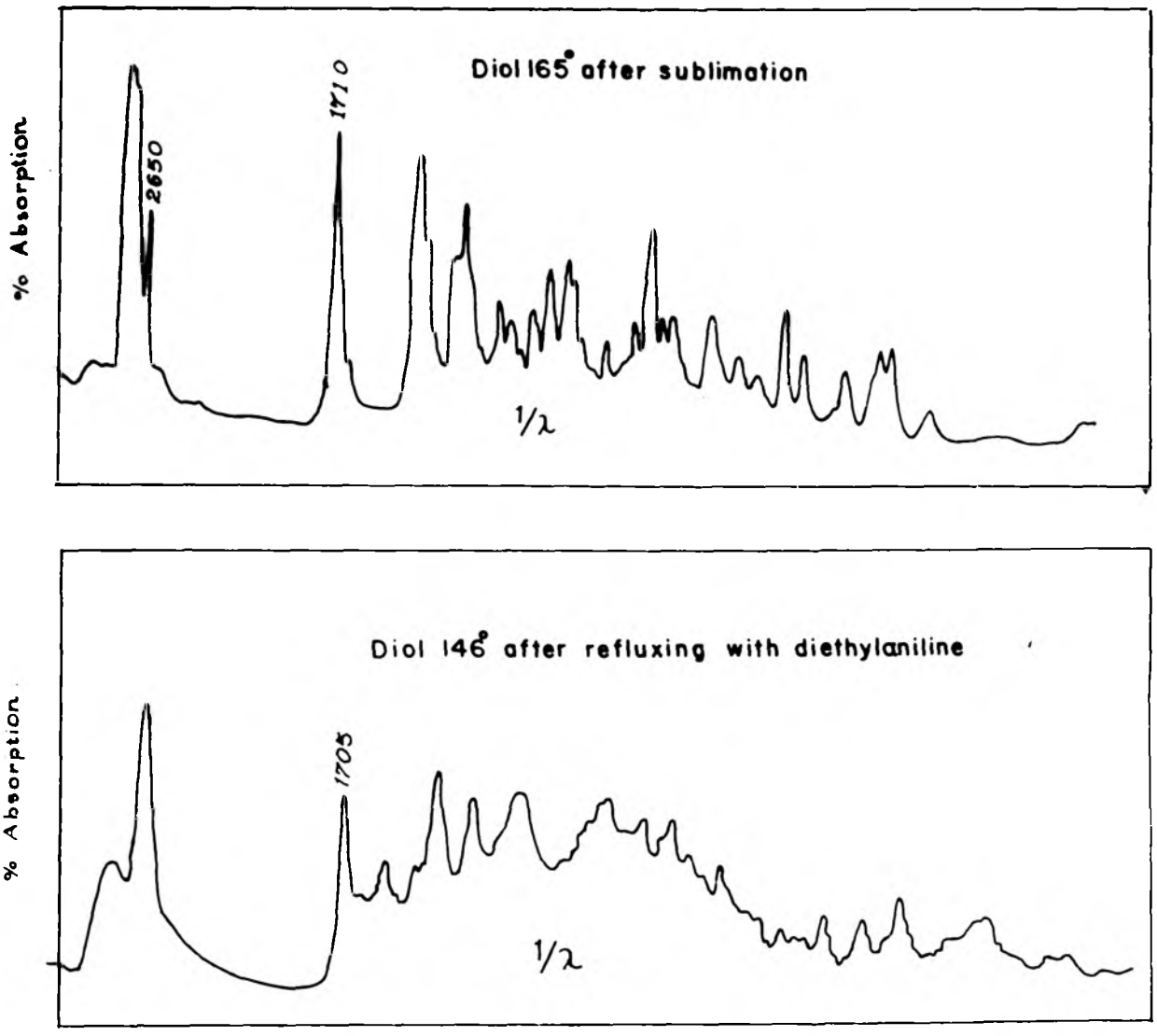
Taking for granted the structure (XXXIII) for the aldehyde, it is possible to speculate on some feasible mechanisms for its formation from Diol 165. As a working hypothesis this diol may be considered to exist in one (XXIa) of the two possible hydrogen bonded forms (XXIa and XXIb) in which the 18-hydroxyl group is bonded to the 20-oxygen.



XXI A

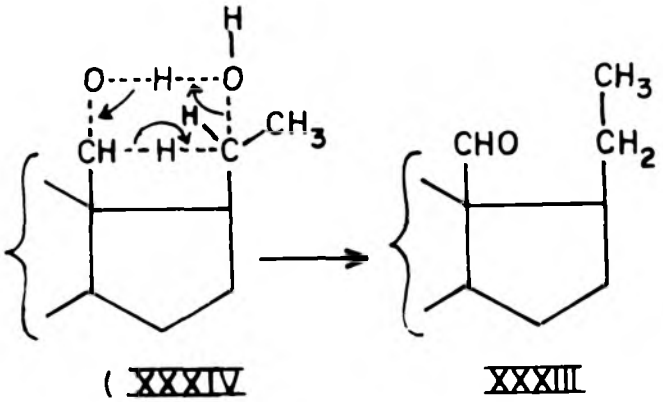


XXI B


Diol 165° after sublimation
% Absorption
2650
1710
1/2
Diol 146° after refluxing with diethylaniline
% Absorption
1705
1/2

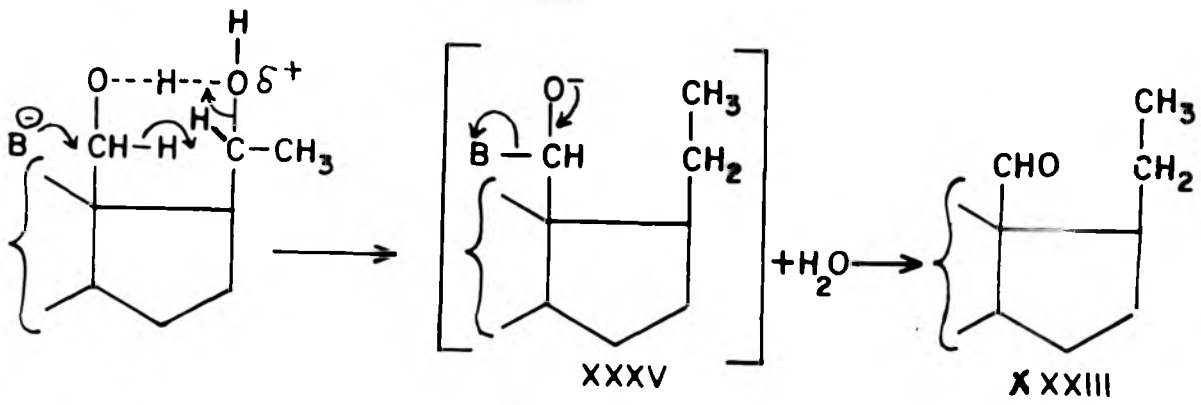
The electron deficiency on the oxygen at position 20 caused by hydrogen bonding would tend to delocalize the bond electrons of one of the C-H bonds at position 18 towards position 20 leading to a six-membered transition state (XXXIV).

A 1,4-hydride shift may then occur from the carbon 18 to carbon 20 by a purely thermal mechanism.



(XXXIV XXXIII

Alternatively, the above hydride transfer may be a base-catalysed reaction initiated by the electron sink at the oxygen on carbon 20.

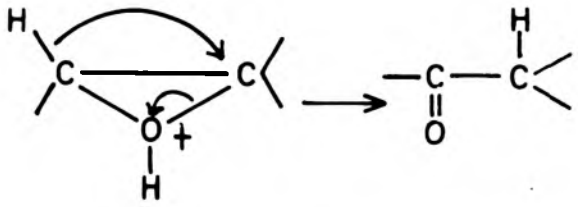


XXXV XXXIII

The transient tetrahedral intermediate (XXXV) would then form the pyramidal carbonyl function by expulsion of the base. The base in this reaction may be presumed to be the 3β-N-dimethyl group of a second molecule of the diol.

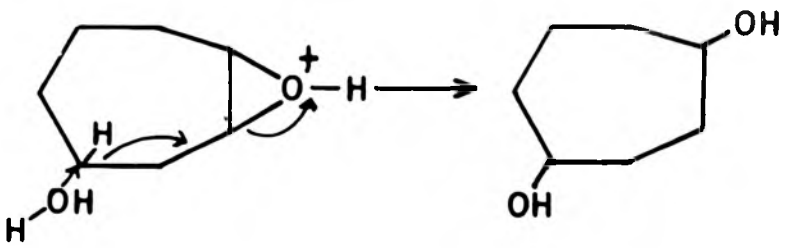
The driving force for the hydride shift is presumably contributed by the electron deficiency on the oxygen at carbon 20 and the release of steric strain at position 18 caused by the transition of a sp^3 tetrahedral carbon to a planar sp^2 state.

A literature search at this point revealed that 1,4-hydride shifts even if known, are rather rare. 1,2-hydride shifts, on the other hand, are extremely common, the proton catalysed rearrangement of 1,2-epoxides to carbonyl compounds being an example -

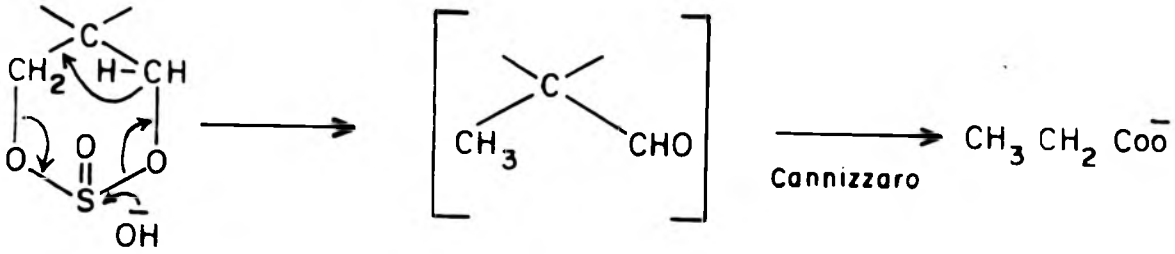


Stereochemical and conformational factors sometimes lead to 1,3-hydride shifts in the epoxide ring opening. Cope *et al.*[83] reported the formation

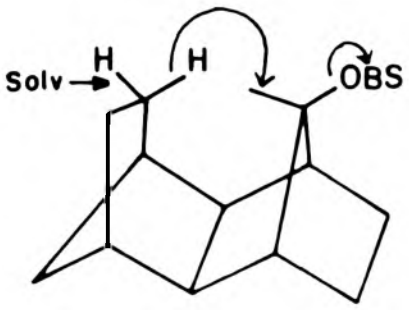
of cycloheptane 1,4-diols from cycloheptene oxide by such a mechanism.



1,3-hydride transfers are also known to occur in the cyclic sulfites of 1,3-glycols under strongly basic conditions.[84]

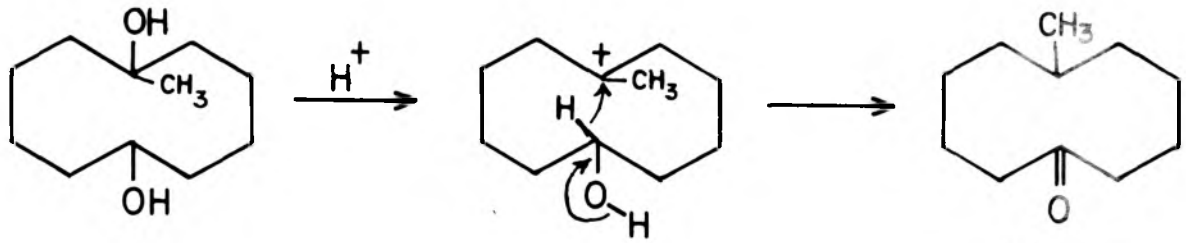


A case of 1,5-hydride transfer has been reported by Winstein and Hansel[85] in the solvolysis of the brosylate of decahydrodimethanonaphthalene (XXXVI).



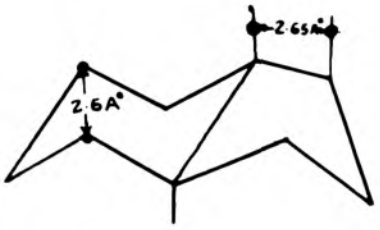
XXXVI

Transannular interactions in a methyl cyclodecane 1,6-diol (XXXVII) cause a 1,6-hydride shift in an acid catalysed rearrangement to a methyl cyclodecanone (XXXVIII).[86]



XXXVIII

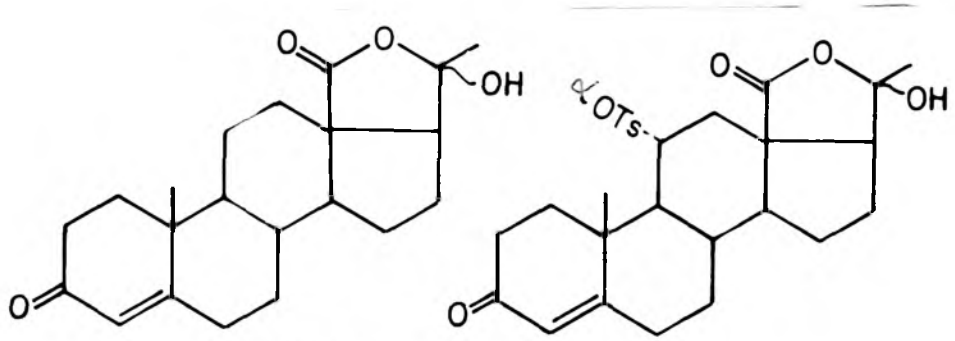
The proximity of the angular methyl position 18 and the side chain position 20 gives rise to some interesting steric interactions which are manifested in some 18,20-disubstituted steroids. The distance between the corresponding positions on a trans-fused hydrindane system (as measured on Barton's model) appears to be 2.65A° which is of the same order as that between the positions 1 and 3 in a cyclohexane ring in the chair conformation.



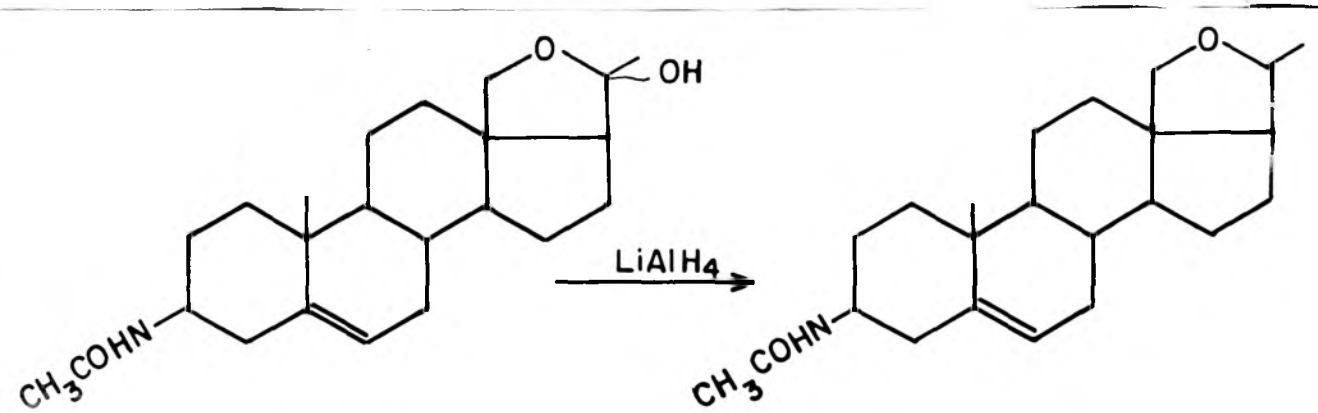
In the steroids an additional compression from the other angular methyl group 19 tends to bring these carbons even closer.

Some observations made in this laboratory as well as elsewhere illustrate these interactions.

It has been mentioned that 18-hydroxy-20-carbonyl systems with the exception of Compounds (XXA and XXB) exist almost exclusively in the 20-hemiketal form. Progesterone-18-carboxylic acid (XXXIX) and its 11α tosyloxy derivative (XL) are insoluble in bicarbonate and exist in 18-20 lactol form. On reduction with lithium aluminium hydride Compound (XXVII) yielded 3 β-acetamido-18,20α-oxido-5-pregnene (XLI) instead of the expected diol.[71]



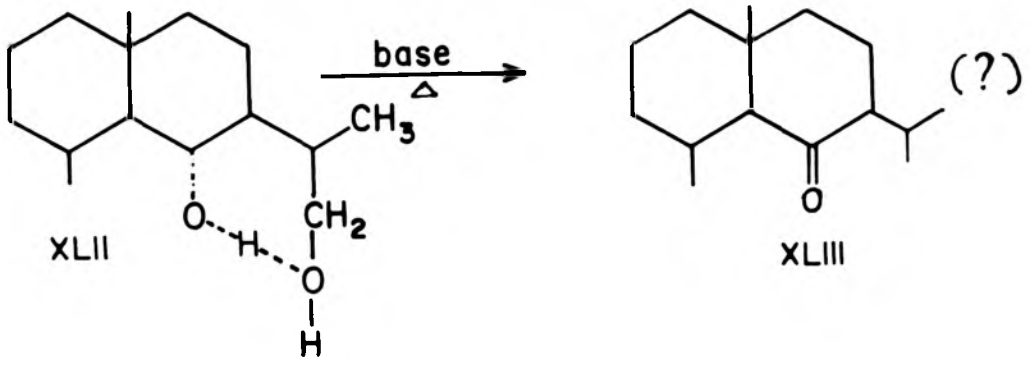
XXXIX XL



XXVII XLI

At least from a conformational standpoint a hydride transfer from position 18 to position 20 does not appear to be beyond the range of feasibility.

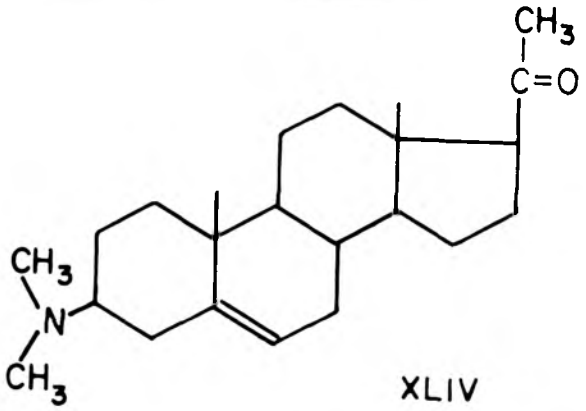
It was necessary to search for a model compound, preferably a hydrogen bonded 1,4-diol system where the stereochemical features were comparable with those of a steroidal 18,20β diol. Shaligram *et al.*[87] in this laboratory obtained a 1,4 diol in the selinane system (XLII) by lithium aluminium hydride reduction of santanolide 'C'. Compound(XLII) exhibited a strong intramolecular hydrogen bonding between the hydroxyl groups, (λ max. 3200 cm^{-1}). On vacuum sublimation the Diol XLII was recovered unchanged. On refluxing, however, with a high boiling base such as diethyl aniline, an impure product was obtained. This product had a new absorption band at 1705 cm^{-1}.



The 1,4-hydride transfer reaction in the Diol 165 is thus likely to be a base-catalysed or base-assisted process.

Pyrolysis of Diol 146

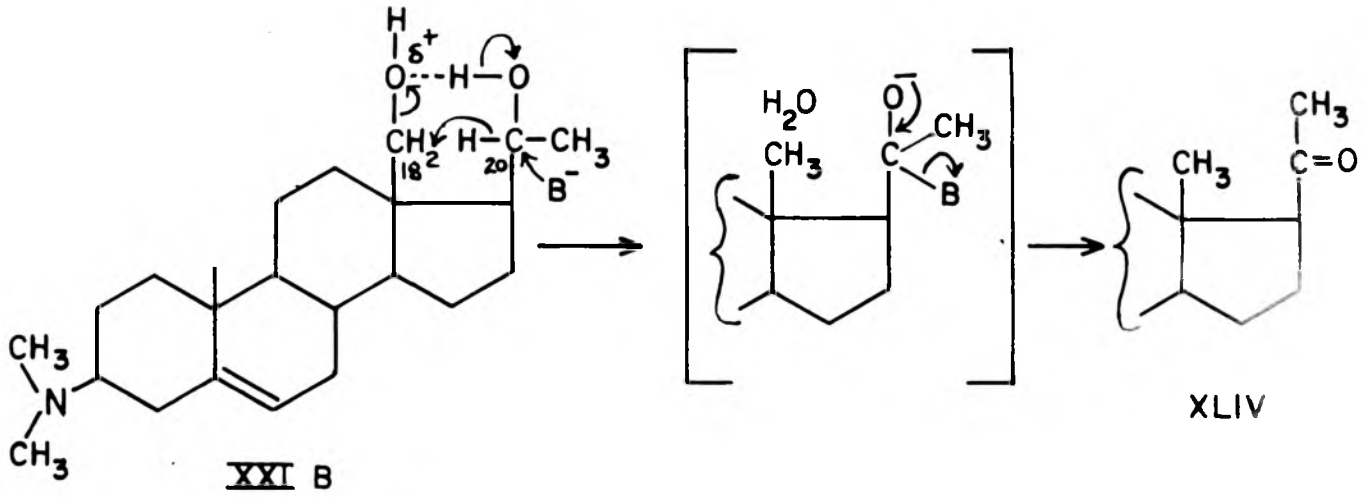
When Diol 146 was subjected to vacuum sublimation at 110-120° under the vacuum of the aspirator pump, it was noticed that for a steroidal diol containing three polar functional groups, the compound exhibited a surprising degree of volatility. Even at 100° it showed signs of sublimation. The sublimate from Diol 145 melted with a wide range (118-130°) and contained mostly the unchanged diol alongwith small amounts of a carbonyl compound having I.R. absorption at 1705 cm^{-1}. Somewhat better yields of the carbonyl compound were obtained by refluxing the diol overnight with diethyl aniline. The crude carbonyl compound was obtained in small quantities and no further studies were possible on its structure. But in the I.R. spectra it showed no bands at 2660 cm^{-1} (p. 105a) characteristic of the C-H of an aldehyde function. The I.R. spectra were not inconsistent with Structure (XLIV) for the product



XLIV

which is expected to be identical with holaphylline, an alkaloid isolated by from some African variety of kurchi.[88]

Following the same line of argument which led to the assignment of Structure XXIa for Diol 165, one may consider for Diol 146 the Structure XXIb in which the hydroxyl group at position 20 is bonded with the oxygen at position 18. The hydride transfer now may occur in the reverse direction from position 20 to position 18.



XXI B

XLIV

It may be argued that the contribution to the driving force for the hydride transfer due to the release of steric strain by the transformation of a sp^3 carbon to a sp^2 carbon would be minor significance in XXIb and consequently, the reaction is rather sluggish with Diol 146 as compared to that with Diol 165.

The assignment of structures XXIa and XXIb for the Diols 165 and 146 are, of course, based on the seemingly improbable hypothesis that a single hydrogen

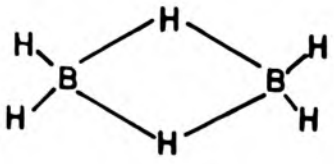
bond can give rise to two stable isomers. In case of stable hydrogen bonded systems such as proteins or nucleic acids, multiple hydrogen bonds impart the secondary, tertiary and quarternary structures, a certain degree of stability. Sometimes these structures, the integrity of which are, intimately connected with enzymatic and other specific biological properties are remarkably stable. Ribonuclease, for instance, is known to retain enzymatic activity even in 8 M urea solution - a concentration known to disrupt the hydrogen bonds in most other proteins.[89]

The energy of hydrogen bonds have been estimated to lie between 4-9 Kcal.[90] These calculations are in many cases based on the shifts in the stretching frequencies in the I.R. spectra (Δ Vs).

Even if the maximum energy for a hydrogen bond is assigned to that between the oxygens at positions 18 and 20, there remains an energy gap of 20 to 25 Kcal. which has to be met before the isomers can acquire the observed stability. Conformational features such as non-bonded interactions and steric compression, which would unquestionably account for a part of the energy deficit has to be stretched too far, if they were to account for the entire energy gap.

On the other hand, as far as the investigator is aware, no reliable direct methods exist for the calculation of enthalpy or free energy of the intramolecular hydrogen bond. Furthermore, from the data of Badger and Bauer[91] it appears that ΔH and ΔVs do not exhibit a simple linear relationship and at higher ΔVs small changes in ΔVs are associated with large changes in H.

As an extreme example of stability of hydrogen bridged compounds, the case of diborane may be taken into account although boron being electron deficient and approximately of the same order of electronegativity as that of hydrogen[92] does not form hydrogen bonds in the strictest sense of the term. But the monomeric borane BH_3, dimerises to B_2H_6 so completely that BH_3 has never been detected spectroscopically. The association equilibrium of $2BH_3 \rightleftharpoons B_2H_6$ has been estimated to have $-\Delta H^{o} = +28$ Kcal.[93] The structure of diborane is shown to have a bridge configuration in which two hydrogen atoms occupy unique positions equidistant from two boron atoms in the form of the so-called "three-cornered bonds".



Shapiro *et al.*[94] found that the frequency of absorption of the bridging hydrogens is shifted by 1000 cm^{-1} from the normal frequency range of B-H of 2500-2600 cm^{-1}.

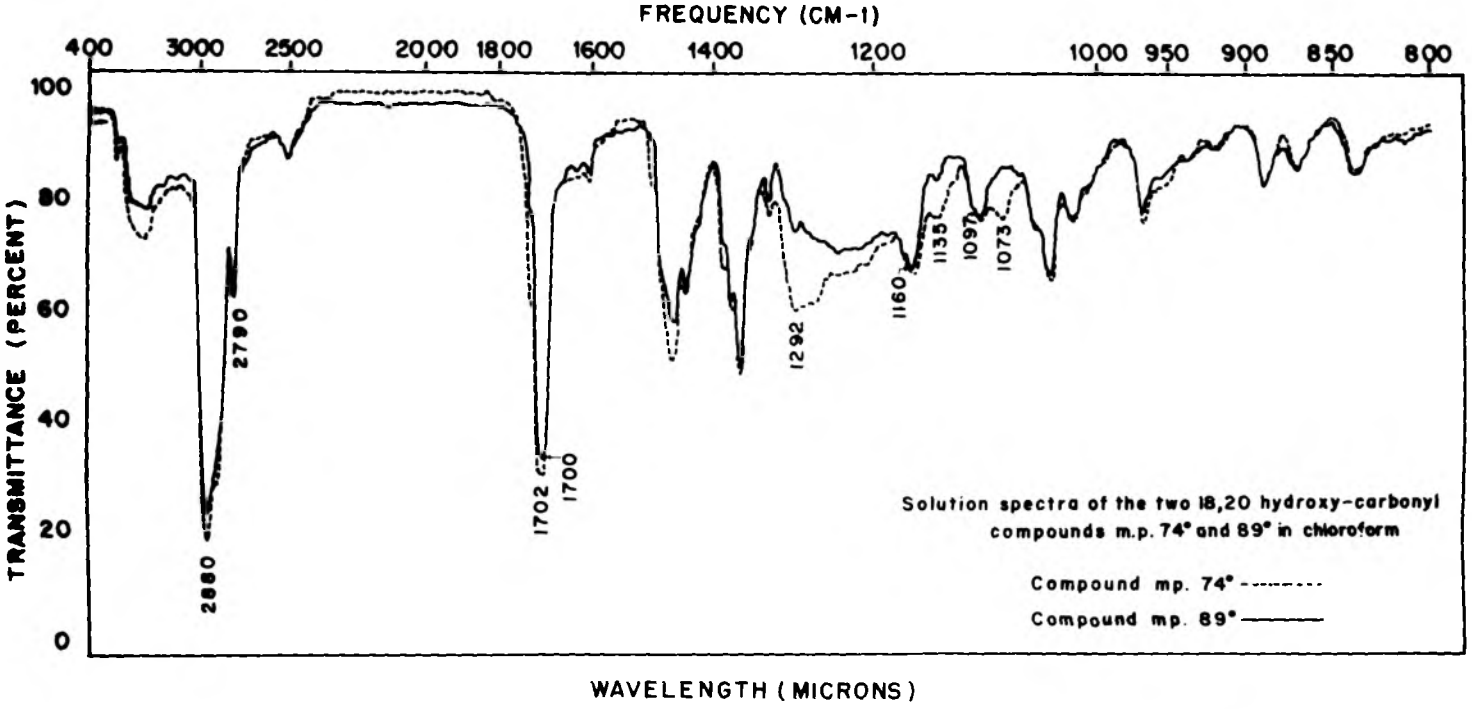
It is, unfortunately, not possible to extend the analogy of diborane too far in this case.

Assuming, however, that hydrogen bonded isomers can uniquely exist in Compound XXI, it becomes necessary to speculate on how the Diols, 165 and 146, could arise from the two steroids, XXA and XXB, respectively and exclusively, without forming mixtures.

In this connection, the structures of the Schiff's base (XVIII) and its nitrous acid reaction products, particularly Compounds XXA and XXB, were subjected to a re-examination to determine whether some inherent distinctive features of these compounds might have been overlooked. From the data, it was clear that as far as the Schiff's base (XVIII) was concerned, the structure seemed to be firmly established.

About the structures XXA and XXB, the logical possibility suggested itself that if XXA is 18-hydroxy-20-oxo-compound, XXB may be a 20-hydroxy-18 aldo compound.

A solution I.R. spectra of both these compounds were obtained in chloroform and carbon tetrachloride. The essential features of the spectra were similar in both of


FREQUENCY (CM-1)
400
3000
2500
2000
1800
1600
1400
1200
1000
950
900
850
800
100
80
60
40
20
0
TRANSMITTANCE (PERCENT)
2880
2790
1702
1700
1292
1160
1135
1097
1073
Solution spectra of the two 18,20 hydroxy-carbonyl compounds m.p. 74° and 89° in chloroform
Compound mp. 74°
Compound mp. 89°
WAVELENGTH (MICRONS)

these solvents and the spectra in chloroform have been reproduced (page 117a). The broken lines represent the spectra of Compound XXA (m.p. 74°) and the solid lines that of the Compound XXB (m.p. 89°).

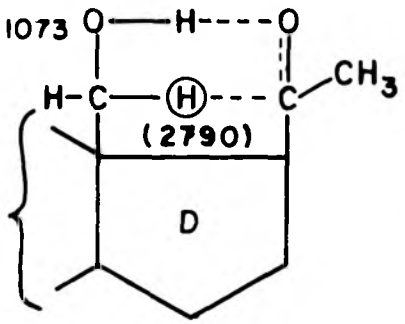
Both showed the characteristics of a bonded hydroxy carbonyl system (diffuse hydroxy peaks at 3400 cm^{-1} and C=O peaks at 1702, 1700 cm^{-1} respectively) indicating that in solution these compounds exist mostly in the bonded form XXA or XXB. Both showed a sharp band at 2790 cm^{-1}.

As a distinctive feature Compound XXA shows a band at 1073 cm^{-1} and an intensification of bands at 1135 and 1290 cm^{-1}. The assignment of the band at 1073 cm^{-1} for the C-O bending frequency of the bonded primary hydroxyl group at position 18 does not appear to be unjustified. In Compound XXB the sharpened absorption bands at 1097 and 1160 cm^{-1} may perhaps be assigned to the bonded secondary hydroxyl at position 20.

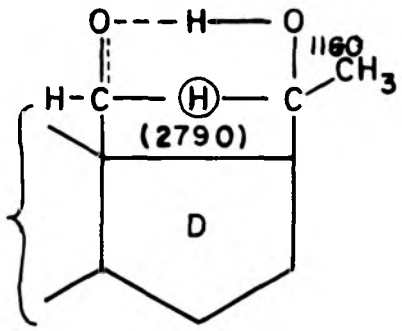
The band at 2790 cm^{-1} may be due to the stretching of the C-H bonds at position 18 (circled) in both the compounds. In Compound XXA a delocalisation of bond electrons due to hydrogen bonding may be responsible for a shift of this C-H stretching absorption to a lower frequency. In Compound XXB the normal C-H stretching frequency of an aldehyde at 2650-2720 cm^{-1} may have been

shifted in the reverse direction. Mention may be made in this connection of the observation of Pinchas[95] that in the spectra of ortho-substituted benzaldehydes the characteristic C-H stretching absorptions of the aldehyde are shifted to higher frequencies.

It could be just a matter of coincidence that the C-H stretching vibrations of an alcoholic group in Compound XXA and that of an aldehyde group in Compound XXB appear at an identical frequency of 2790 cm^{-1}.



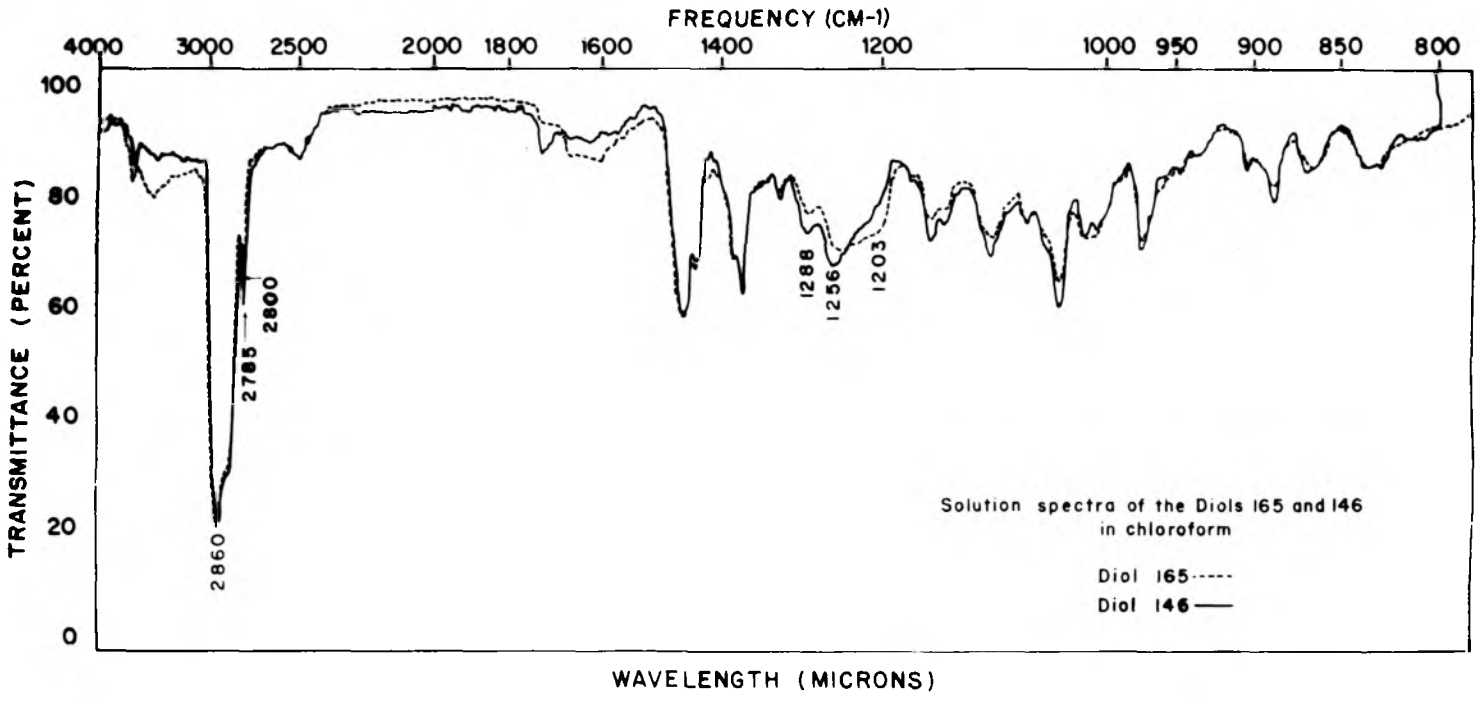
XX A



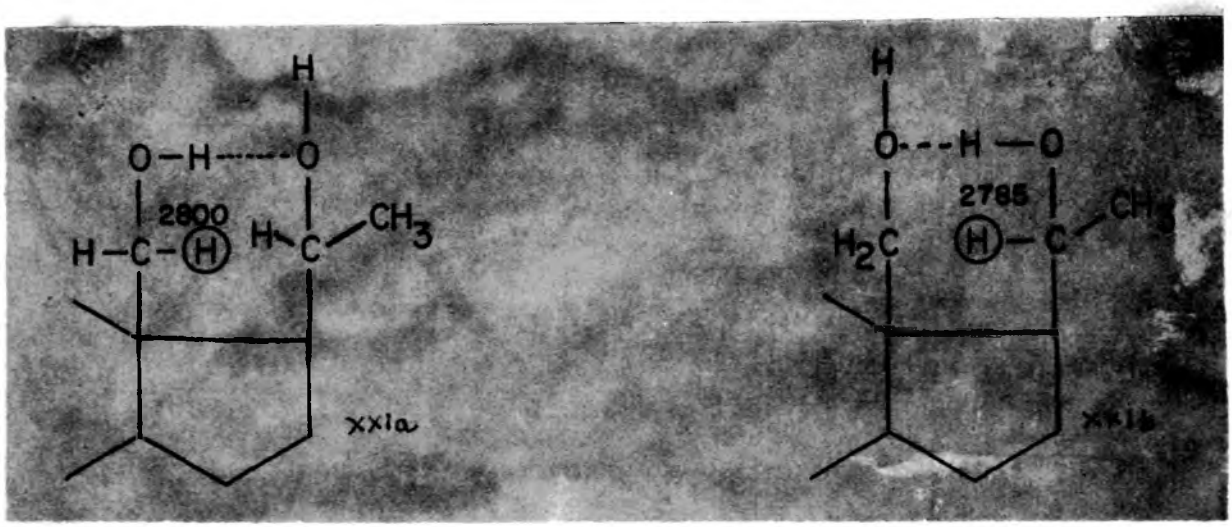
XX B

The formation of Compound XXB may be accounted for by a hydride transfer during the course of the nitrous acid reaction.

The solution spectra of the Diol 165 (broken line) and the Diol 146 (solid line) in chloroform (page 118a) show that these compounds are more closely related than the parent compounds XXA and XXB. It is interesting to observe that the hydroxyl bands have completely disappeared and probably shifted to coincide with the CH_2 (shoulder


FREQUENCY (CM-1)
4000
3000
2500
2000
1800
1600
1400
1200
1000
950
900
850
800
100
80
60
40
20
0
TRANSMITTANCE (PERCENT)
2860
2785
2800
1288
1256
1203
Solution spectra of the Diols 165 and 146
in chloroform
Diol 165-----
Diol 146——
WAVELENGTH (MICRONS)

at 2860 cm^{-1}) vibration in the 3000 cm^{-1} region. Diols 165 and 146 had sharp peaks at 2800 and 2785 cm^{-1} which may be due to the C-H of the hydrogens (circled) at positions 18 and 20 involved in the hydride transfer.

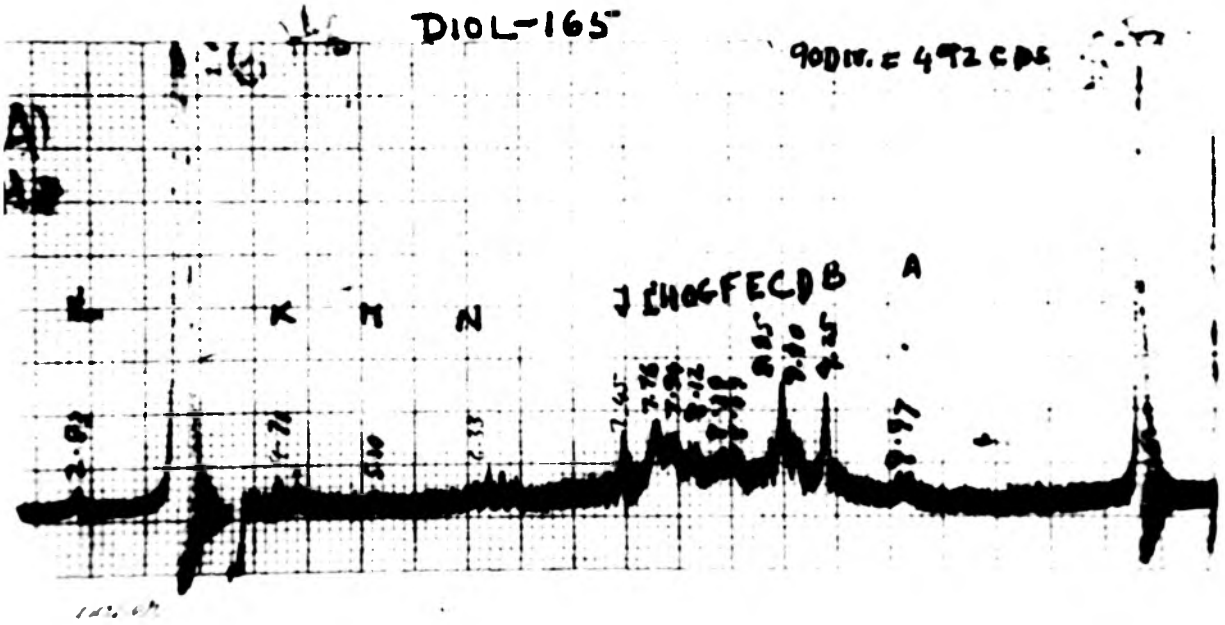


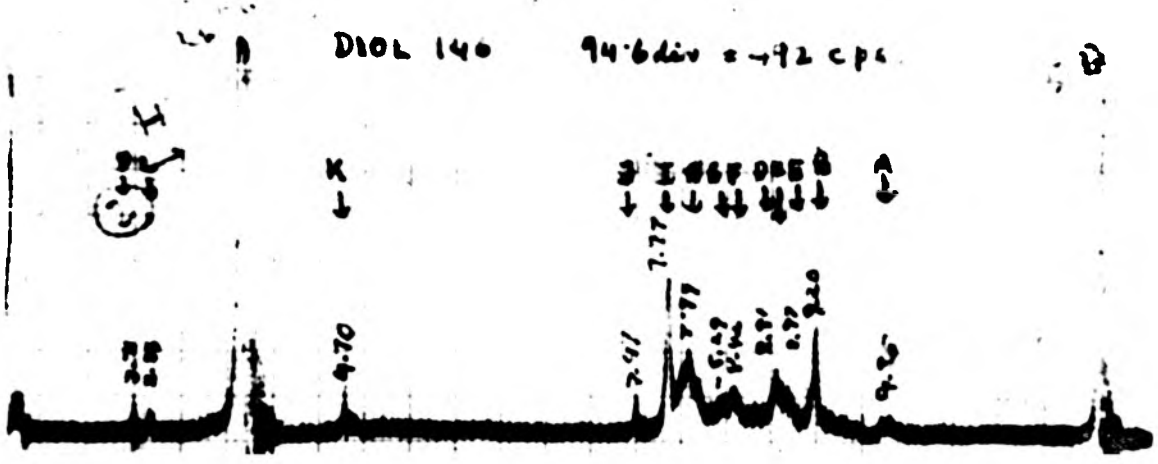
Some minor differences in the relative intensities of the peaks in the 1200-1300 cm^{-1} region can also be observed. From a comparison of the solution spectra of Diols 165 and 146 with those of XXA and XXB it appears that the hydrogen bonding is much stronger in the case of the diols than that in the hydroxy-carbonyl systems. This anomaly is due to the fact that steric factors favour the formation of hydrogen bridge in the case of the former compounds in which the oxygens are located on tetrahedral carbons, than in the

case of the latter where the planarity of the carbonyl carbons make the stereochemistry less favourable for bridge formation, inspite of the increased electronegativity of the carbonyl oxygen.

Of all the 18-hydroxy-20-oxo- steroids studied in this laboratory,[71] only the Compound XX exhibited hydrogen bonding in the I.R. spectra. This may be due to an intermolecular attack of the nucleophilic 3β N-dimethyl group on the electron-deficient carbonyl carbon thereby modifying the planar sp^2 character to a certain extent and rendering the stereochemistry more favourable for hydrogen bonding (p. 121)

Diols 165 and 146 which show almost identical I.R. spectra exhibit a surprising degree of difference in their n.m.r. spectra (page 120a). The spectra were taken in $CDCl_3$ with benzene as the reference compound on a Model HR 60 Spectrometer of the Varian Associates Ltd. at 60 mcs. The spectra were of poor quality and no attempts have been made to analyse them in detail. However, among the common features of both the spectra are the bands (approx. τ values in parenthesis), B(9.2), C(8.81), composite bands D-E (8.79 to 9.10), F-G(8.30-8.50), H, (7.94), I (7.78), J(7.47), K(4.71) and L(2.83) which may perhaps tentatively be assigned to the protons of 21-methyl, 18-methyl, ring methylenes, tertiary hydrogens


DIOL-165
90 DIV. = 492 CPS
K
M
N
JIHGFEDCB
A

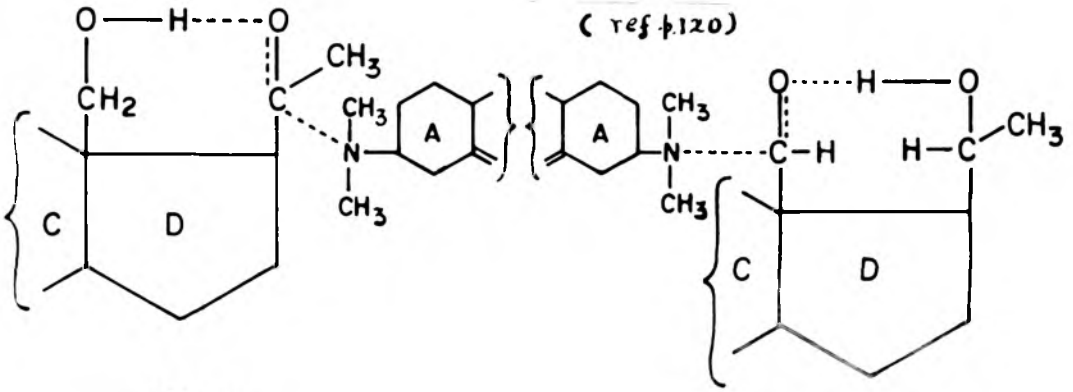

DIOL 140
94·6 div = 492 cps
K
A

at positions 8, 9, 14 and 17, the methylene hydrogens at C_{18} (carrying oxygen), 3α-hydrogen adjacent to nitrogen, the vinyl hydrogen at position 6 and to the traces of chloroform present as an impurity in $CDCl_3$, respectively. Both the spectra show a band at a high τ region (9.8-9.9).

Besides these common features, the spectra of Diol 165 show three additional bands M (5.30), N(6.33 a quadruplet J-7.50 cps.) and O (8.12) which are absent from the spectra of Diol 146 which show a new band P (2.71) and intensification of bands I and H.

With the limited data available it is, unfortunately, not possible to make assignments for all the observed bands. Introduction of deuterium at positions 20 and 18 by reduction of the parent hydroxyl carbonyl compounds (XXA) and (XXB) with lithium aluminium deuteride may be of assistance in the assignment of the bands due to the protons in the 18-20 region.

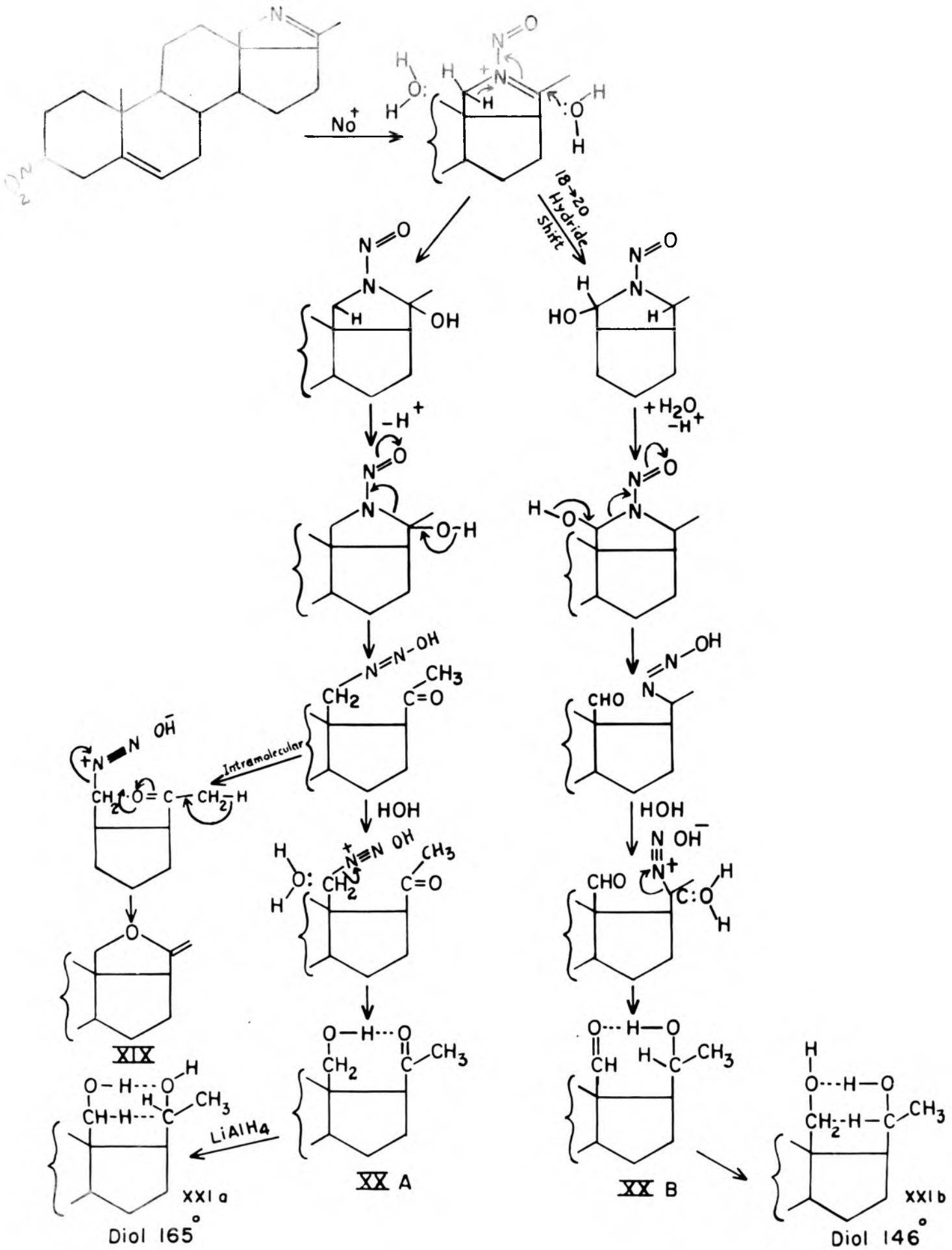
Accepting the postulate of hydrogen-bonded isomers, it is possible to formulate the entire sequence of reactions starting from the Schiff's base (XVIII), (Plate 6).



XX A

XX B

Plate 6


No+
18→20 Hydride Shift
-H+
+H2O -H+
Intramolecular
HOH
LiAlH4
XIX
XX A
XX B
XXI a
XXI b
Diol 165°
Diol 146°

The investigator is aware that although the above speculations lead to a consistent hypothesis for explaining the observed experimental facts, they do not necessarily constitute acceptable proof for the existence of hydrogen bonded isomers. The energy gap appears to be far too great. Alternative formulations for the Diols 165 and 146, on the other hand, such as a 20-21 diol, or a 17-20 diol, do not fit in with the optical rotation and are not likely to form as strong a hydrogeh bonded system as that in a seven-membered ring where the O-H-O system can have a linear configuration.[89]

Judging from the differences in the n.m.r.spectra of the two diols, it will relevant to consider whether one of the hydroxycarbonyl compounds, XX (and consequently one of the diols) could be formed by a deep-seated rearrangement of the homo-steroid type caused by a shift of the 13-17 bond or the 16-17 bond during the nitrous acid reaction. In fact, such possibilities cannot be ruled out completely from the data. However, the physical data, and the similarly in the I.R. spectra of the diols especially in the finger-print region seem to contraindicate the possibility that the basic skeleton in one case has undergone a drastic rearrangement. If it is argued that both the compounds (XXA) and (XXB) are ring-enlargement products, one has still to settle the question of hydrogen-bonded isomeric diols leading to the same diacetate. Some more work would be needed to settle this point. With the data available the investigator cannot offer any other explanation which would fit in with the observed facts.

EXPERIMENTAL

Transformations of Conessimine

N-Chlorination of Conessimine (III)

To 200 mg. of conessimine (III) (0.59 mole) in 50 ml. of dry methylene chloride was added 80 mg. of N-chlorosuccinimide (0.60 m mole) in small lots with stirring in the course of 10-12 minutes. Stirring was continued for a further period of 20 minutes. The methylene chloride solution was washed thoroughly with water to remove succinimide formed during the reaction and any unreacted N-chlorosuccinimide. The solvent layer responded to starch-iodide test characteristic of a N-chloro compound. After drying the solution over anhydrous sodium sulphate it was evaporated carefully *in vacuo* keeping the temperature of the bath below 35°. After evaporation of the solvent a crystalline solid separated in the flask (217 mg.). It was recrystallised from ethylacetate.

Physical constants

Melting point 135° (decomposed)

Optical rotation $[\alpha]_D$ + 30° (C = 0.018)

Analysis

	C.	H.	N.	Cl.
$C_{23}H_{37}N_2Cl$ requires	73.40	9.84	7.44	9.31%
Found:	73.20	9.76	7.40	9.40%

Infrared absorption spectra

ν_{λ} max. 735 cm^{-1} (N-Cl)

The physical data was consistent with the structure (XVII).

Dehydrochlorination of N-chlorocconessimine

To 200 mg. of the N-chlorocompound (XVII) was added freshly prepared sodium-ethoxide (1 g. of sodium/ 50 ml. of ethanol) and the mixture refluxed for 30 minutes. The solution lost its property to respond to the starch-iodide test indicating the completion of the reaction. During refluxing sodium chloride separated out in the flask. At the end of the reaction ca 50 ml. of water was added. The colourless precipitate was extracted out with chloroform and the chloroform extract washed free from alkali and dried over anhydrous sodium sulphate. On evaporation the chloroform extract yielded a crystalline material (195 mg.). It was recrystallised from ethylacetate.

Physical constants

Melting point 146°

Optical rotation $[\alpha]_D$ = 20.2° (ethanol C=0.019).

Analysis

		C.	H.	N.	N-CH_3
$C_{23}H_{35}N_2$	requires	81.17	10.60	8.23	8.80 %
	Found:	81.12	10.48	8.11	8.65 %

Infrared absorption spectrum

$1/\lambda$ max. 1647 cm^{-1} (N=C).

Estimation of Nitrogen by the Van Slyke Procedure

By employing the usual nitrite-acetic acid procedure of Van Slyke for amino-N determination it was established that the Schiff's base gave off one atom of nitrogen (Amino nitrogen - Found: 4.12% calcd. for one amino group - 4.2 %).

Attempted acid-hydrolysis of the Schiff's base

To 100 mg. of the Schiff's base was added 20 ml. of 2 N sulphuric acid and the solution heated on a water bath for 24 hours. The reaction mixture was made alkaline and the precipitated base recovered in chloroform. On working up the chloroform extract the crude Schiff's base was obtained (93 mg.). It was crystallised from ethyl-acetate, m.p. 145-46°. Mixed m.p. with the starting material showed no depression (145-46°).

Benzoyl derivative of the Schiff's base (XVI)

To 73 mg. of Schiff's base suspension in 20 ml. of 5% sodium hydroxide was added 0.4 ml. of benzoyl-chloride with stirring. After stirring for 30 minutes more of benzoyl chloride (0.75 ml.) was added in three lots at 15-minute intervals. Stirring was continued for an hour. The gummy precipitate which settled at the bottom was recovered in ether.

The step consisting of an addition of pyridine and hydrochloric acid in the procedure of Cerny and Sorm was omitted.

The ether extract yielded a gummy material (43 mg.) on evaporation. The material could not be crystallised even after column chromatography over alumina.

Infrared absorption spectrum of the derivative
(Spectrum not reproduced)

The infrared spectrum of the gummy material showed the following characteristics:

1. Original band at 1647 cm^{-1} for N=C absent.
2. Band at 1528 cm^{-1} for NH at position 18.
3. Band at 1652 cm^{-1} for C=O at position 18.
4. Band at 1695 cm^{-1} for C=O at position 20.
5. Band at 1360 cm^{-1} for CH_2 at position 21.

Conversion of the Schiff's base (XVIII) to the 18-20 Schiff's base of 18-amino-3,20-dioxo-4-pregnene (VIII)

The Schiff's base (XVIII) (500 mg.) was treated with 300 mg. of cyanogen bromide in benzene in the manner described for conimine (page **66**). Attempts to crystallise the neutral dicyano compound recovered in chloroform did not succeed. Hydrolysis of the dicyano compound was accomplished by refluxing it in 20 ml. methanol containing 500 mg. KOH for 24 hours. The reaction mixture was diluted

with 100 ml. water and the precipitated base was recovered in chloroform. The gum (207 mg.) obtained by working up of the chloroform extract failed to crystallise even after a column chromatography over alumina. The purified product (108 mg.) analysed for 3.97% N-methyl and exhibited I.R. absorption bands at 3280 cm^{-1}, 1648 cm^{-1} indicating that a partial demethylation has taken place at the 3 nitrogen position.

The gummy material was subjected to N-chlorination in the usual manner with 45 mg. of N-chlorosuccinimide in 25 ml. of methylene chloride. The N-chloro compound obtained after evaporation of the solvent was refluxed with 10 ml. of anhydrous alcohol in which 60 mg. of sodium had been dissolved. After the completion of the dehydrochlorination reaction, the mixture was diluted with water and extracted with chloroform. The chloroform extract was washed, dried and evaporated and the gummy residue was hydrolysed on a water bath for two hours with 25 ml. of 2 N sulphuric acid. The crude Schiff's base (VIII) was liberated on treatment with excess sodium hydroxide and extracted with chloroform. After purification through the perchlorate method (page ***70***) the Schiff's base was obtained in a purified form and recrystallised from ethyl acetate (48 mg. m.p. 180-81°). The identity of the product was established by a comparison of its physico-chemical properties, comparative I.R. spectra and a mixed melting point determination with the 18-20 Schiff's base of

18 amino-3,20-dioxo-4-pregnene (VIII) obtained from conessine.

Action of Nitrous Acid on the Schiff's Base (XVIII)

To 600 mg. of Compound (XVIII) in 50 ml. of 25% acetic acid, 500 mg. of sodium nitrite in 5 ml. of water were added. The mixture was stirred for 5 minutes after which the reaction was arrested by adding 5 N sodium hydroxide. A colourless precipitate separated out. It was extracted with chloroform and worked up in the usual manner to yield a gummy material (585 mg.). It was kept in ethylacetate at 5° for 12-14 hours, when a crystalline mass (XIX) separated out as platelets (134 mg., m.p. 180°). On evaporation of the mother liquor to three-fourth s of its original volume and storage at 0° for 24 hours, another crop (14 mg.) of Compound (XIX) separated out, m.p. 179-180°. The mother liquor from (XIX) was evaporated to half its original volume and maintained at room temperature for 8-10 hours when clusters of needles separated out (150 mg., m.p.73°). When the mother liquors were concentrated further, two types of crystals separated out in the flask simultaneously. Because of the difference in the appearances of two products, one as platelets and the other as needles, they could be separated mechanically.

In a subsequent experiment the mother liquors from Compound (XIX) were subjected to column chromatography over alumina (basic, grade II). The results are presented in Table X.

TABLE X

Chromatography of the nitrous-acid reaction products of Compound XVIII after separation of Compound XIX

Fr.No.	Volume of the solvent	Weight (mg)	Nature of the product after evaporation
1	25 ml.	23	gummy
2	"	38	"
3	"	21	"
4	"	13	"
5	"	10	"
6	"	9	"
7	"	6	"
8	"	12	"
9	"	10	"
10	"	10	"
11	"	10	"
12	"	49	"
13	"	8	"
14	"	1	"
15	"	-	-

Recovery 220 mg.

Fractions 2, 3, 4 and 5 were pooled and crystallised from ethyl acetate (XXB), m.p. 89°.

Fractions 9, 10, 11, 12 and 13 were pooled and crystallised from ethylacetate (XXA), m.p. 74°.

Characterisation of Compound (XIX)

Physical constants

Melting point 180°

Optical rotation $[\alpha]_D$ = - 8.25° (C = 0.018).

Analysis	C.	H.	N.	N-CH_3	Active H
$C_{23}H_{35}NO$ requires	80.94	10.26	4.10	8.80	- %
Found:	80.46	10.30	3.92	8.55	nil %

Infrared absorption spectra

$1/\lambda$ max. 892 cm^{-1} for C = CH_2; and the band at 1647 cm^{-1} for N = C was absent.

Chemical Studies

(i) Ozonolysis

A dry stream of ozone was passed through a solution of 100 mg. of the Compound (XIX) in 15 ml. of dry ethylacetate at -20° for 1½ hours. The issuing gases were passed through a cold water trap. At the end of ozonolysis, the cold water in the trap was treated with a saturated solution of dimedone in water. On warming the solution on a water bath the dimedone derivative of formaldehyde was obtained which was crystallised from aqueous ethanol; m.p. 189°. Mixed m.p. with dimedone derivative of formaldehyde - 188-89°.

The ozonolysis mixture was evaporated *in vacuo* at 35° to a gum and decomposed by treatment with 10-15 ml. of water for 3 hours in a current of nitrogen and the issuing gases were bubbled through a cold water trap. No further volatile carbonyl compounds were detected in the cold water trap. The residue was extracted with chloroform and the chloroform extract on evaporation yielded 44 mg. of a gummy product. The I.R. spectrum of the product showed a broad peak in the carbonyl region which was resolved with calcium chloride prism into a sharp band at 1770 cm^{-1} (γ-lactone).

Hydrolysis of Compound XIX

An aliquot of 100 mg. of Compound (XIX) was taken in 10 ml. of 10 N sulphuric acid, heated on a steam-bath for 3 hours and left at room temperature overnight. The reaction mixture was then diluted with water, basified and extracted with chloroform. The chloroform extract was worked up in the usual manner to yield 92 mg. of a colourless gum. It was crystallised from ethylacetate.

The first crop (26 mg., m.p. 178-79°) was identified as the unreacted starting material. The mother liquor after filtration through a short column of alumina yielded 61 mg. of a crystalline residue which

was recrystallised from ethyl acetate to yield a mixture of crystals of (XXA) and (XXB) (m.p. 74° and 89°).

Characterisation of Compound XXA

Physical constants

Melting point 74°

Optical rotation $[\alpha]_D$ - 46.3° (C = 0.020).

Analysis	C.	H.	N.	N-CH_3
$C_{23}H_{37}NO_2$ requires	76.88	10.31	3.90	8.85 %
Found:	76.41	10.19	3.78	8.43 %

Infrared absorption spectrum

Solid state (nujol mull)

$1/\lambda$ max. 3575 and 3200 cm^{-1} (for OH)

1689 cm^{-1} (C=O) (page 96a)

Solution (chloroform) taken on a Perkin Elmer Model 221 Spectrometer. (page 118a)

$1/\lambda$ max. 3400 cm^{-1}, 2790, 1702 cm^{-1}.

The physical data were consistent with the Structure XXA for the compound.

Characterisation of Compound XXB

Physical constants

Melting point 88°

Optical rotation $[\alpha]_D$ + 47° (C = 0.018).

Analysis	C.	H.	N.	N-CH_3
$C_{23}H_{37}NO_2$ requires	76.88	10.31	3.9	8.80 %
Found:	76.49	10.28	3.85	8.42 %

Infrared absorption spectra

Solid state (nujol mull)

$1/\lambda$ max. 3575 cm^{-1} and 3200 cm^{-1}, 1691 cm^{-1} (C=O). (p 96a)

Solution (chloroform) taken on a Perkin Elmer Model 221 Spectrophotometer. (p. 118a)

$1/\lambda$ max. 3400 cm^{-1}, 2790 cm^{-1}, 1700 cm^{-1}, 1290 cm^{-1}, 1073 cm^{-1}.

Reduction of Compound XXA with Lithium Alumium Hydride

To a slurry of lithium aluminium hydride (160 mg.) in dry ether (50 ml.) at 0° a solution of Compound(XXA) (100 mg.) in 10 ml. ether was added dropwise with stirring. After one hour at 0°, the solution was maintained at room temperature for another hour and then heated at reflux temperature of ether for 2 hours. At the end of the reaction, excess $LiAlH_4$ was slowly decomposed first with moist ether and then water. After removal of the ether layer, the aqueous phase was extracted thrice with ether and the ether extracts combined. The ether extract after drying was evaporated to a semicrystalline mass (98 mg.). It was crystallised from ethyl acetate after filtration through a short column of alumina.

Physical constants

Molecular formula $C_{23}H_{39}NO_2$

Melting point 165°

Optical rotation $[\alpha]_D$ - 63° (C = 0.02).

Analysis

	C.	H.	N.	
$C_{23}H_{39}NO_2$ requires	76.45	10.8	3.88	%
Found:	76.21	10.8	3.78	%

Infrared absorption spectra

Solid state (nujol mull).

$1/\lambda$ max. 3200 cm^{-1} (OH, bonded) resolved with LiF prism (page 99a).

$1/\lambda$ max. 3219 cm^{-1} and 3185 cm^{-1}. Solution spectra taken in chloroform with a Model 221 Perkin Elmer Spectrophotometer (p.118a).

The physical data were consistent with the Structure XXIa.

Reduction of Compound XXB with Lithium Aluminium Hydride

Compound (XXB) (100 mg.) was subjected to reduction by $LiAlH_4$ as described before for the reduction of Compound (XXA). The crude reduction product (97 mg.) was also crystallised from ethylacetate.

Physical constants

Molecular formula $C_{23}H_{39}NO_2$

Melting point 146°

Optical rotation $[\alpha]_D$ - 62.3° (C 1 = 0.019).

Infrared absorption spectrum

Solid state (nujol mull)

$1/\lambda$ max. at 3200 cm^{-1} resolved by LiF prism into 3219 cm^{-1} and 3185 cm^{-1}. (p.99a)

Solution spectra taken in chloroform with a Model 221 Perkin-Elmer Spectrophotometer. (p. 118a)

Analysis

		C.	H.	N.
$C_{23}H_{39}NO_2$	requires	76.45	10.80	3.88 %
	Found:	76.30	10.71	3.66 %

The physical data were consistent with the Structure (XXI).

***

S U M M A R Y

S U M M A R Y

The shrub Holarrhena antidysenterica or Indian "kurchi" is known to contain many alkaloids having the pregnane type of basic steroid nucleus. It was considered desirable to investigate whether some constituents of kurchi could be utilised as starting materials for the synthesis of 18-substituted steroids.

1. The neutral non-saponifiable material from kurchi bark giving a positive Liebermann reaction was examined to determine whether the plant stores any of the neutral hypothetical precursors of the pregnane alkaloids in the bark in appreciable amounts. The neutral petroleum ether extract from the bark was separated into three major fractions based on their polarity by a solvent-solvent distribution between hexane and 90% ethanol. The non-polar fractions after saponification yielded two crystalline compounds. These were identified as lupeol and β-sitosterol. The fractions of intermediate polarity after fractional crystallisation and chromatography yielded lupeol and two more crystalline Liebermann - positive compounds, a Compound 'C', $C_{30}H_{50}O$, m.p. 179-80°, $[\alpha]_D$ + 33° and a Compound 'D', m.p.123-25°, $[\alpha]_D$ - 32°.

2. Methods for the isolation of conessine, isoconessimine and conessimine three major alkaloids from kurchi were developed. The bark was extracted with a mixture of ether, alcohol and ammonia in the proportions of 8:1:1 respectively. The bases were precipitated as hydrochlorides and separated into water soluble and water-insoluble sulphates following the method of Siddiqui and Pillay. The bases from the water soluble sulphate fraction were chromatographed to yield conessine and fractions enriched in conessimine and isoconessimine. The pure bases, conessimine and isoconessimine, were obtained by a Craig-distribution of these fractions in chloroform and phosphate-citrate buffer (pH 7.0) and by working up the aqueous phase.

3. Conessine was partially demethylated to the disecondary base, conimine, by von Braun cyanogen bromide procedure. Conimine was converted to the internal Schiff's base of 18-amino-3,20-dioxo-4-pregnene essentially according to the procedure of Jeger and co-workers.

Microbiological transformation of this Schiff's base with Aspergillus niger led to the formation in poor yields of a basic hydroxylated gummy product which did not show the $\Delta^{4,3}$ keto chromophore in the u.v. spectrum.

On treatment with nitrous acid, the internal Schiff's base yielded 18-hydroxyprogesterone.

Conessine on treatment with hydrogen peroxide yielded two N-oxides, the 3-mono-N-oxide and the di N-oxide. The 3-mono-N-oxide was converted to conessimine by demethylation at the bridge nitrogen with the von Braun cyanogen bromide procedure, reduction of the resulting N-oxide N-cyano compound by sodium borohydride and aluminium chloride and hydrolysis.

4. Isoconessimine was converted to the crystalline N-chlorocompound by treatment with N-chlorosuccinimide The N-chloro compound was dehydrochlorinated with sodium ethoxide and the Schiff's base was hydrolysed by acid to 3-oxo-4-conenine characterised through its crystalline 2,4-dinitrophenyl hydrazone.

5. Conessimine was N-chlorinated to the crystalline N-chloro derivative which was dehydrochlorinated to the internal Schiff's base of 18-amino 3β N-dimethyl-20-oxo-5-pregnene. The structure of this Schiff's base was established by benzoylation and by its conversion in five stages to the known internal Schiff's base of 18-amino-3,20-dioxo-4-pregnene obtained from conessine and conimine.

On treatment with nitrous acid the internal Schiff's base from conessimine yielded a mixture of

three crystalline compounds: 3βN-dimethyl 18-20 oxido-5,20(21) pregnadiene and two other compounds tentatively identified as 18-hydroxy, 3β N-dimethyl-20-oxo-5-pregnene (XXA) and 20-hydroxy 3β N-dimethyl-18-aldo-5-pregnene (XXB) respectively. On reduction with lithium aluminium hydride XXA and XXB yielded two diols melting at 165° and 146° respectively. These two diols had the same rotation, almost superimposable I.R. spectra and yielded the identical diacetate on acetylation. On pyrolysis, however, they yielded two distinctly different carbonyl compounds. The implications of such unusual behaviour of these two diols are discussed.

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