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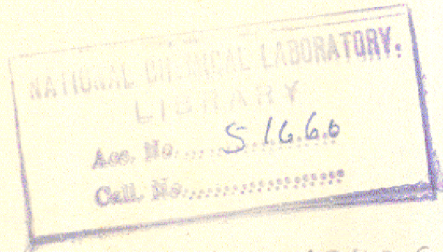
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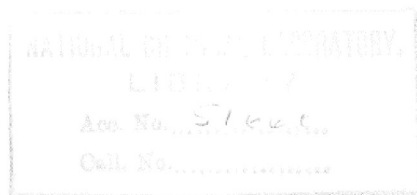


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Investigations on Steroids

*A Thesis Submitted to the
University of Bombay*

*For the Degree of
Doctor of Philosophy
(In Chemistry)*

by

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November 1967

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I am deeply indebted to the Director of this Laboratory for permitting me to submit this work in the form of thesis and to the Council of Scientific and Industrial Research for the award of Junior Research Fellowship.


Candidate

November, 1967

National Chemical Laboratory
Poona-8.

Statements to be submitted by the Candidate under the rule 0.413

Statement-I

A critical survey of relevant literature available is presented in collated and condensed form in the thesis. These portions in addition to being of interest in their own right, will provide the necessary background for the appraisal of the work described. The present work has been clearly indicated separately and consists of bonafide record of the experimental work carried out by the candidate. Detailed information about the new facts observed and how they have advanced the knowledge of the subject have been fully dealt with in the summary of the thesis.

Statement-II

The candidate has, with the concurrence of the University Teacher, worked on "Investigations on Steroids", suggested by Dr. P. K. Bhattacharyya.

In keeping with the general practice in the reporting of scientific observations, due acknowledgement has been made wherever the work described is based on the findings of other investigators. A complete bibliography of the books and journals consulted is given at the end of the thesis.

P. K. Bhattacharyya
Signature of the Guide

[Signature]
Signature of the Candidate

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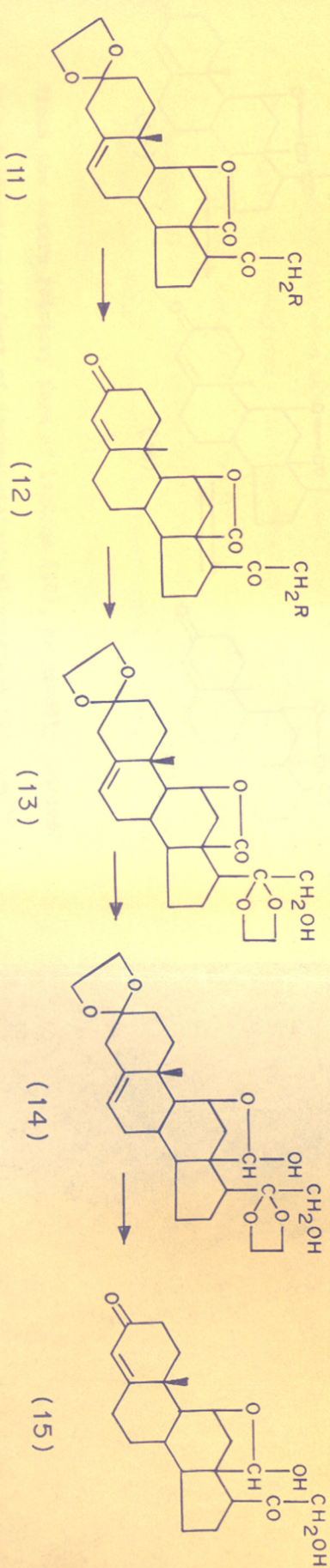
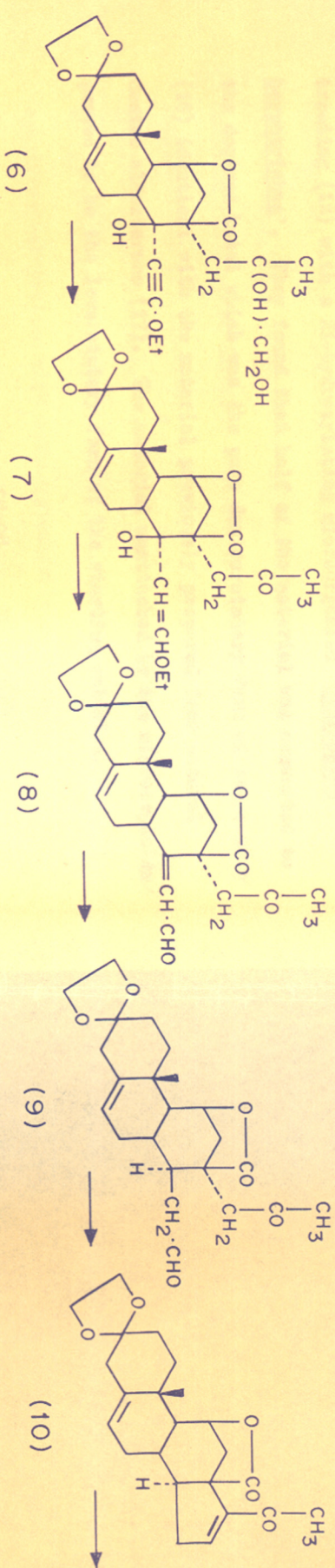
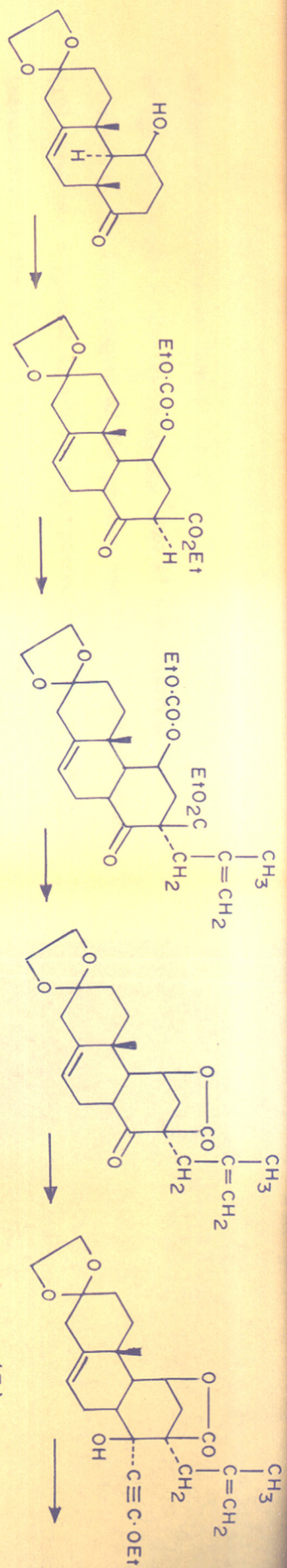
INTRODUCTION

The knowledge acquired in the late 1920's, that adrenalectomized animals could be kept alive for a while by giving them cortical extracts provided a stimulus for extensive chemical investigations of the adrenocortical hormones. In 1938, T. Reichstein¹ succeeded in extracting desoxycorticosterone from adrenals and in 1942, Miescher, Fischer and Meystre² announced the preparation of desoxycorticosterone glycoside of high therapeutic significance. At this stage it was becoming increasingly evident that the adrenal cortex produced another hormone exerting both a potent action on mineral metabolism as well on prolongation of life in adrenalectomized animals. This hormone was finally isolated by Simpson et al.³ in pure crystalline form from 'the amorphous fraction' remaining after separation of the various crystalline adrenocortical substances. This substance was named "aldosterone" after its structure was established³.

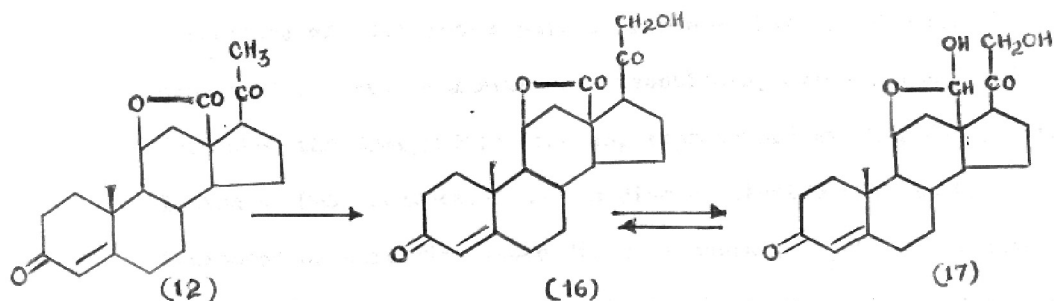
The discovery of aldosterone provided an attractive challenge to chemists to develop methods of its synthesis. In 1955, Wettstein et al.⁴ accomplished the total synthesis of dextro and levo aldosterone, starting from Sarett's ketal (1). Carboxylation of Sarett's ketal (1) with diethyl carbonate gave the ketoester (2), alkylated with methylallyl iodide to yield compound (3). The carbonyl group was reduced with sodium borohydride, whereupon alkaline hydrolysis, lactonization and

oxidation with chromium trioxide in pyridine furnished the keto-lactone (4). Treatment with ethoxyethynyl-magnesium bromide gave (5), converted by osmium tetroxide in pyridine to a mixture of epimeric triols, represented by (6); selective reduction of the triple bond with palladium-calcium carbonate in pyridine and oxidation with periodic acid gave compound (7), smoothly converted by phosphorus tribromide or thionyl chloride to a mixture of cis-trans isomeric aldehydes (8), hydrogenated by palladium to give mainly the aldehyde (9). Cyclization with benzoic acid and trimethylamine in hot xylene afforded the pregna-5:16 diene 18:11 lactone (10), hydrogenated with palladium to pregn^a-5-ene 18:11 lactone (11, R=H); this was converted by the Ruscig sequence: $-\text{CH}_2-\text{CO}-\text{CO}_2\text{Me} \longrightarrow \text{CHI}.\text{CO}.\text{CO}_2\text{Me} \longrightarrow \text{CH}_2\text{I} \longrightarrow \text{CH}_2\text{OAc}$, into compound (11, R=OAc), hydrolysed by dilute acetic acid to the mixture of dextro and levo rotatory forms of 18:11 lactone acetate (12, R=OAc). The free (+) lactone (12, R=OH), obtained by hydrolysis with perchloric acid was converted with ethylene glycol-toluene-p-sulphonic acid into 3:20 bisketal (13), reduced by lithium aluminium hydride to the cyclohemiacetal-3:20-bisketal (14), hydrolysed by hydrogen chloride in tetrahydrofuran to the lactol form of dextro and levo rotatory mixture of aldosterone (15). This synthesis is schematically represented in Chart-I.

Extensive further investigations by Wettstein and co-workers⁵ of Ciba led to the modified and improved synthesis of (+)aldosterone.



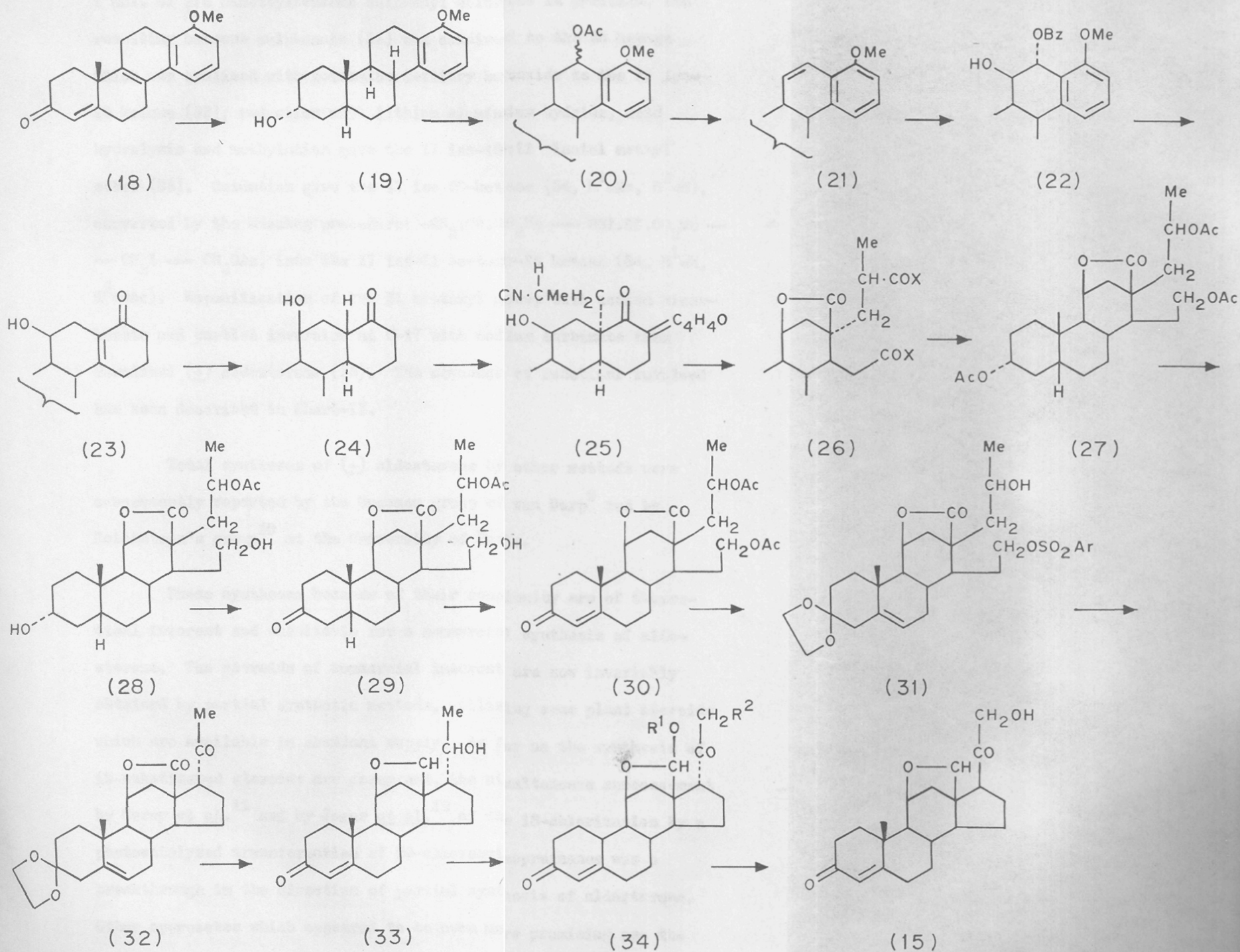
The first total synthesis leading to dextro rotatory aldosterone was reported by Vischer et al.⁶ In the earlier synthesis of (+) aldosterone by Wettstein et al.⁴ one of the intermediates involved in the later stages of the process was the (+) methyl ketone (12) which was subjected to oxalic ester condensation for the eventual introduction of the 21-hydroxyl function. However, the chemical method was lengthy and Vischer et al.⁶ tried the alternative scheme of treating (12) with a 21-hydroxylating microorganism 'Ophiobolus herpetrichus'. They found that half of the material was converted to the desired ketol which was the pure dextrorotatory form of compound 9 (16) identical with the material previously prepared from natural dextro aldosterone (17). The material unattacked by the microorganism proved to be the levorotatory form of the starting material.



Since the dextro rotatory form of lactone (16), originally obtained as a degradation product of dextrorotatory aldosterone (17) had been reconverted into aldosterone (17) by Reichstein et al.⁷ this microbiological resolution, coupled with the process for the synthesis of

dextro and levo forms of (12), constituted a total synthesis of dextro rotatory aldosterone (17).

Another stereospecific total synthesis of (+) aldosterone by W. S. Johnson and his collaborators⁸ started from tetracyclic ketone (18). The tetracyclic ketone (18), by successive reduction with i) palladium-hydrogen ii) sodium borohydride and iii) potassium-liquid ammonia-ethanol gave the (+) D-homo-18 nor-5 β -androst-13:15:17 trien 3 α -ol (19). Oxidation with lead tetraacetate yielded the 12 ξ -acetate, (20), converted by boiling acetic acid into the Δ^{11} compound (21), whose 11 β :12 β -epoxide by fission with benzoic acid furnished the 11 β :12 α diol 12-benzoate (22). Reduction with lithium-liquid ammonia-ethanol gave (23), hydrogenated with palladium to (+) 17 α -exo-D-homo-18 nor-5 β -androstane-3 α :11 β diol (24). Stereospecific α -alkylation with methacrylonitrile of the 17-furfurylidene derivative of (24) gave a pair of isomers (25), which could be separated or used as a mixture. Ozonolysis, oxidation and hydrolysis furnished the 20-epimeric diacids, represented by (26, X=OH). Acetylation at C-3, conversion to the diacid chloride (26, X=Cl) and treatment of this with keten dimethyl acetal, CH₂=C(OMe)₂, gave the diketone (26, X=Me), rearranged by trifluoroacetic acid to the triacetate (27). Selective alkaline hydrolysis gave the monoacetate (28), selectively oxidized with N-bromoacetamide to the 3-ketone-monoacetate (29). Acetylation, bromination and dehydrobromination with lithium chloride in dimethyl formamide afforded the $\Delta^{4,3}$ ketone diacetate (30), which was converted to the Δ^5 3:3 ketal, saponified and the primary hydroxyl group selectively acylated with



1 mol. of 2:5 dimethylbenzene sulphonyl chloride in pyridine. The resulting benzene sulphonate (31) was oxidized to the 20 ketone which was cyclized with potassium tertiary butoxide to the 17 iso-20 ketone (32), reduction with lithium aluminium hydride, acid hydrolysis and methylation gave the 17 iso-18:11 lactol methyl ether (33). Oxidation gave the 17 iso 20-ketone (34, $R^1=Me$, $R^2=H$), converted by the Buschag procedure: $-CH_2.CO.CO_2Me \rightarrow CHI.CO.CO_2Me \rightarrow -CH_2I \rightarrow -CH_2OAc$, into the 17 iso-21 acetoxy-20 ketone (34, $R^1=H$, $R^2=OAc$). Saponification of the 21 acetoxyl group with sodium bicarbonate and partial inversion at C-17 with sodium carbonate then furnished (+) aldosterone (15). The sequence of reactions involved has been described in Chart-II.

Total syntheses of (+) aldosterone by other methods were subsequently reported by the Organon group of van Dorp⁹ and by Reichstein's group¹⁰ at the University of Basle.

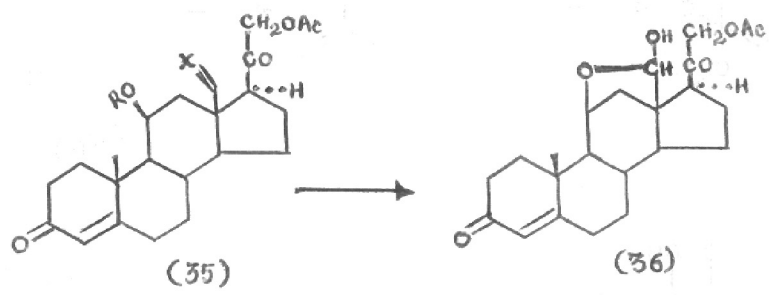
These syntheses because of their complexity are of theoretical interest and unsuitable for a commercial synthesis of aldosterone. The steroids of commercial interest are now invariably obtained by partial synthetic methods, utilizing some plant steroids which are available in abundant supply. As far as the synthesis of 18-substituted steroids are concerned, the simultaneous announcement by Corey *et al.*¹¹ and by Jeger *et al.*¹² of the 18-chlorination by a photocatalysed transformation of 20-chloroaminopregnanes was a breakthrough in the direction of partial synthesis of aldosterone. Other approaches which appeared to be even more promising are the

syntheses of aldosterone by Barton and Beaton¹³ and by Heusler et al.¹⁴

Barton, developed a three-stage partial synthesis of aldosterone from corticosterone acetate using a photocatalysed transfer. A nitroso radical from the 11 β position was migrated to position 18.



In this elegant synthesis the 11 β -hydroxyl group in corticosterone acetate (35, R=H, X=H₂) was nitrated with nitrosyl chloride. The resulting nitrite (35, R=NO, X=H₂) was converted to aldosterone acetate oxime (35, R=H, X=N.OH) by irradiation. The oximino group was removed by treatment with nitrous acid to give aldosterone-21 acetate (36) in 10-12 per cent yields.



Heusler et al.¹⁴ used another 1:4 intramolecular transfer reaction in which a 20 β hydroxypregnane was transformed into an 18:20 epoxide. On lead tetraacetate oxidation both 3 α , 11 α or 3 β , 11 β -diacetoxo 5 β pregnan-20 β -ol (37) gave 18:20 epoxide (38). The epoxide was cleaved by boron trifluoride-acetic anhydride to the 3 α , 11 α (or β), 18:20 β -tetraacetates (39) which on saponification and oxidation furnished the 18:20 lactone (40) of 3, 11, dioxo-20 β -hydroxy-5 β -pregnan-18 oic acid. Bromination followed by

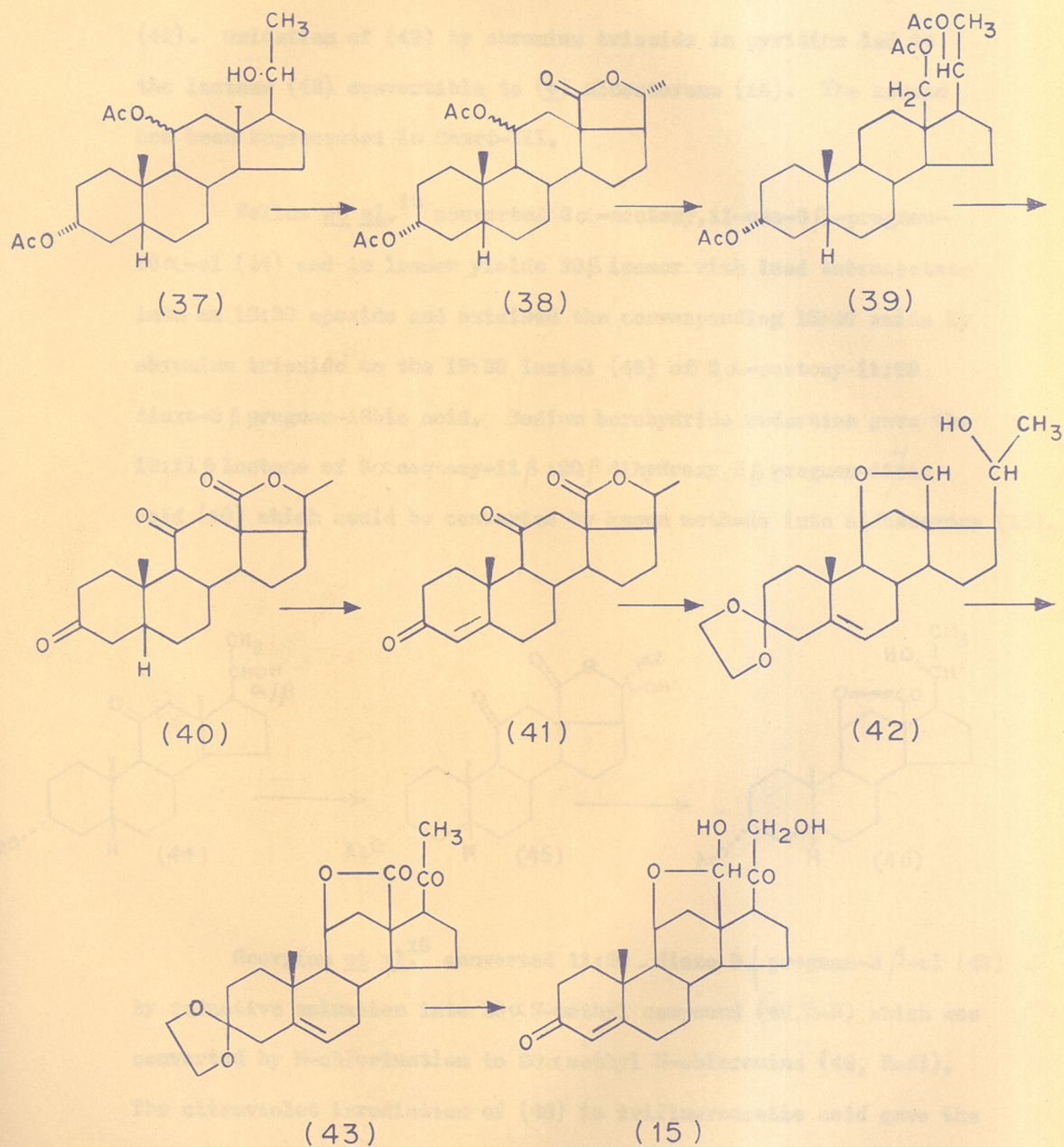
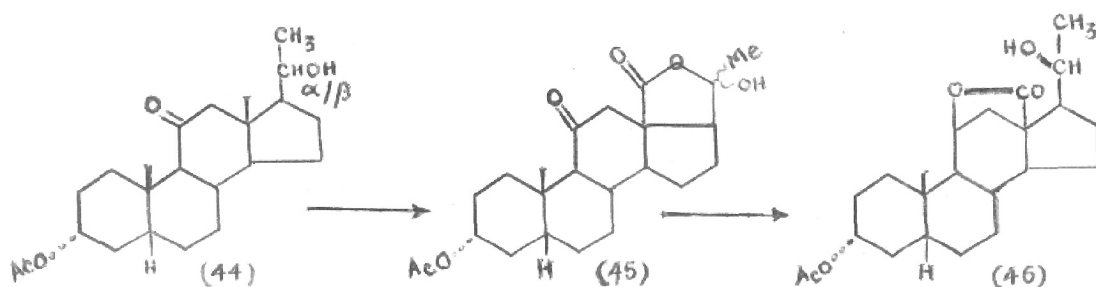


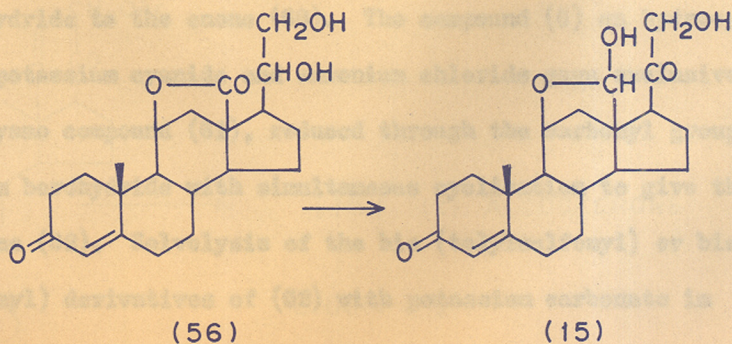
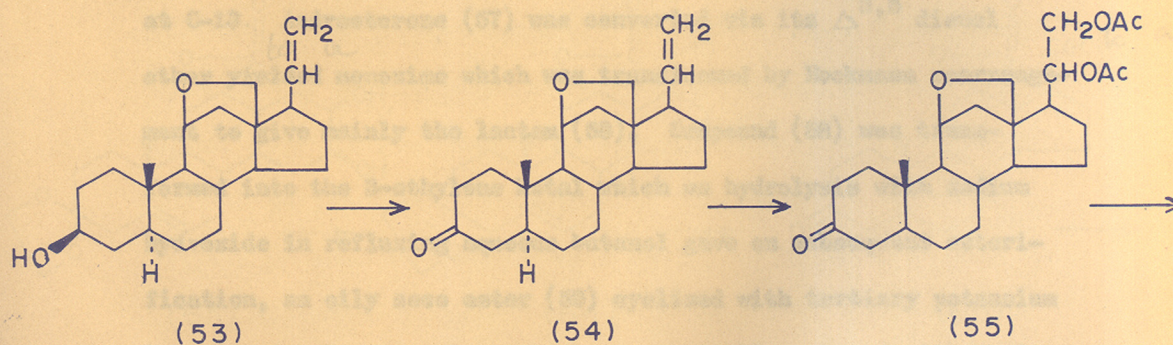
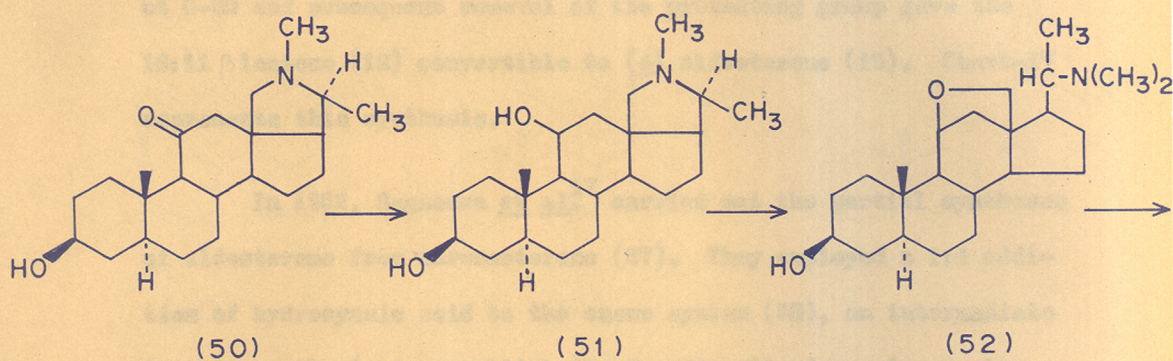
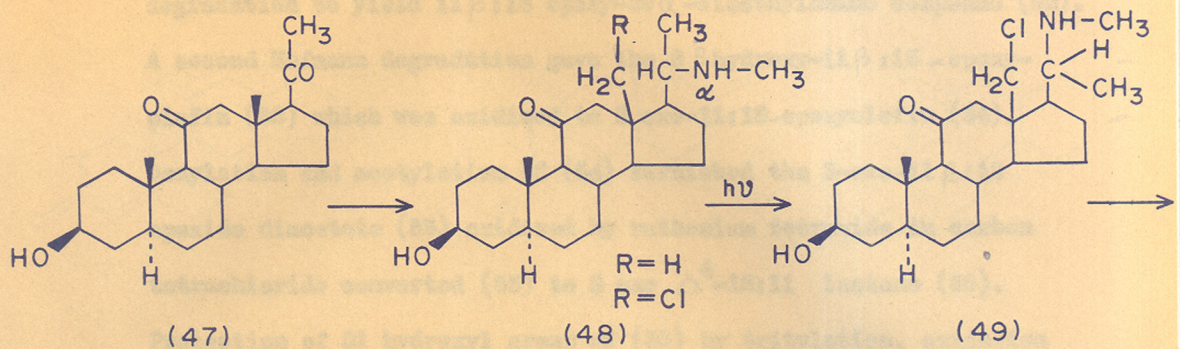
CHART III

dehydrobromination gave (41). Sodium borohydride reduction of the 3-ketal of compound (41) in sodium hydroxide yielded compound (42). Oxidation of (42) by chromium trioxide in pyridine led to the lactone (43) convertible to (+) aldosterone (15). The scheme has been represented in Chart-III.

Velluz et al.¹⁵ converted 3 α -acetoxy, 11-oxo-5 β -pregnan-20 α -ol (44) and in lesser yields 20 β isomer with lead tetraacetate into an 18:20 epoxide and oxidized the corresponding 18:20 oxide by chromium trioxide to the 18:20 lactol (45) of 3 α -acetoxy-11:20 dioxo-5 β pregnan-18oic acid. Sodium borohydride reduction gave the 18:11 β lactone of 3 α -acetoxy-11 β :20 β dihydroxy 5 β pregnan-18oic acid (46) which could be converted by known methods into aldosterone (15).

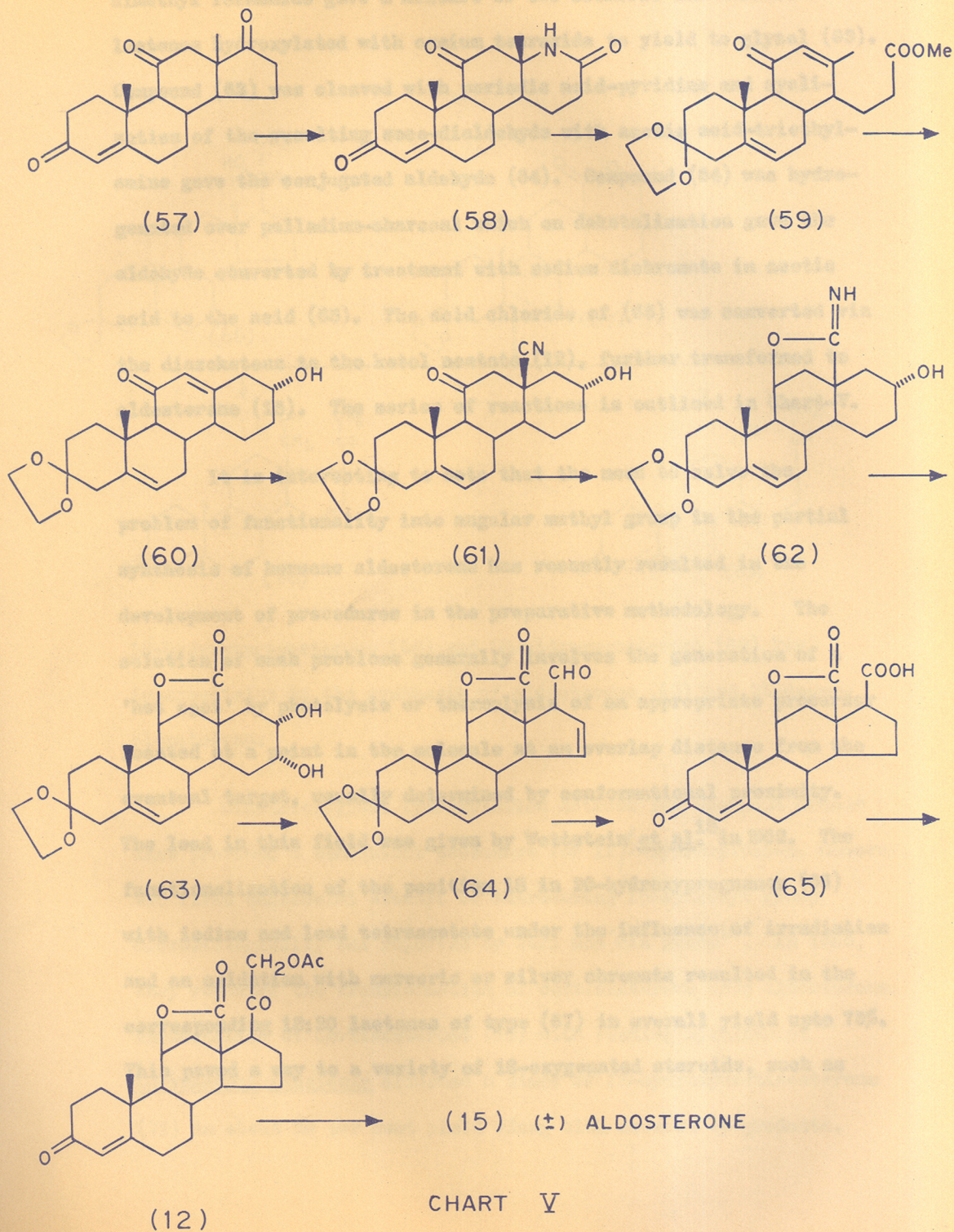


Georgian et al.¹⁶ converted 11:20 -dioxo 5 β pregnan-3 β -ol (47) by reductive amination into 20 α N-methyl compound (48, R=H) which was converted by N-chlorination to 20 α methyl N-chloramine (48, R=Cl). The ultraviolet irradiation of (48) in trifluoroacetic acid gave the 18-chloride (49) which was cyclized by alkali to 11-oxoconamine (50). Reduction with lithium aluminium hydride afforded 11 β -hydroxyconamine (51). The corresponding quaternary hydroxide underwent Hofmann



degradation to yield 11 β :18 epoxy-20 α -dimethylamino compound (52). A second Hofmann degradation gave the 3 β hydroxy-11 β :18-epoxyolefin (53) which was oxidized to 3-oxo-11:18-epoxyolefin (54). Osmylation and acetylation of (54) furnished the 3-oxo-11 β :18 epoxide diacetate (55) oxidized by ruthenium tetroxide in carbon tetrachloride converted (55) to 3-oxo Δ^4 -18:11 lactone (56). Protection of 21 hydroxyl group of (56) by tritylation, oxidation at C-20 and subsequent removal of the protecting group gave the 18:11 β lactone (12) convertible to (+) aldosterone (15). Chart-IV represents this synthesis.

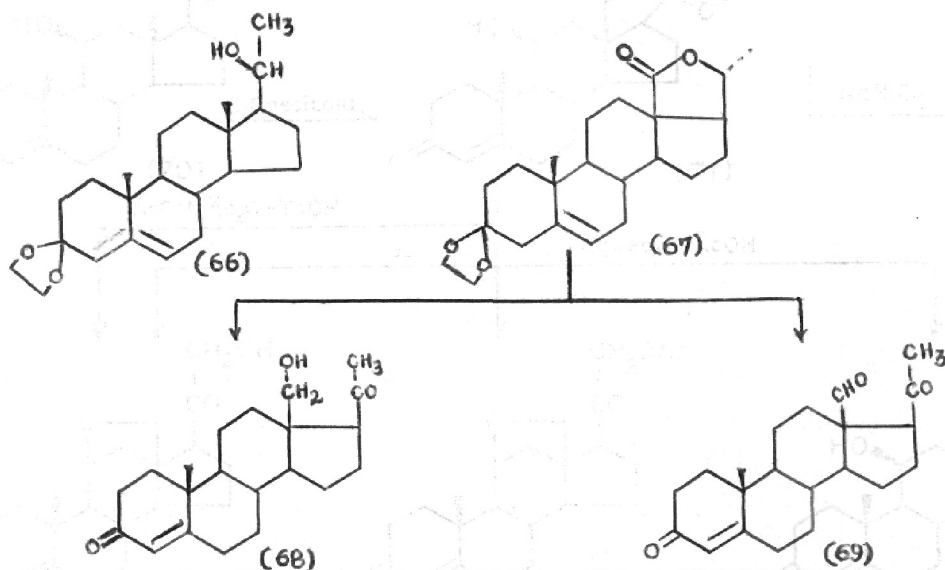
In 1962, Sugawara et al.¹⁷ carried out the partial syntheses of aldosterone from adrenosterone (57). They employed a 1:4 addition of hydrocyanic acid to the enone system (60), an intermediate in this synthesis, to establish the functionalized angular carbon at C-13. Androsterone (57) was converted via its $\Delta^{3,5}$ dienol ether ^{to a} yielded monoxime which was transformed by Beckmann rearrangement to give mainly the lactam (58). Compound (58) was transformed into the 3-ethylene ketal which on hydrolysis with sodium hydroxide in refluxing aqueous butanol gave on subsequent esterification, an oily seco ester (59) cyclized with tertiary potassium butoxide to give dienolone which was partially reduced with sodium borohydride to the enone (60). The compound (6) on hydrocyanation with potassium cyanide and ammonium chloride gave exclusively the 13 β cyano compound (61), reduced through the carbonyl group with sodium borohydride with simultaneous cyclization to give the imide lactone (62). Solvolysis of the bis (tolylsulfonyl) or bis (methylsulfonyl) derivatives of (62) with potassium carbonate in



dimethyl formamide gave a mixture of two isomeric unsaturated lactones hydroxylated with osmium tetroxide to yield to glycol (63). Compound (63) was cleaved with periodic acid-pyridine and cyclization of the resulting seco-dialdehyde with acetic acid-triethylamine gave the conjugated aldehyde (64). Compound (64) was hydrogenated over palladium-charcoal which on deketalization gave the aldehyde converted by treatment with sodium dichromate in acetic acid to the acid (65). The acid chloride of (65) was converted via the diazoketone to the ketol acetate (12), further transformed to aldosterone (15). The series of reactions is outlined in Chart-V.

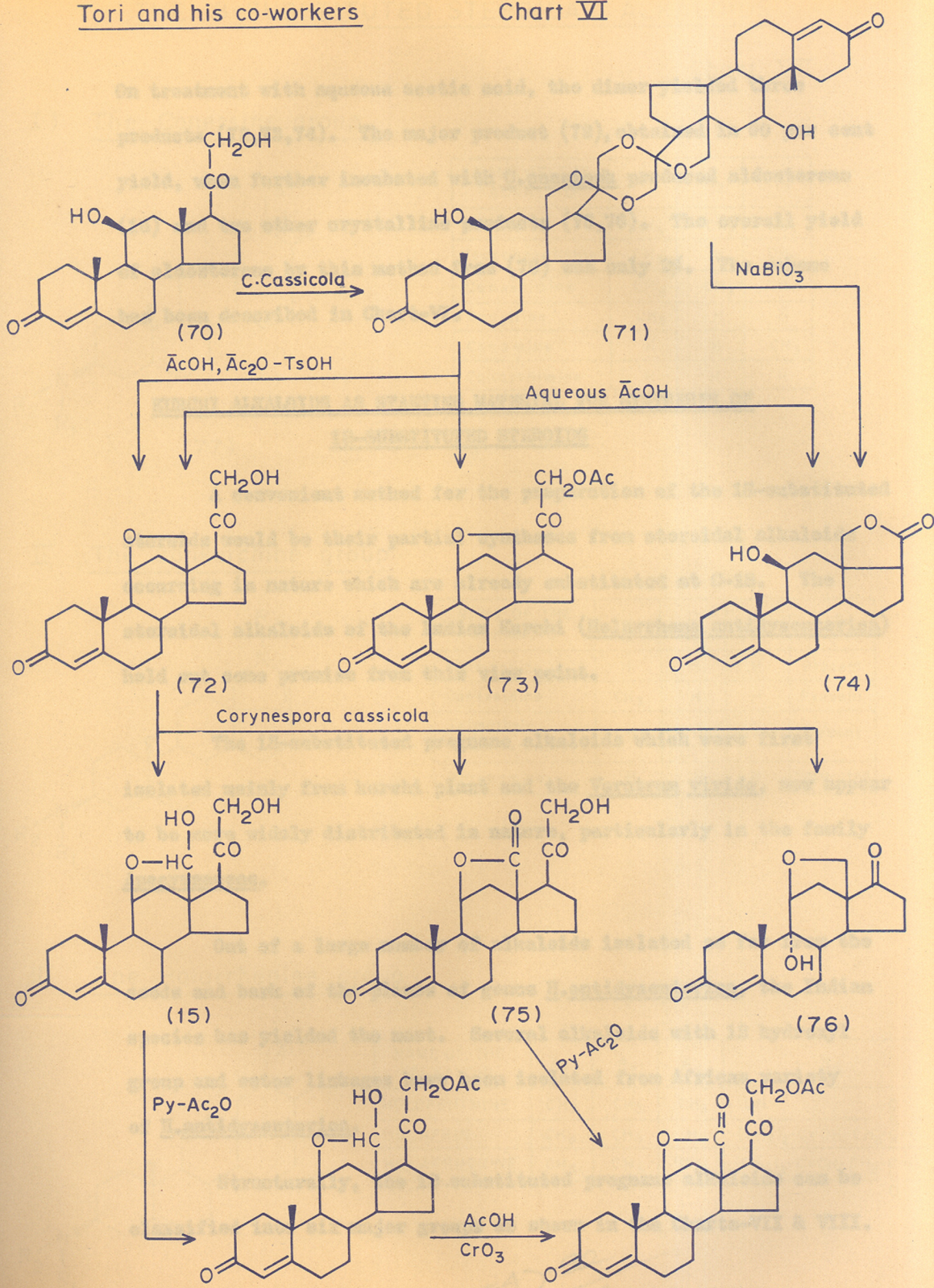
It is interesting to note that the need to solve the problem of functionality into angular methyl group in the partial synthesis of hormone aldosterone has recently resulted in the development of procedures in the preparative methodology. The solution of such problems generally involves the generation of a 'hot spot' by photolysis or thermolysis of an appropriate precursor located at a point in the molecule at an overlap distance from the eventual target, usually determined by conformational proximity. The lead in this field was given by Wettstein et al.¹⁸ in 1962. The functionalization of the position 18 in 20-hydroxypregnanes (66) with iodine and lead tetraacetate under the influence of irradiation and an oxidation with mercuric or silver chromate resulted in the corresponding 18:20 lactones of type (67) in overall yield upto 75%. This paved a way to a variety of 18-oxygenated steroids, such as

(18:20) about 20 per cent yield along with several by-products.



18-hydroxyprogesterone (68) and 18-aldoprogesterone (69) key intermediates in the synthesis of aldosterone (65) and 21-deoxy-aldosterone.

Microbiological hydroxylation methods for the past decade have provided a convenient procedure for 11 β -hydroxylation. However, the first successful attempt of introducing 18-hydroxyl group microbiologically was reported by Tori and his group¹⁹. In 1965, they reported the transformation of corticosterone into aldosterone. Fermentation of corticosterone (70) with resting mycelium of Corynespora cassicola afforded a dimer of 18-hydroxycorticosterone (71) in about 20 per cent yield along with several by-products.



On treatment with aqueous acetic acid, the dimer yielded three products (72,73,74). The major product (72), obtained in 60 per cent yield, when further incubated with C. classicon produced aldosterone (15) and two other crystalline products (75,76). The overall yield of aldosterone by this method from (70) was only 2%. The scheme has been described in Chart-VI.

KURCHI ALKALOIDS AS STARTING MATERIAL FOR SYNTHESIS OF
18-SUBSTITUTED STEROIDS

A convenient method for the preparation of the 18-substituted steroids would be their partial syntheses from steroidal alkaloids occurring in nature which are already substituted at C-18. The steroidal alkaloids of the Indian Kurchi (Holarhena antidysenterica) hold out some promise from this view point.

The 18-substituted pregnane alkaloids which were first isolated mainly from kurchi plant and the Veratrum viride, now appear to be more widely distributed in nature, particularly in the family Apocyanaceae.

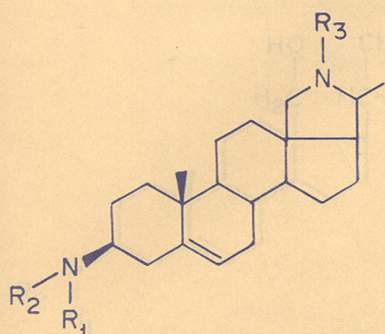
Out of a large number of alkaloids isolated so far from the seeds and bark of the plants of genus H. antidysenterica, the Indian species has yielded the most. Several alkaloids with 12 hydroxyl group and ester linkages have been isolated from African variety of H. antidysenterica.

Structurally, the 18-substituted pregnane alkaloids can be classified into six major groups as shown in the Charts-VII & VIII.

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Chart VII

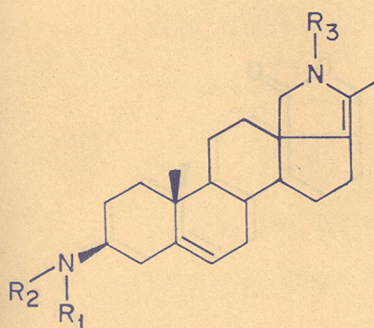
I) The Conessine group



(77)

NAME OF THE ALKALOID	R ₁	R ₂	R ₃
Conessine	CH ₃	CH ₃	CH ₃
Isoconessimine	CH ₃	H	CH ₃
Conessimine	CH ₃	CH ₃	H
Conimine	CH ₃	H	H
Conamine	H	H	CH ₃
Conarrhimine	H	H	H

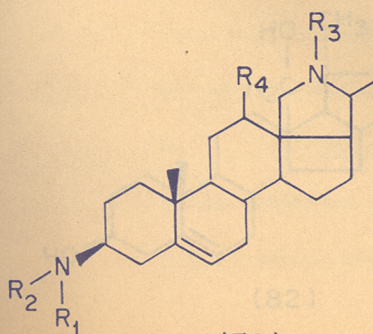
II The Konkurchine group



(78)

NAME OF THE ALKALOID	R ₁	R ₂	R ₃	Δ ⁵
Konkurchine	H	H	H	Present
Conessidine	CH ₃	H	H	Present
Trimethyl-konkurchine	CH ₃	CH ₃	CH ₃	Present
Kurchamine	H	H	H	Saturated

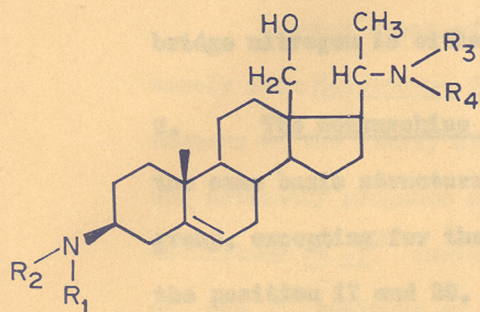
III The Holarrhenine group



(79)

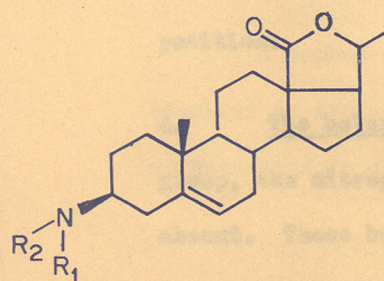
NAME OF THE ALKALOID	R ₁	R ₂	R ₃	R ₄
HOLORRHININE	CH ₃	CH ₃	CH ₃	OH
HOLAFRIN	CH ₃	CH ₃	H	(CH ₃) ₂ -C=CH-CH ₂ -C(=O)-O
HOLARRHENINE	CH ₃	CH ₃	CH ₃	(CH ₃) ₂ -C=CH-CH ₂ -C(=O)-O

Chart VIII

IV) The Holarrhimine group

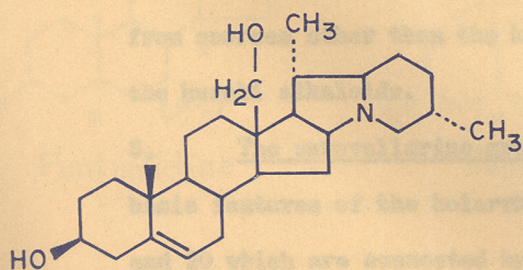
(80)

NAME OF THE ALKALOID	R ₁	R ₂	R ₃	R ₄	CONFIGURATION AT 3
Holarrhimine	H	H	H	H	β
3N-Methyl-holarrhimine	CH ₃	H	H	H	β
20N-Methyl-holarrhimine	H	H	CH ₃	H	β
Tetramethyl-holarrhimine	CH ₃	CH ₃	CH ₃	CH ₃	β
Holarrhidine	H	H	H	H	α

V) The Paravallaris alkaloids

(81)

NAME OF THE ALKALOID	R ₁	R ₂
Paravallarine	CH ₃	H
Methyl-paravallarine	CH ₃	CH ₃

VI) The Veratrine alkaloid

(82)

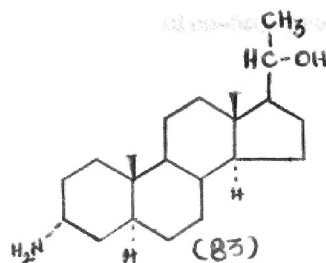
Isorubijervine

1. The conessine group: This group is characterized by the presence of 3 α or 3 β -amino group, either unsubstituted/by one or two methyl groups in a 5-pregnene nucleus and an 18:20 imino bridge nitrogen is either unsubstituted or methylated.
 2. The conkurchine group: The alkaloids of this group have the same basic structural features as those of the conessine group, excepting for the presence of an extra double bond between the position 17 and 20. In kurchamine, the 5:6 double bond is absent, but 17:20 double bond is present.
 3. The holarrhenine group: The bases of this group are structurally similar to the conessine alkaloids excepting for the presence of a free or esterified hydroxyl group at the 12 position.
 4. The holarrhimine group: In the alkaloids of holarrhimine group, the nitrogen bridge across the positions 18 and 20 is absent. These bases have 18-hydroxy 3 α or 3 β and 20 α -diamino 5-pregnene as the basic skeleton where both the amino groups may be unsubstituted or either partially or completely substituted by methyl groups.
- Two other groups of 18-substituted steroidal alkaloids from sources other than the kurchi can be classified along with the kurchi alkaloids.
5. The paravallarine group: These alkaloids have most of the basic features of the holarrhimines excepting for the positions 18 and 20 which are connected by a lactone bridge.

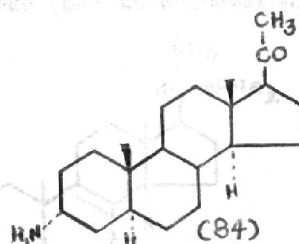
6. The isorubijervine group: This group contains the 18 hydroxy-5 pregnene nucleus with two fused heterocyclic rings attached to ring D.

Another group of azosteroids, isolated from *Holarrhena* species namely from *Holarrhena floribunda*, *Funtumia latifolia* and other members of the family apocyanaceae is the funtamine group, which has the necessary pregnane structure but without the 18-substitution. McPhillamy *et al.*²⁰ isolated D(+)-glycoside of 20 β -aminopregn-5 α -3 β -ol, the Δ^5 -analogue of funtumphyllamine-A (86) from the extract of the roots of *Cornopharyngia pachysiphon*. The glycoside was found to exert considerable hypotensive activity when tested intravenously in dogs, however, this action was accompanied by a marked respiratory depressant effect.

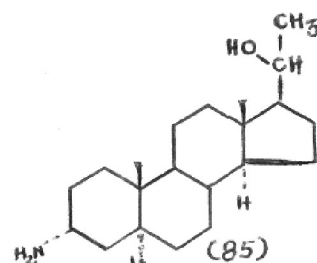
A number of alkaloids of this group have been either synthesized or isolated^{21,22}. Funtumidine is a saturated primary amine-secondary alcohol and funtamine (84) is the related ketone.



Funtumidine



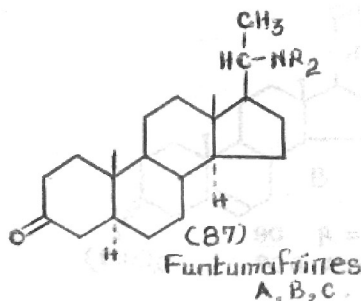
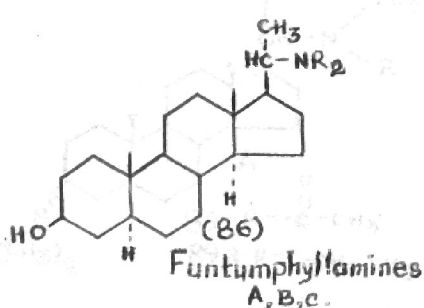
Funtamine



20-Isofuntumidine

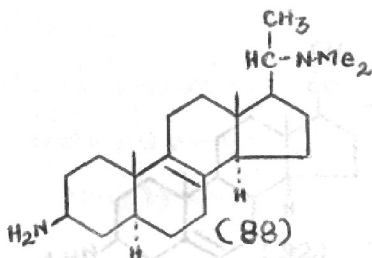
The presence of an alkaloidal principle in *Holarrhena*

The funtumphyllamines, A, B & C (86, R=H or CH₃) are 20 α amino, -20 α methyl amino and 20 α dimethylamino 5 α pregnan-3 β -ols respectively.



Funtumafrines (87) are the 3-ketones (R=H or CH₃) related to funtumphyllamine B and C respectively and are obtained from these compounds by oxidation. Funtumafrine C has been prepared previously by Cerny *et al.*²³

Chonemorphine, a steroidal alkaloid was first reported from Chonemorpha macrophylla G. Don (family Apocynaceae) by Das and Pillay²⁴. Chatterji *et al.*²⁵ isolated chonemorphine from C. macrophylla as well as from C. penangensis. Based on the physico-chemical data Chatterji *et al.*²⁵ assigned the structure as 3 β -amino-20 α -dimethylamino-5 α -pregn-8-ene (88) to chonemorphine.



The presence of an alkaloidal principle in Sarcococca pruniformis was first reported by Chopra and Handa²⁶. Recently,

The kurchi alkaloids and their interrelationship:

The ditertiary base, conessine, was isolated in crystalline form more than 100 years back, by Haines³⁰. The first systematic investigations however on the alkaloids of H. antidyserterica were undertaken by Siddiqui and co-workers^{31-A,B,C} only thirtyfive years ago. They isolated several alkaloids from the seeds and bark of Indian kurchi, and named them as iso-conessimine, conessimine, holarrhimine, conimine, conamine, conarrhimine etc. They were also able to demonstrate some interesting interrelationships among these alkaloids. 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, isoconessimine and di-secondary base conimine. They also recorded an interesting observation that the completely unsubstituted base, conarrhimine was converted to holarrhimine by refluxing with moist ethylacetate^{31C}. Now that the chemistry of kurchi alkaloids has been firmly established, it is difficult to visualize a plausible mechanism for such an unusual transformation. It may be mentioned in this connection that the purity of conarrhimine isolated by Siddiqui and Siddiqui was not rigorously established.

The bases of the conkurchine group were isolated by Bertho³² and later by Tschesche and co-workers^{33-A,B}, and were correlated with the conessine group by hydrogenation of the easily reducible 17:20 double bond.

Holarrhenine, isolated by Pyman³⁴ in 1919 is 12 hydroxy-conessine. The holarrhenine group of alkaloids have been isolated so far only from the African variety of kurchi. Oxidation of the 12 hydroxyl group followed by the hydrogenolysis of ethylene dithio-ketal of the resulting 12 oxe compound converted this class into the conessine group.

Alkaloids of the holarrhimine group have been isolated and characterized in 1932 by Siddiqui and Pillay^{31,A,B,C} and more recently by Bertho³²³ and by Tschesche et al. & Labler et al.^{35,A-H}

The Czechoslovakian workers converted dihydrotetramethyl holarrhimine to dihydroconessine by refluxing over thionyl chloride, which resulted in an intramolecular displacement of the 18-hydroxyl group by the 20-dimethylamino group and demethylation. Bhattacharyya et al.³⁶ converted conessine to conessimine by protecting the 3 tertiary amine by N-oxide formation, followed by demethylation on the pyrrolidine nitrogen and reduction. The interrelationship of these alkaloids is summarized in Chart IX.

The kurchi alkaloids have also been correlated with the paravallarines by conversion of holarrhimine to methyl paravallarine by Mansa Ram and Bhattacharyya³⁷.

From these relationships it is clear that if the constitution of any one of the alkaloids could be established, the structure of any other alkaloid could 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.³⁸⁻⁴⁸

SORM AND COWORKERS

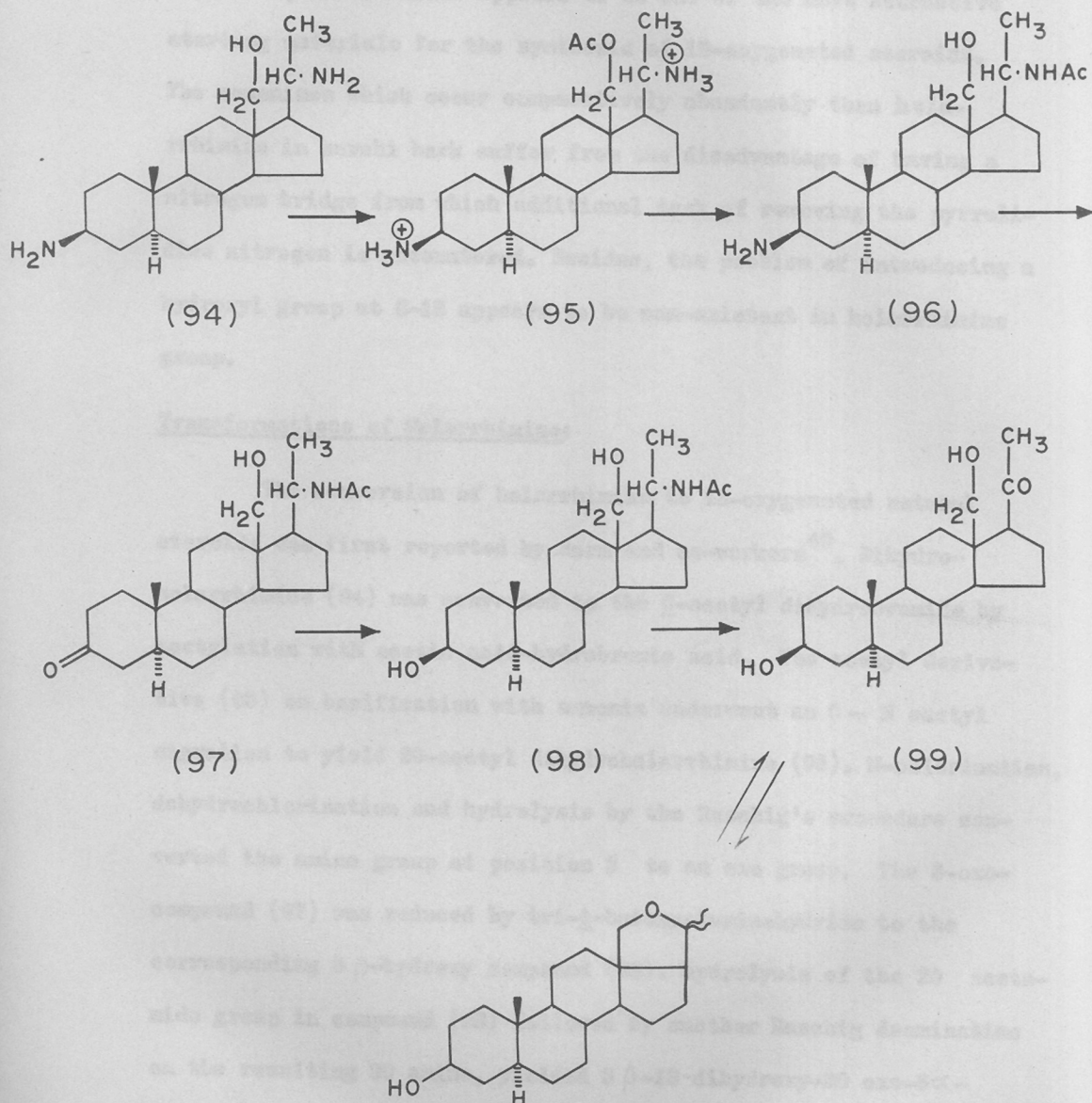


CHART X

From a cursory examination of the structures of kurchi alkaloids, holarrhimine appears to be one of the most attractive starting materials for the synthesis of 18-oxygenated steroids. The conanines which occur comparatively abundantly than holarrhimine in kurchi bark suffer from the disadvantage of having a nitrogen bridge from which additional task of removing the pyrrolidine nitrogen is encountered. Besides, the problem of introducing a hydroxyl group at C-18 appears to be non-existent in holarrhimine group.

Transformations of Holarrhimine:

The conversion of holarrhimine to 18-oxygenated natural steroids was first reported by Sorn and co-workers⁴⁹. Dihydroholarrhimine (94) was converted to the O-acetyl dihydrobromide by acetylation with acetic acid-hydrobromic acid. The acetyl derivative (95) on basification with ammonia underwent an O - N acetyl migration to yield 20-acetyl dihydroholarrhimine (96). N-chlorination, dehydrochlorination and hydrolysis by the Ruschig's procedure converted the amino group at position 3 to an oxo group. The 3-oxo-compound (97) was reduced by tri-t-butoxyaluminumhydride to the corresponding 3 β -hydroxy compound (98). Hydrolysis of the 20 acetamide group in compound (98) followed by another Ruschig deamination on the resulting 20 amine, yielded 3 β -18-dihydroxy-20 oxo-5 α -pregnane (99) which existed in the tautomeric hemiketal form. The sequence has been outlined in Chart-X.

In a parallel series of degradations, holarrhimine was converted into 18-hydroxy progesterone by Sorn and Labler.⁵⁰

SORM AND COWORKERS

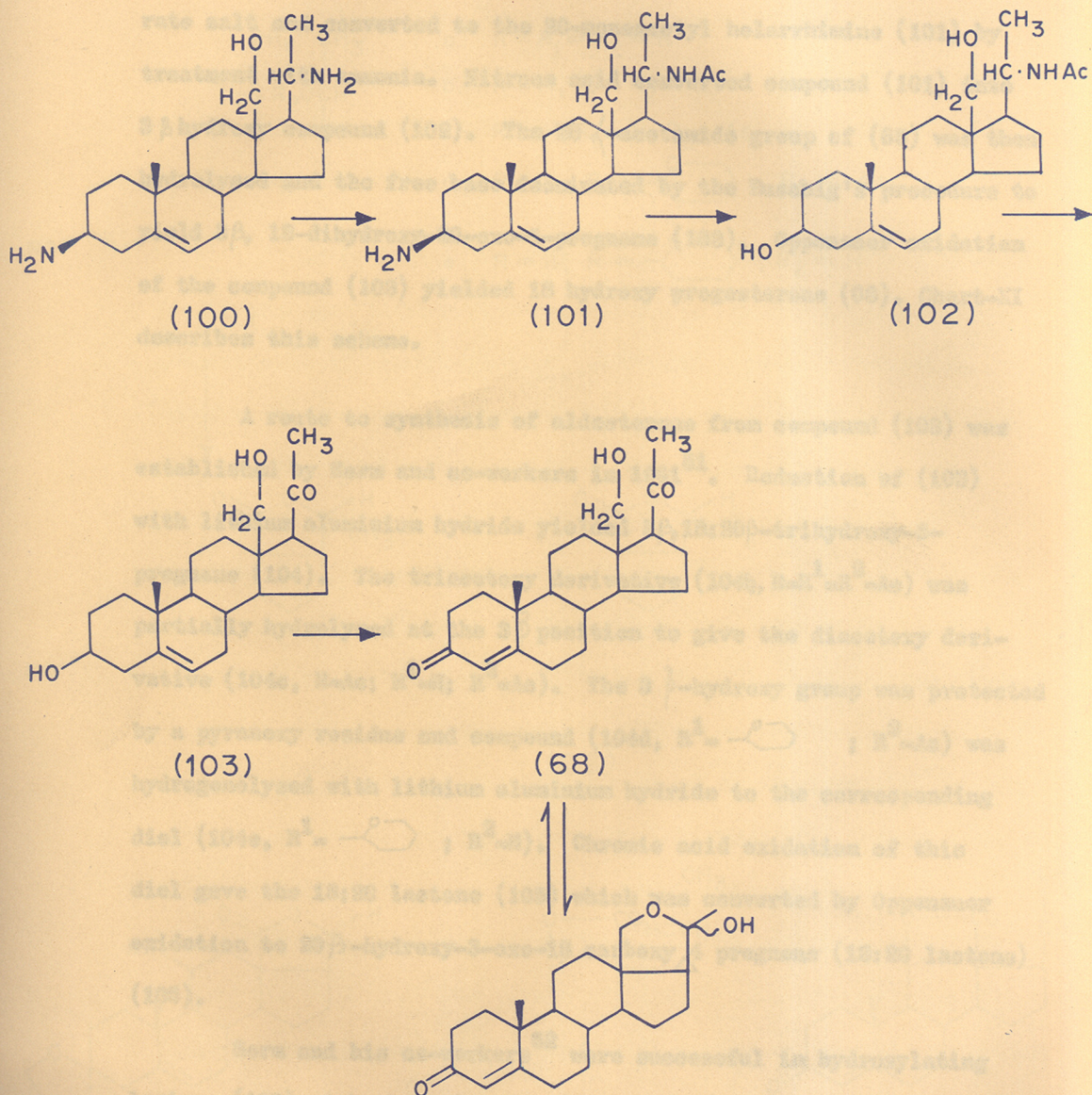


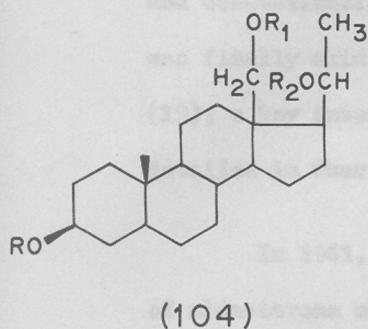
CHART XI

SORM AND CO-WORKERS

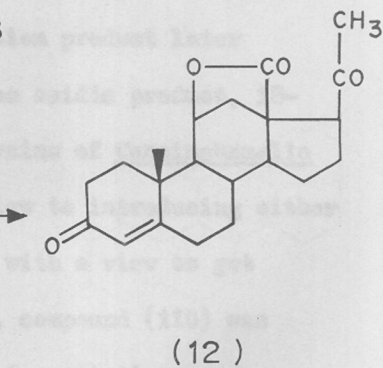
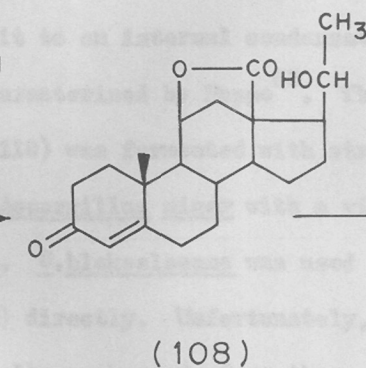
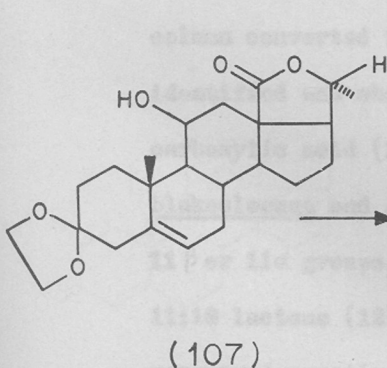
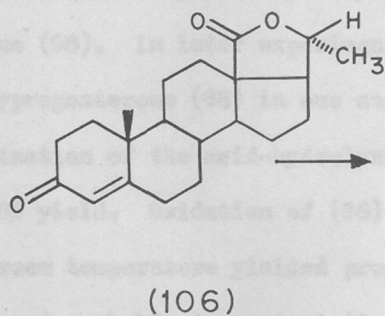
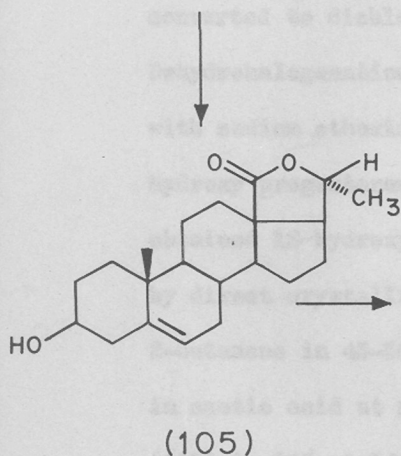
The O-acetyl derivative was prepared from holarrhimine (100) by treatment with acetic acid-perchloric acid, isolated as diperchlorate salt and converted to the 20-monoacetyl holarrhimine (101) by treatment with ammonia. Nitrous acid converted compound (101) into 3 β hydroxy compound (102). The 20/-acetamido group of (65) was then hydrolysed and the free base desaminated by the Ruschig's procedure to yield 3 β , 18-dihydroxy-20-exo-5-pregnene (103). Oppenauer oxidation of the compound (103) yielded 18 hydroxy progesterone (68). Chart-XI describes this scheme.

A route to synthesis of aldosterone from compound (103) was established by Sorm and co-workers in 1961⁵¹. Reduction of (103) with lithium aluminium hydride yielded 3 β ,18:20 β -trihydroxy-5-pregnane (104). The triacetox derivative (104b, R=R¹=R²=Ac) was partially hydrolysed at the 3 β position to give the diacetox derivative (104c, R=Ac; R¹=H; R²=Ac). The 3 β -hydroxy group was protected by a pyranox residue and compound (104d, R¹=; R²=Ac) was hydrogenolysed with lithium aluminium hydride to the corresponding diol (104e, R¹=; R²=H). Chromic acid oxidation of this diol gave the 18:20 lactone (105) which was converted by Oppenauer oxidation to 20 β -hydroxy-3-oxo-18 carboxy Δ^4 pregnene (18:20 lactone) (106).

Sorm and his co-workers⁵² were successful in hydroxylating lactone (106) with the help of *Rhizopus nigricans*, at C-11 position. The 11 α hydroxy compound they obtained, was oxidized to the corresponding 11 ketone, the 3-keto group was protected by dioxolane formation and the 11 ketone function was reduced to 11 β hydroxyl group with

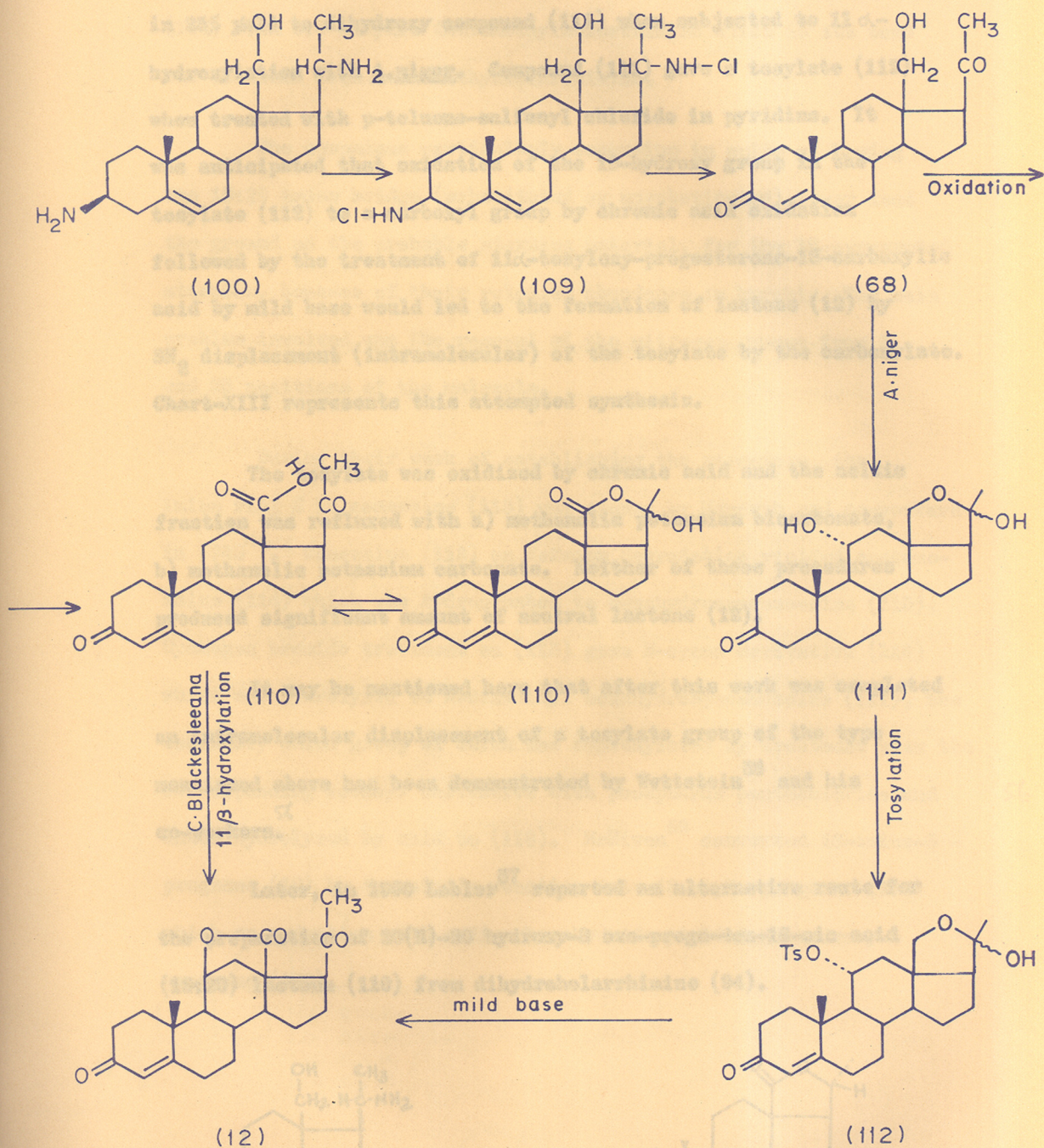


- a. $R = R_1 = R_2 = H$
- b. $R = H, R_1 = R_2 = Ac$
- c. $R = H, R_1 = H, R_2 = Ac$
- d. $R = H, R_1 = \text{cyclohexyl}, R_2 = Ac$
- e. $R = H, R = \text{cyclohexyl}, R_2 = H$



sodium borohydride to get compound (107). The 18:20 lactone was opened by base and the 11:18 lactone was formed on acidification and deketalization gave (108). The 20 β -hydroxyl group in (108) was finally oxidized by chromic acid to yield the 20-oxo compound (12), a key intermediate in the synthesis of aldosterone as detailed in Chart-XII.

In 1961, Godse⁵³ of this laboratory attempted the synthesis of aldosterone starting from holarrhimine. Holarrhimine (100) was converted to dichloroderivative (109) with N-chlorosuccinimide. Dehydrohalogenation of compound (109) was carried out by refluxing with sodium ethoxide followed by acid hydrolysis to obtain 18-hydroxy progesterone (68). In later experiments Godse *et al.*⁵⁴ obtained 18-hydroxyprogesterone (68) in one step from holarrhimine by direct crystallization of the acid-hydrolysed product from 2-butanone in 45-50% yield. Oxidation of (68) with chromium trioxide in acetic acid at room temperature yielded progesterone-18-oic acid (110) in 30% yield and probably to neutral 18-aldehyde (69) to some extent. Attempted purification of the aldehyde (69) through alumina column converted it to an internal condensation product later identified and characterized by Pappo⁵⁵. The acidic product, 18-carboxylic acid (110) was fermented with strains of Cunninghamella blakesleeana and Aspergillus niger with a view to introducing either 11 β or 11 α groups. C.blakesleeana was used with a view to get 11:18 lactone (12) directly. Unfortunately, compound (110) was recovered practically unchanged, from these fermentations.

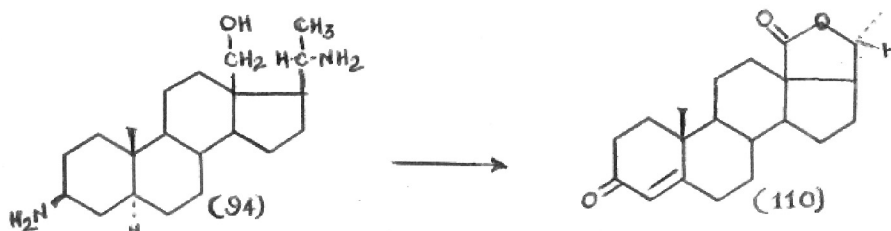


18-Hydroxyprogesterone, on the other hand, was converted in 25% yield to dihydroxy compound (111) when subjected to 11 α -hydroxylation with A.niger. Compound (111) gave a tosylate (112) when treated with p-toluene-sulfonyl chloride in pyridine. It was anticipated that oxidation of the 18-hydroxy group in the tosylate (112) to a carboxyl group by chromic acid oxidation followed by the treatment of 11 α -tosyloxy-pregesterone-18-carboxylic acid by mild base would lead to the formation of lactone (12) by SN_2 displacement (intramolecular) of the tosylate by the carboxylate. Chart-XIII represents this attempted synthesis.

The tosylate was oxidized by chromic acid and the acidic fraction was refluxed with a) methanolic potassium bicarbonate, b) methanolic potassium carbonate. Neither of these procedures produced significant amount of neutral lactone (12).

It may be mentioned here that after this work was completed an intramolecular displacement of a tosylate group of the type mentioned above has been demonstrated by Wettstein⁵⁶ and his co-workers.⁵⁶

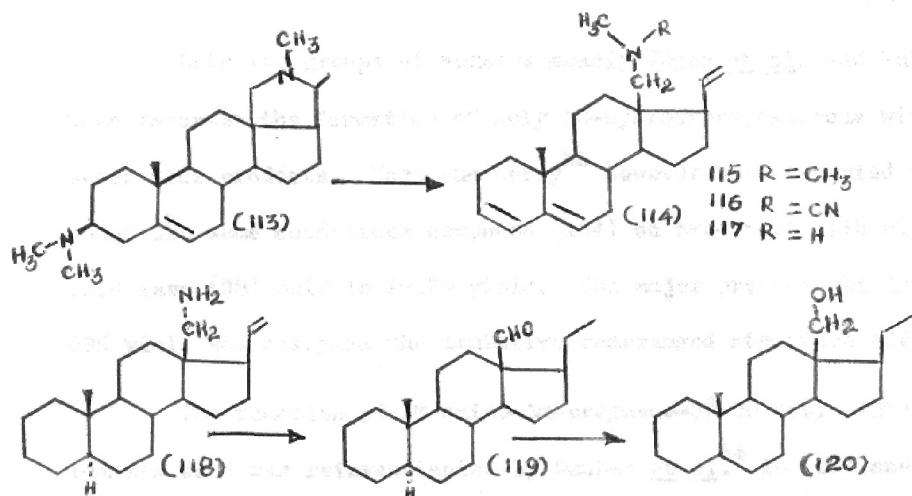
Later, in 1960 Labler⁵⁷ reported an alternative route for the preparation of 20(R)-20 hydroxy-3 oxa-pregn-4-en-18-oic acid (18:20) lactone (110) from dihydroholarrhizine (94).



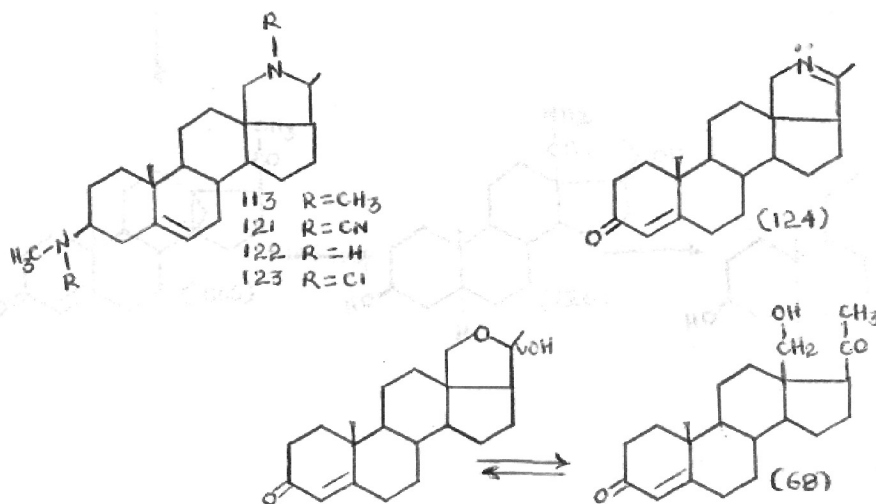
Cerny and his co-workers⁵⁸ in 1962 synthesized the lactone (110) from 5:6 dihydro tetramethylholarrhimine which in its turn was obtained from tetramethylholarrhimine.

The conamines particularly conessine in spite of having the 18:20 imino bridge (substituted or unsubstituted) never lost the ground as the probable starting material, for the 18-oxygenated steroids, because of their relative abundance in kurchi. The main problem involved was the removal of the nitrogen groups from 3 and 20 positions of the molecule.

During their work of establishing the structure, the imino ring E in conessine (113) was opened by Haworth and co-workers in 1953⁴⁴. Conessine (113) on Hofmann degradation yielded apoconessine (114) which was hydrogenated to hexahydroapoconessine (115). Cyanogen bromide treatment on (115) gave N-cyano derivative (116) which was hydrolysed to N-desmethyl hexahydroapoconessine (117), the secondary amino group of which was demethylated by treatment with the corresponding benzoyl derivative with phosphorus pentachloride and later hydrolysed by acid to (118). McNiven⁵⁹ converted 18-amino-5 -pregnane (18) by Sommelet's reaction into 18-ex,5 -pregnane (119) which on reduction with sodium borohydride yielded 18-hydroxy-5 pregnane (120).



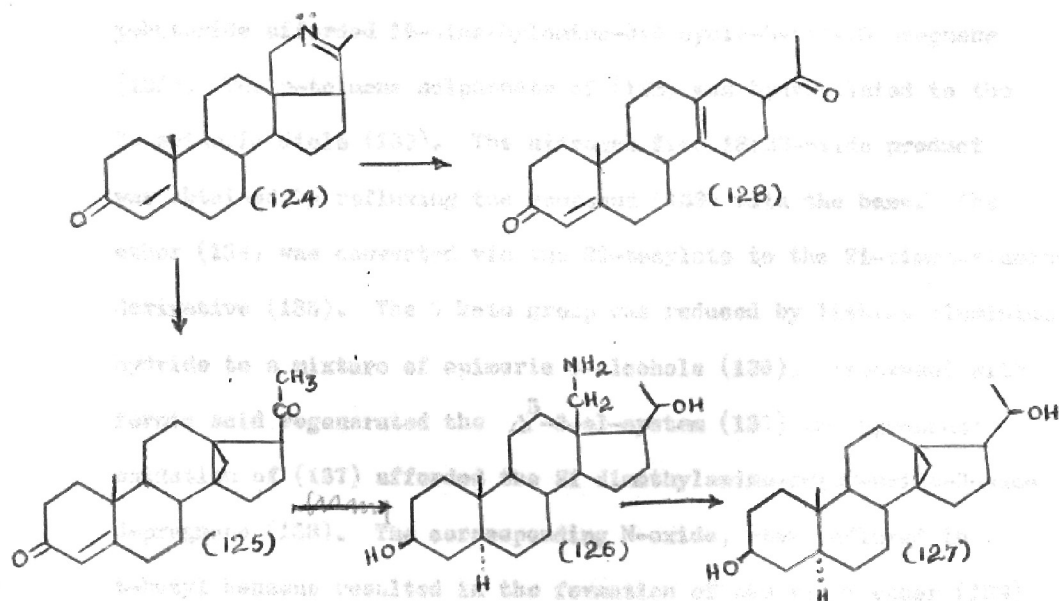
Cerny and Sorm⁶⁰ in 1959, reported the degradation of dihydroconessine to 18-benzoylamino-5 α -pregnan-3 β -ol-20-one. Jeger and his colleagues⁶¹ and shortly after that, Kulkarni⁶² synthesized 18-hydroxyprogesterone, independently from conessine by following an essentially similar route. All the three pathways, although reported independently, involved in principle, the degradation of tertiary amino groups of the alkaloid by cyanogen bromide to secondary amino groups which subsequently can be degraded via the chloramines. The Schiff's base, $\Delta^4,20$,N-imino 3 keto, pregna-4-diene (124), on treatment with nitrous acid as in later two cases yielded 18-hydroxyprogesterone (68).



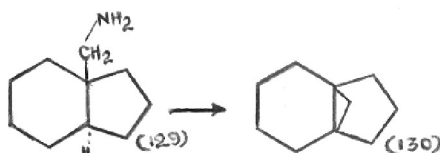
Both the groups of workers namely Jeger *et al.* and Kulkarni have reported the formation of only 18-hydroxyprogesterone without other side products. Hora and Cerny⁶³ however have reported that under the same conditions compound (124) on treatment with nitrous acid gave (68) only in 10.5% yield. The major product obtained in 69% yield was assigned the tentative rearranged structure (128).

The reaction of 18 amino-5 α -pregnane-3 β -20 diol (126) with nitrous acid was reinvestigated by Dauben *et al.*⁶⁴ in 1964 and in agreement with Hora and Cerny's results, it was found that the major product was not 18-hydroxy derivative of (126) but a cyclosteroid (127).

The structure of the major deamination product for the reaction of pyrolline (124) was also reexamined and it was shown to have a similar 14-18 cycle structure (125).

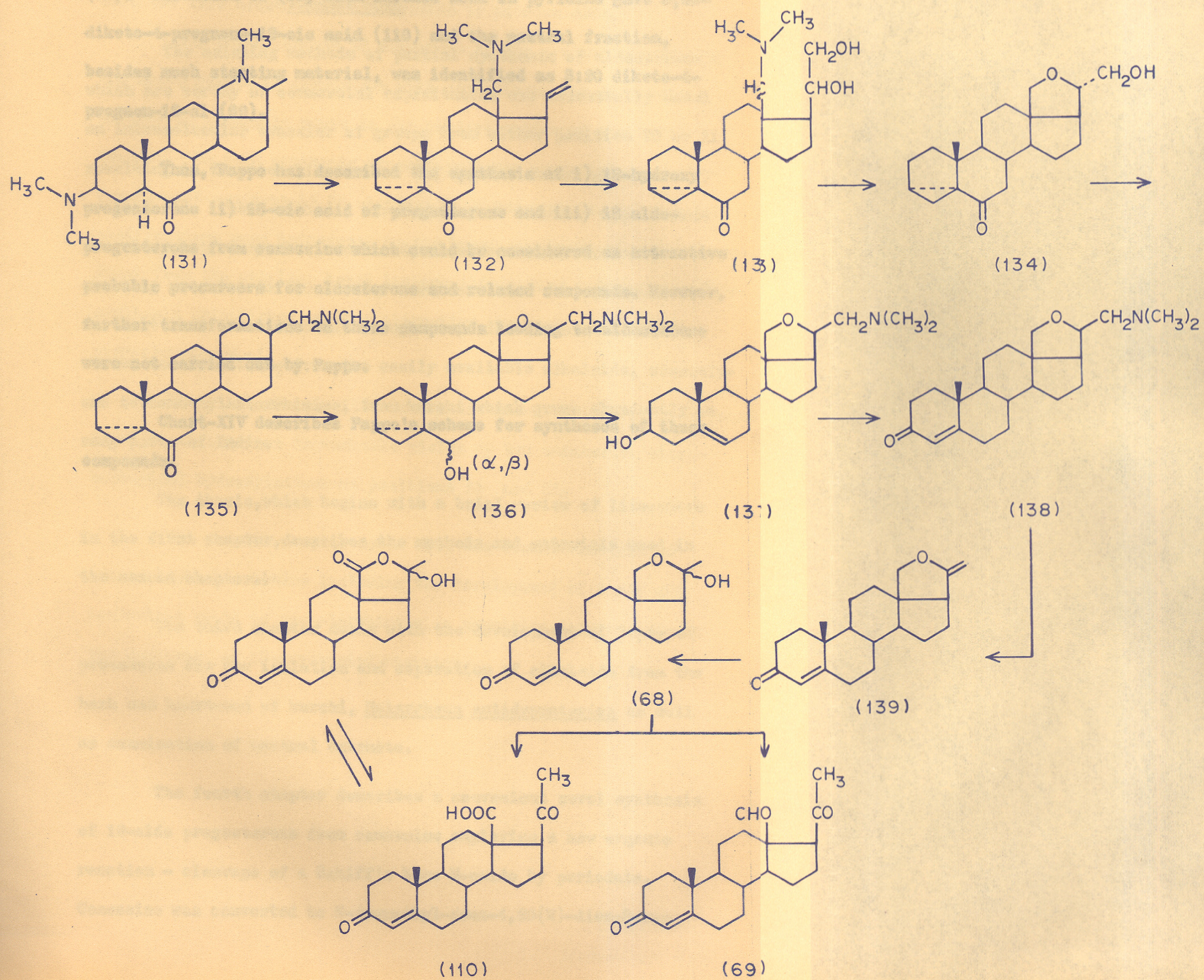


The authors extended this reaction on a model compound having angularly substituted group on simple fused ring system (129) when tricyclo (4:3:1:0^{1,6}) compound (130) was formed in 74% yield.



In 1959, Pappo⁵⁵ reported a practical method for the conversion of the readily available alkaloid conessine to C-18 progesterones and other related compounds.

Conessine (113) on treatment with sodium borohydride and aluminium chloride gave a 6-boron complex which was oxidized by chromic acid to 3 β -N-dimethyl-6-oxo-18:20N methyl-5 α -pregnane (131). The dimethiodide of (131) under Hofmann degradation with potassium *t*-butoxide afforded 18-dimethylamino-3:5 cyclo-5-oxo-20 pregnene (132). The *p*-toluene sulphonate of (132) was hydroxylated to the 20 epimeric diols (133). The nitrogen free 18:20-oxide product was obtained by refluxing the compound (133) with the base. The ether (134) was converted via the 21-tosylate to the 21-dimethylamino derivative (135). The 6 keto group was reduced by lithium aluminium hydride to a mixture of epimeric 6-alcohols (136). Treatment with formic acid regenerated the Δ^5 -3-ol-system (137) and Oppenauer oxidation of (137) afforded the 21 dimethylamino-18:20-oxido-3-oxo-4-pregnene (138). The corresponding N-oxide, when refluxed in *t*-butyl benzene resulted in the formation of the vinyl ether (139).



which was hydrolysed by dilute acids to 18-hydroxy progesterone (68). Oxidation of (68) with chromic acid in pyridine gave 3,20-diketo-4-pregnene-18-oic acid (110) and the neutral fraction,

The existing methods of partial synthesis of aldosterones besides much starting material, was identified as 3:20 diketo-4-pregnene-18-al (69).

an intramolecular transfer of groups from either position 20 or 11

resulted. Thus, Pappo has described the synthesis of i) 18-hydroxy

progesterone ii) 18-oic acid of progesterone and iii) 18-aldo-

progesterone from conessine which could be considered as attractive

probable precursors for aldosterone and related compounds. However,

further transformations on these compounds leading to aldosterone

were not carried out by Pappo. easily available steroids, conessine

and tetramethylolarrhizane, from karebi which grows abundantly in

Chart-XIV describes Pappo's scheme for syntheses of these compounds.

by periodate methods. The N-oxide of this Schiff's base was cleaved by periodate to yield progesterone-18-aldehyde which was converted into the free aldehyde PREGNEN-20-AL yields by nitroreduction.

The existing methods of partial synthesis of aldosterone by which are worthy of commercial exploitation are essentially based on intramolecular transfer of groups from either position 20 or 11 resulting in functionalization of the angular methyl group at position 18. These methods, however elegant they may appear from the viewpoint of synthetic chemist, require rather expensive starting material in the form of corticosterone derivatives. It was felt that there is still enough scope for exploration of new routes to aldosterone from the easily available alkaloids, conessine and tetramethylholarrhinine, from kurchi which grows abundantly in many parts of India.

The thesis, which begins with a brief review of literature in the first chapter, describes the methods and materials used in the second chapter.

The third chapter deals with the development of improved procedures for the isolation and separation of alkaloids from the bark and heartwood of kurchi, Holarrhena antidysenterica as well as examination of neutral extracts.

The fourth chapter describes a convenient novel synthesis of 18-aldo progesterone from conessine employing a new organic reaction - cleavage of a Schiff's base N-oxide by periodate. Conessine was converted to N-desmethyl-cona-4,20(N)-dien-3-one

by known methods. The N-oxide of this Schiff's base was cleaved by periodate to yield progesterone-18-al-oxime which was converted into the free aldehyde in high yields by nitrous acid.

The establishment of structure of the product obtained by the above method by comparison with progesterone-18-al obtained through an alternate route is the subject matter of the fifth chapter. In this synthesis progesterone was converted to 3-ethylenedioxy-5-pregnene-18,20 -lactone by known methods. Substantial modifications were introduced in the known synthesis of progesterone-18-al from the ethylene ketal lactone eliminating a few steps. This synthesis establishes another attractive preparative route to progesterone-18-al.

The sixth chapter illustrates the conversion of progesterone-18-al to aldosterone in workable yields by two successive microbiological hydroxylations at positions 11 and 21.

The seventh chapter deals with the transformations of tetramethylholarrhimine including the development of a new and convenient route to apoholarrhimine, a promising intermediate in the synthesis of 18-oxygenated steroids.

METHODS AND MATERIALS

Kurchi bark and stem

Kurchi bark as well as stem were procured from Messrs Jadavjee Lallubhai and Co. Bombay, India at the rates of Rs.0.75/kg and Rs.4.00/kg respectively. The authenticity of the materials was established by comparison of the samples obtained through the courtesy of the Forest Department, Maharashtra State, India and was also further confirmed by the Botanical Survey Department, Poona, India.

Isolation of alkaloids

The final scheme for the isolation of the two main alkaloids, conessine and tetramethylholarrhimine consisted of a modified procedure of Labler and Cerny^{35G}.

Removal of nitrogen

The general procedure employed in the removal of partially substituted nitrogen atoms, from the alkaloids was the Ruschig's procedure⁶⁵ of N-chlorination, dehydrochlorination, followed by acid hydrolysis.

N-chloro succinimide was prepared according to the procedure of Tschernijac⁶⁶.

Cyanogen bromide and perbenzoic acid were prepared according to procedure described in "Organic Syntheses"^{67,68}.

Cleavage of the N-oxide of Schiff's base was effected by treating it with sodium metaperiodate. Nitrous acid treatment according to Barton and Beaton¹³, of the cleaved product yielded

nitrogen-free steroid. These will be described in detail in the Experimental Section.

For the deamination of tertiary base in tetramethyl holarrhine via its 3 β -mono-N-oxide the method of Cope *et al.*⁶⁹ has been followed. The pyrolytic cleavage of the N-oxide followed by Hofmann's degradation resulted into a nitrogen-free steroidal ether.

Ketalization and deketalization

Wherever necessary, the carbonyl groups of ketones were protected by forming the corresponding ethylene dioxyketals with ethylene glycol and p-toluene sulphonic acid, in toluene⁷⁰. Deketalizations were carried out by using either 80 per cent acetic acid⁷¹ or glacial acetic acid and fused sodium acetate with varying time intervals depending upon the nature of the groups⁷².

Oxidation

Silver chromate used for the oxidation was prepared by the reaction between silver nitrate and potassium chromate⁷³. Silver chromate was activated for 6 hours at 220° before use.

Lead tetra acetate was prepared as per the procedure described in "Organic Syntheses"⁷⁴.

To detect the steroids on chromatograms, Jones' reagent was prepared according to the procedure described in literature⁷⁵.

Chromic acid oxidation was carried out by using chromium trioxide in aqueous pyridine. In addition, when the paper chromatograms were sprayed with the blue tetrazolium reagent were heated for 40 min at 60° the

Reduction

Reduction of carbonyl functions was carried out by lithium aluminium hydride.

Chromatographic materials

Alumina (Basic and Neutral) was supplied by the Fine Chemicals Project of this laboratory. It was made into requisite grade by adding appropriate amounts of water according to the procedure of Lederer and Lederer⁷⁶.

Silica gel for column as well as for thin layer chromatography was procured from Fine Chemicals Project of this laboratory.

Paper chromatography

Descending chromatography on Whatman No.1 paper was carried out at 26-28° with the following solvent systems:

Bush B₃ (Bush⁷⁷): Benzene-pet-ether (67:33) and methanol-water (80:20).

Bush B₄ (Bush⁷⁷): Benzene-methanol-water (100:50:50).

The steroids were extracted from paper chromatograms with methylene chloride.

Detection of steroids

To detect the steroids on chromatograms, the paper chromatograms were sprayed with a mixture of 1 ml of 0.2 per cent blue tetrazolium in ethanol and 40 ml of 10 per cent sodium hydroxide in water.

In addition, when the paper chromatograms following spraying of the blue tetrazolium reagent were heated for 40 min at 60° the compounds having a Δ^{4-3} keto group gave a yellow fluorescence under ultraviolet (300 mμ, Bush⁷⁸). Aldosterone was detected by its

fluorescence as well as positive blue tetrazelium reaction.

Optical rotations

Libermann-Burchard colour reaction⁷⁹

Optical rotations were determined in chloroform solution. To the steroidal material in approximately 0.5 ml of chloroform solution, using 1 cm tube, the concentrations are reported in the form was added one or two drops of test reagent prepared by chilling

5 ml acetic anhydride in a 10 ml flask and adding 0.5 ml of concentrated sulphuric acid. The formation of a transient-purple colour,

The ultraviolet absorption spectra were taken in ethanol changing to blue, green, pink or purple indicated the presence of steroids with a Beckman Quartz Spectrophotometer model DU.

The infrared spectra were recorded in nujol mull, unless

Zimmermann's colour reaction⁸⁰

The method, first used for determination of 17-ketosteroids, and later modified by Emmens *et al.*⁸¹ and Koch *et al.*⁸² for 3 or 20 ketosteroids consists of treating the steroid in alcohol with

m-dinitrobenzene in alcoholic potash. The presence of 17, 3 or 20 keto group is shown by the formation of transient violent coloration. 6, 7 and 12 ketones do not give the above test.

Dragendorff's reagent⁸³

Potassium bismuth iodide known as Dragendorff's reagent has been widely used for precipitating alkaloids. Stock solution of potassium bismuth iodide (KBI_4) was prepared according to Block's procedure⁸³. The reagent is prepared from the stock solution by mixing 20 ml water, few drops of 6N KCl, 2 ml stock solution of KBI_4 and 5 ml 6N HCl. With alkaloids, it gives a buff coloured precipitate.

Melting points

All melting points are uncorrected and have been taken in a Gallenkamp melting point apparatus.

Optical rotations

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

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 mull, unless otherwise stated, on a Perkin-Elmer infracord, model 173 E or Perkin-Elmer model 221 spectrometer.

Proton magnetic resonance spectra

Proton magnetic resonance spectra were recorded on a Varian A-60 spectrometer in ¹deuteriochloroform or carbon tetrachloride/chloroform solutions using tetramethylsilane as the internal standard. All the chemical shifts are expressed in terms of τ values.

An authentic sample of d-aldosterone was kindly supplied by Dr. J. J. Chart of Ciba Pharmaceutical Company, New Jersey, U.S.A.

Progesterone for the reactions was obtained from M/s. Wyeth Laboratories, Bombay, India as a research gift.

Isolation and Separation of alkaloids from
Kurchi bark

Various procedures have been described in literature regarding the extraction and isolation of kurchi alkaloids. A brief comparative survey of a few relatively simple procedures is made here.

Siddiqui and Pillay³¹ A-D isolated alkaloids from the bark of Holarrhena antidysenterica in pure form in 1932. The dried bark was ground, soaked for a week with a mixture of ether, ethanol and ammonia and percolated. A second extract was drawn three days after soaking the bark with a fresh solvent mixture. Hydrochloric acid gas was then bubbled through the extracts which precipitated the alkaloidal hydrochlorides together with a bulk of ammonium chloride. The supernatant solvent mixture was basified with ammonia and was used for further extractions. Eight such percolations were carried out. The total hydrochlorides were dissolved in water and treated with a solution of sodium sulphate which precipitated the insoluble alkaloidal sulphates. These insoluble sulphates contain holarrhimine as the major alkaloid. Bases from water-soluble sulphates were recovered by basification and extraction with chloroform; this portion was rich with conessine and other related alkaloids. The total yield of alkaloids by this procedure is 2.1 per cent.

Haworth's group³⁸ used soxhlet extraction for obtaining the total alkaloids. The bark was mixed with calcium oxide and extracted with ethanol on a soxhlet extraction unit. The yields of total alkaloids were 1.3 per cent.

This way, by sacrificing only 1 per cent of the total alkaloids

was obtained at the end of the process.

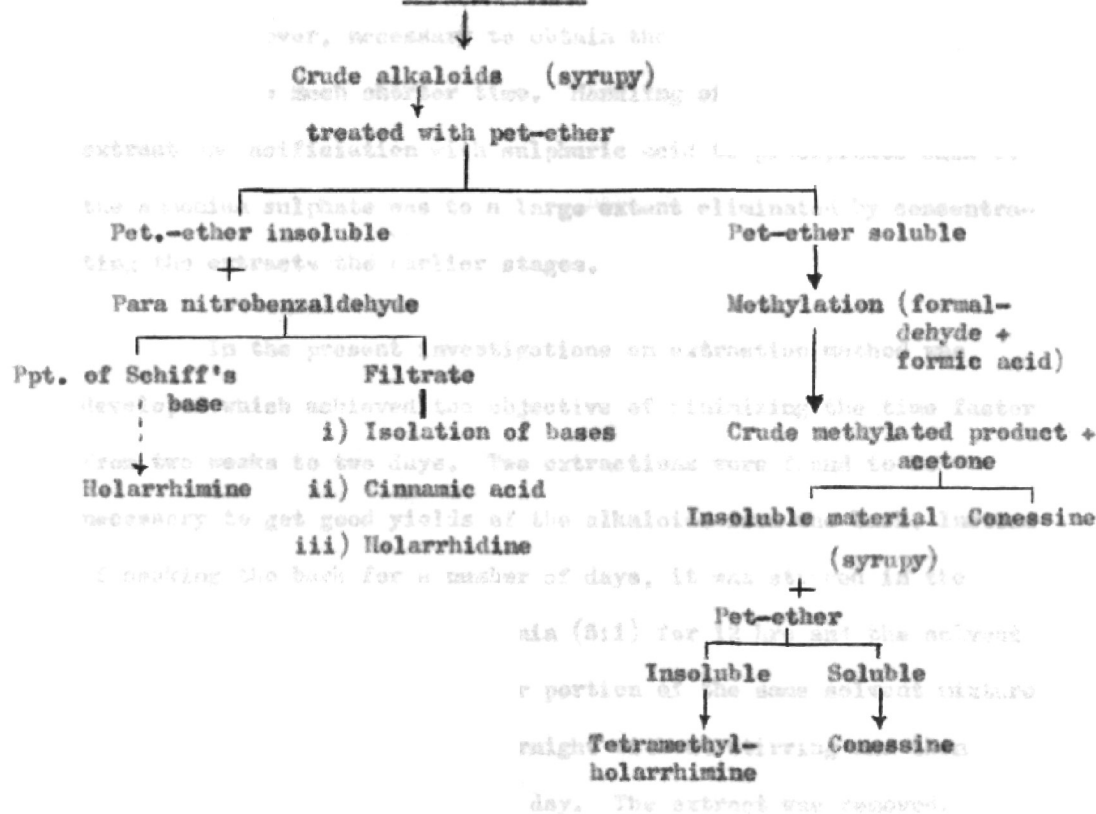
The third procedure was that reported by Tschesche and Petersen^{33A} who used chloroform in addition to the solvent mixture used by Siddiqui and Pillay. A single percolation after soaking the bark for eight days appeared to be enough to procure the total alkaloids in somewhat satisfactory yields. The concentrated extract was dissolved in chloroform and the alkaloids were extracted with aqueous hydrochloric acid. The hydrochlorides were basified and reextracted in chloroform. The chloroform solution was concentrated and chromatographed over basic alumina. The chromatographic fractions contain overlapping mixtures of alkaloids which were further separated by aqueous extraction of the alkaloids from their hydrochlorides at pH near about their pK values. The pH was adjusted by the addition of dilute ammonia solution. The total yield of alkaloids reported was 1.1 per cent.

An extraction procedure developed in this laboratory by Kulkarni⁶² was essentially on the lines of Siddiqui. Siddiqui and Pillay percolated kurchi bark eight times with a mixture of ether, alcohol, ammonia as described earlier. In the procedure developed by Kulkarni the time consuming method of percolation was replaced by an extraction procedure. Instead of soaking the bark for a number of days, it was stirred in the solvent mixture for four hours and the solvent layer was taken off. Six such extractions were necessary to extract most of the alkaloids from the bark. It was found that four extractions could yield 93 per cent of the total extractable alkaloids (37, 36, 12 and 8 per cent) and practically all holarrhimine. In this way, by sacrificing only 7 per cent of the total alkaloids

which remained in the bark, it was possible to achieve the economy in solvents and time. The extracts were further processed according to Siddiqui's method.

Another simpler method was reported by Labler and Cerny³⁵ G who extracted the bark with ethanolic ammonia (10 kgs - 30 lit. ethanol + 3 litres ammonia). The total time required for two such extractions was two weeks. The extract was then acidified in the cold with 30 per cent sulphuric acid. Bulk of ammonia was precipitated as filterable ammonium sulphate, the precipitate was filtered out and washed with ethanol repeatedly. The extract was concentrated and shaken with chloroform. The aqueous acidic layer was treated with crushed ice and basified with 20 per cent sodium hydroxide solution. The bases which separated were extracted with ether. The ether layer was washed free of alkali and the solvent was evaporated under reduced pressure. The crude bases obtained as a yellow syrup were treated with pet-ether, allowed to stand overnight and then stirred for 8 hrs. The material insoluble in pet-ether was a semi-solid solidifying slowly. The insoluble part was found to contain the holarrhimine group of alkaloids whereas the pet-ether-soluble portion consisted of alkaloids of conessine group. The pet-ether partition of alkaloids and further separation of different alkaloids have been represented in Scheme-I.

SCHEME-I



The concentrated extract was then treated with 20 per cent HCl. A choice was made among these procedures for the extraction of alkaloids. Although the original procedure reported by Siddiqui was sufficient to remove neutral material, aqueous ether layer and modified later by Kulkarni gave better yields of alkaloids, it had to be altered further owing to the use of expensive solvents such as chloroform and ether. An alternative and more economic method was the procedure followed by Labler and Cerny. A modification was, however, necessary to obtain the total alkaloids in good yield and in a much shorter time. Handling of large volumes of total extract and acidification with sulphuric acid to precipitate bulk of the ammonium sulphate was to a large extent eliminated by concentrating the extracts ^{at} the earlier stages.

In the present investigations an extraction method was developed which achieved the objective of minimizing the time factor

from two weeks to two days. Two extractions were found to be

necessary to get good yields of the alkaloids from the bark. Instead of the introductory part of the thesis. Most of the alkaloids on soaking the bark for a number of days, it was stirred in the solvent mixture of ethanol-ammonia (5:1) for 12 hrs and the solvent layer was siphoned off. Another portion of the same solvent mixture was added to the bark, left overnight without stirring and then stirred. Hence the total alkaloids from the bark were extracted and stirred for another 12 hrs next day. The extract was removed, combined with the first extract and concentrated to 1/5th of its original volume at 45-50° under vacuum when most of ammonia was expelled. The recovery of the solvents was satisfactory. The same solvent mixture was used for the next extraction with addition of fresh liquor ammonia.

The concentrated extract was then treated with 20 per cent H_2SO_4 till it became acidic to litmus. The acidic extract was treated with chloroform to remove neutral material. Aqueous acidic layer was basified with 20 per cent sodium hydroxide with external cooling. The precipitated alkaloids were taken in ether, ether layer washed free of alkali and evaporated to yield a syrupy mixture of total alkaloids. Two extractions yielded 2.6 per cent of total alkaloids.

In this modified procedure the stress has been made on two aspects;

- (1) two extractions of the bark require only two days and the yield of the total bases is satisfactory, and
- (2) concentration of the extract at earlier stages helps to recover solvents quantitatively thus making the method somewhat more economical.

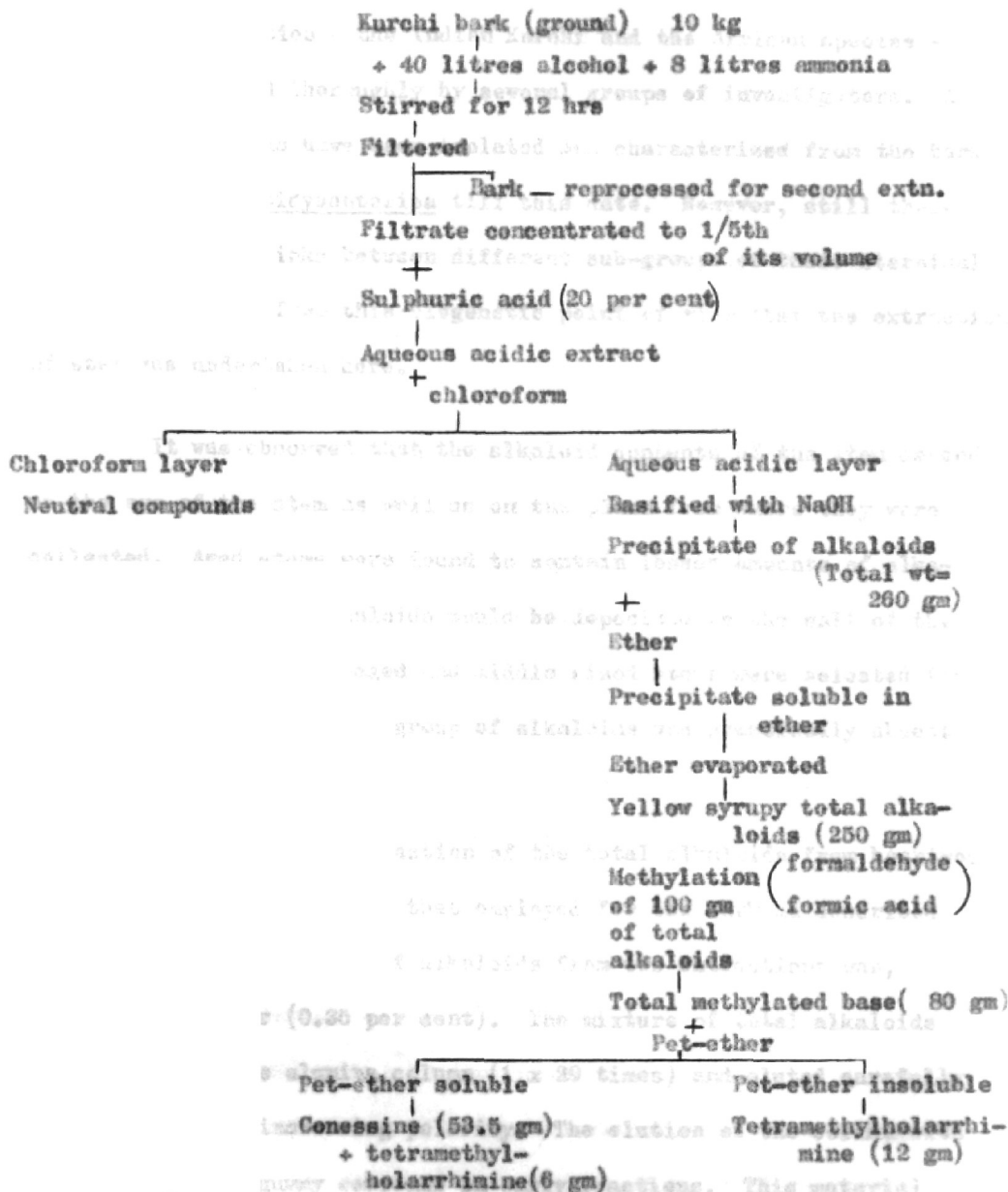
The interrelationship of kurchi alkaloids has been described in the introductory part of the thesis. Most of the alkaloids on methylation, could be converted mainly into conessine and tetramethyl holarrhinine, in quantitative yields. In the present investigations transformations of conessine and tetramethyl holarrhinine have been studied. Hence the total alkaloids from the bark were methylated to get fully methylated alkaloids - conessine and tetramethyl holarrhinine in improved yields. Further separation of these was effected utilizing their differential solubility behaviour towards pet-ether and by column chromatography. Column chromatography of petroleum ether soluble syrup on basic alumina gave pure conessine in first few eluate

(Yield: Conessine, 51.5%; Tetramethylholarrhinine, 5% - based on total methylated alkaloids)

fractions of pet-ether-benzene mixture and then with benzene. Relatively polar compound eluted by benzene containing 1 per cent Methanol was found to be tetramethyl holarrhimine. These have been shown in Scheme-II.

A literature survey revealed that the alkaloids present in

SCHEME-II



(Separated over basic alumina)

(Yield: Conessine, 53.5%; Tetramethylholarrhimine, 6% - based on total methylated alkaloids).

Examination of the stem of *Holarrhena antidysenterica*

A literature survey revealed that the stem of this plant has not been examined for its alkaloidal contents. The seeds and the bark of both the varieties - the Indian Kurchi and the African species - have been examined thoroughly by several groups of investigators. A number of alkaloids have been isolated and characterized from the bark and seeds of *H. antidysenterica* till this date. However, still there are some missing links between different sub-groups of these steroidal alkaloids. It is from this biogenetic point of view that the extraction of stem was undertaken here.

It was observed that the alkaloid contents of the stem depend on the age of the stem as well as on the place from where they were collected. Aged stems were found to contain lesser amounts of alkaloids as more of the alkaloids would be deposited in the wall of the stem i.e. bark. Middle-aged and middle sized stems were selected for the studies. Holarrhimine group of alkaloids was practically absent in this middle-aged stems.

The method of extraction of the total alkaloids from heartwood was essentially the same as that employed for the bark as described earlier. The total yield of alkaloids from two extractions was, however, very poor (0.35 per cent). The mixture of total alkaloids was put over basic alumina column (1 x 20 times) and eluted carefully with solvents of increasing polarity. The elution of the column with pet-ether gave a gummy compound in early fractions. This material

less polar than conessine was found to be homogeneous by TLC on systems containing chloroform, methylethylketone and dimethylamine and chloroform freshly saturated with ammonia.

The infrared spectrum of the compound showed a strong band at 1738 cm^{-1} and a band at 1282 cm^{-1} characteristic of an ester group. However, the compound did not give any precipitate with Dragendorff's reagent, a typical test for alkaloids. The compound was further found to be insoluble in most of the common solvents, acetone, ethyl acetate and chloroform, but soluble in pet-ether. Preliminary studies indicated that the compound was a fatty acid ester.

The column was further eluted with pet-ether-benzene mixture (1:1). The initial three fractions gave a solid which was crystallized from acetone and identified as conessine from its physical constants. Further elution with pet-ether-benzene 50% and then 80% benzene in pet-ether gave more of conessine. The later fractions of benzene exhibited a mixture of three compounds as indicated by TLC. The column was further eluted with 10% ethyl acetate in benzene, which gave a mixture of isoconessimine and conessimine (vide TLC) - a gum which crystallized after passing over a short column of basic alumina. The products were identified by their physical constants.

Further elution of the column with 20% ethyl acetate in benzene (4 fractions) gave a semi-crystalline compound showing two spots on TLC one corresponding to isoconessimine, the other having a different R_f value. On adding ether, a part of the mixture precipitated

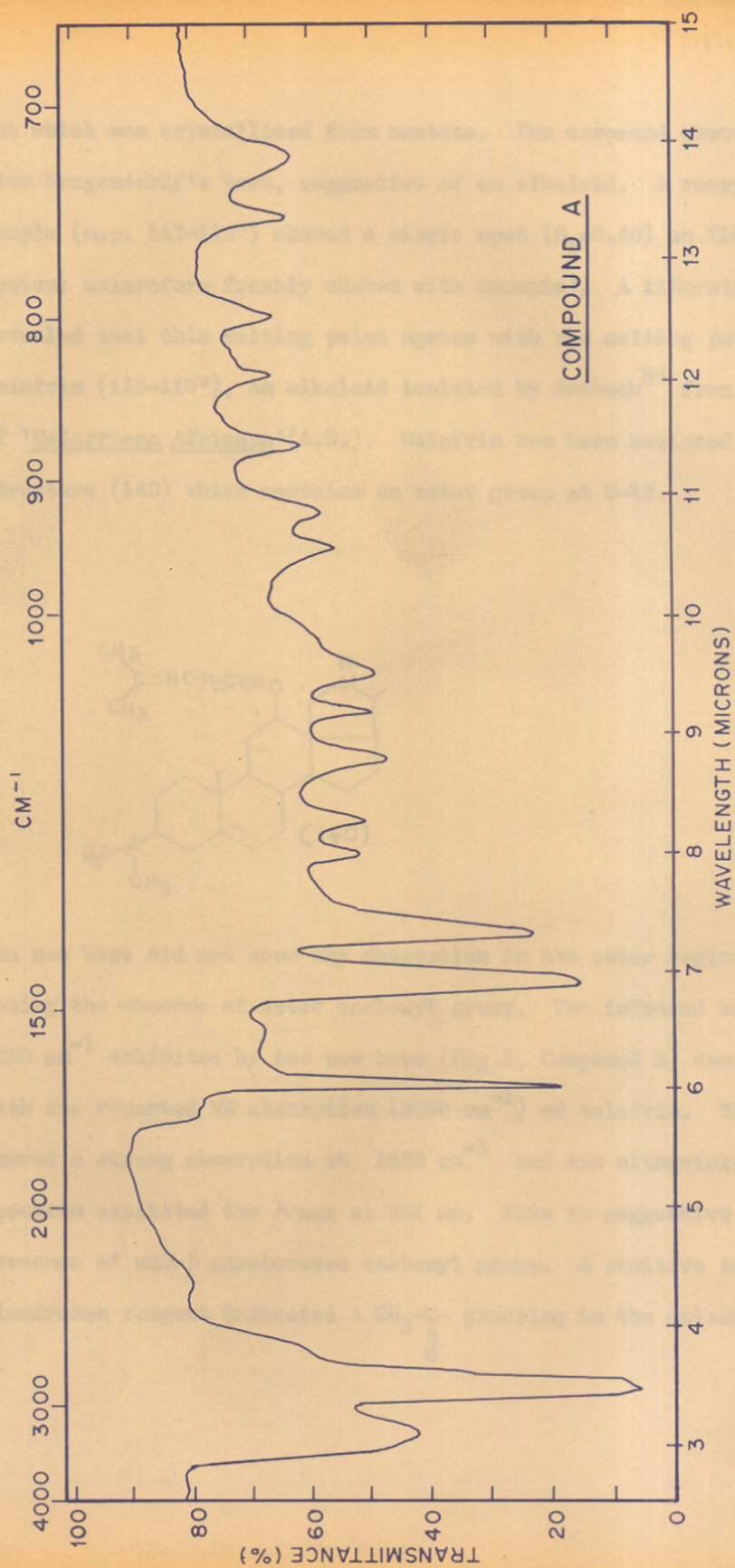
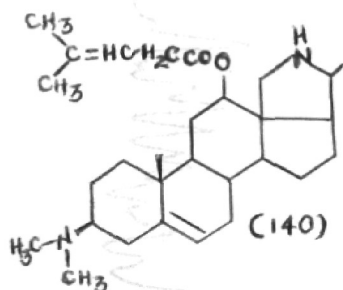


FIG. 1. IR SPECTRUM (NUJOL) OF COMPOUND A.

out which was crystallized from acetone. The compound showed a positive Dragendorff's test, suggestive of an alkaloid. A recrystallized sample (m.p. 117-119°) showed a single spot ($R_f=0.40$) on TLC (solvent system: chloroform freshly shaken with ammonia). A literature survey revealed that this melting point agrees with the melting point of Helafrin (115-116°), an alkaloid isolated by Seebach⁸⁴ from the barks of 'Holarrhena Africana' (A.D.). Helafrin has been assigned the structure (140) which contains an ester group at C-12.



The new base did not show any absorption in the ester region, indicating the absence of ester carbonyl group. The infrared band at 3330 cm^{-1} exhibited by the new base (Fig.1, Compound A) does not agree with the reported NH absorption (3260 cm^{-1}) of helafrin. The base showed a strong absorption at 1668 cm^{-1} and its ultraviolet spectrum exhibited the λ_{max} at 241 mμ. This is suggestive of the presence of an $\alpha\beta$ unsaturated carbonyl group. A positive test with Zimmermann reagent indicated a $\text{CH}_2\text{-C}(=\text{O})$ grouping in the molecule.

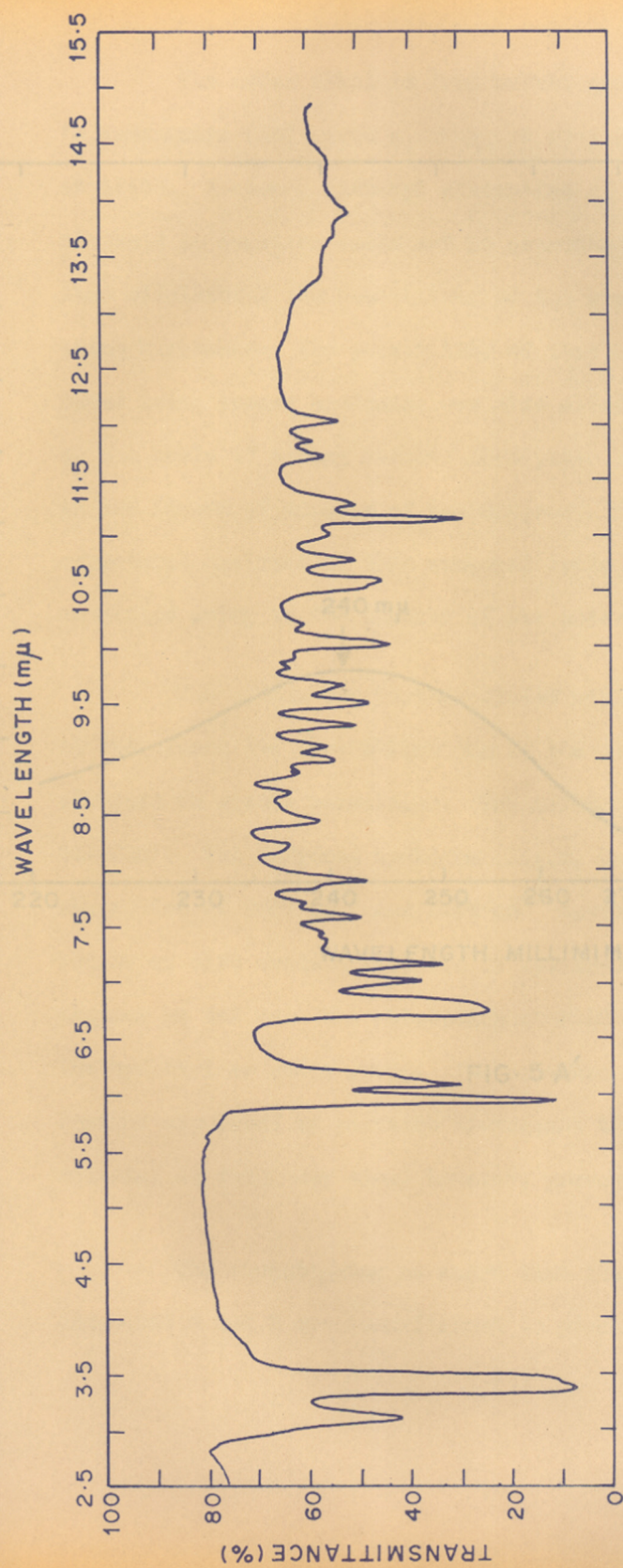


FIG. 2 COMPOUND B

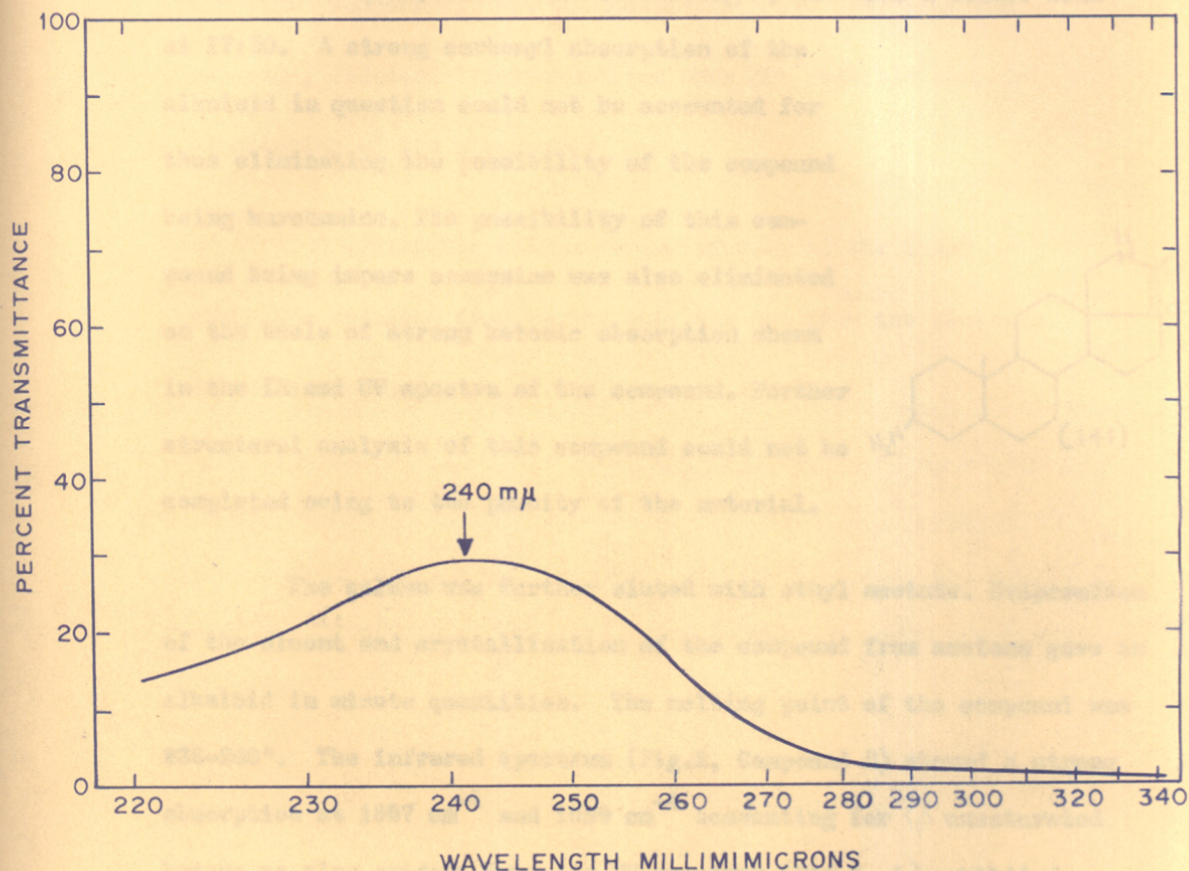
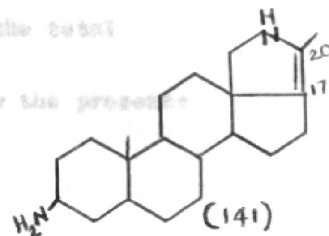


FIG. 5 A'

The other alkaloid from kurchi group melting at 115-17° is kurchamine (141) with a saturated ring B, but with a double bond at 17:20. A strong carbonyl absorption of the material, chemical transformations were not possible towards alkaloid in question could not be accounted for thus eliminating the possibility of the compound being kurchamine. The possibility of this compound being impure conessine was also eliminated on the basis of strong ketonic absorption shown in the IR and UV spectra of the compound. Further structural analysis of this compound could not be completed owing to the paucity of the material.



The column was further eluted with ethyl acetate. Evaporation of the eluent and crystallization of the compound from acetone gave an alkaloid in minute quantities. The melting point of the compound was 236-240°. The infrared spectrum (Fig.2, Compound B) showed a strong absorption at 1667 cm⁻¹ and 1639 cm⁻¹ accounting for β unsaturated ketone as also evident from its UV spectrum (Fig.5, A) exhibited a maximum at 241 m μ . The absorption at 3226 cm⁻¹ in IR indicated either the hydroxyl or amino group. The presence of a ketonic group was further supported by positive Zimmermann test. The paucity of material in this case also, however, prevented further work.

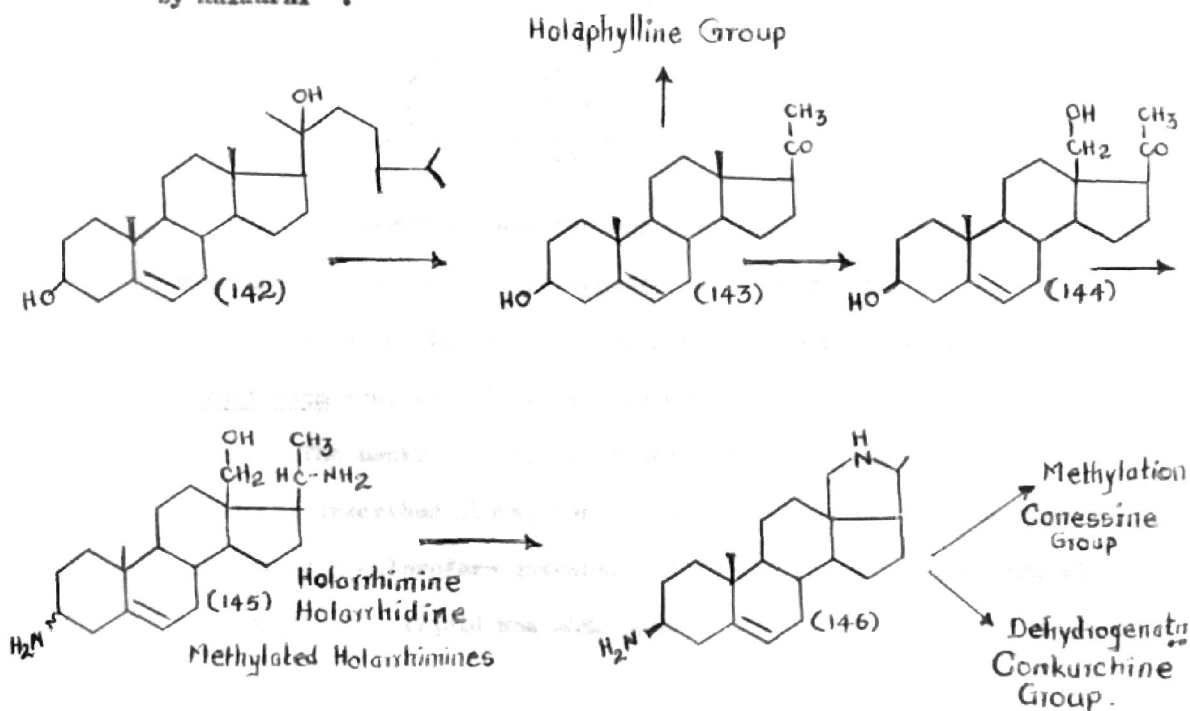
The investigator is aware that the data collected on the compounds A and B are insufficient to propose any tentative structure

for these compounds. Due to considerably low yields of the material, chemical transformations were not possible towards elucidation of the structures.

A systematic column chromatography of the total alkaloids obtained from kurchi bark did not show the presence of these compounds.

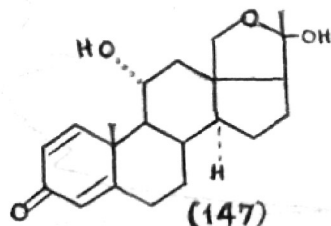
Examination of neutral fraction from kurchi stem extract

Kurchi stem was not examined so far either for its alkaloidal contents or for its steroidal contents. All the steroidal alkaloids isolated so far from H. antidysenterica wall have the pregnane nucleus with C-21 atoms. 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 pathways⁸⁵. It has been established that in the adrenal gland⁸⁶ in the animals the origin of the C-21 steroids is by oxidative cleavage of the isoprenoid side chain of cholesterol initiated by a hydroxylation at C-20. Extending further the concept of biogenetic parallelism in plants and animals for the formation of C-21 steroids, the following tentative scheme for the biogenesis of kurchi alkaloids was formulated by Kulkarni⁸².



The plant accumulates the pregnane alkaloids in larger quantities in the bark than in the stem. Does it store any neutral precursors such as 20-hydroxy steroids, pregnenolone or 18-hydroxy progesterone particularly in the stems?

Neutral steroids from kurchi bark extract were isolated by Siddiqui and Pillay⁸⁷ as early as in 1933. Later in 1963, Tschesche et al.⁸⁸ reported the isolation of a non-nitrogenous C-21 steroid from neutral extract of the kurchi bark which they named as holadyson.⁽¹⁴⁷⁾ It has the molecular formula $C_{21}H_{28}O_4$ and on the basis of its physico-chemical studies the following structure has been assigned to it.



Its structure was further confirmed by its partial synthesis starting from 11 α -acetoxy-progesterone by Tschesche⁸⁸.

A systematic investigation of the neutral components of kurchi stem was, therefore, initiated.

The neutral part, separated during the extraction of alkaloids, as described above, was treated with excess water to remove alcohol from chloroform extract. Chloroform was then concentrated when a greenish oily liquid was obtained.

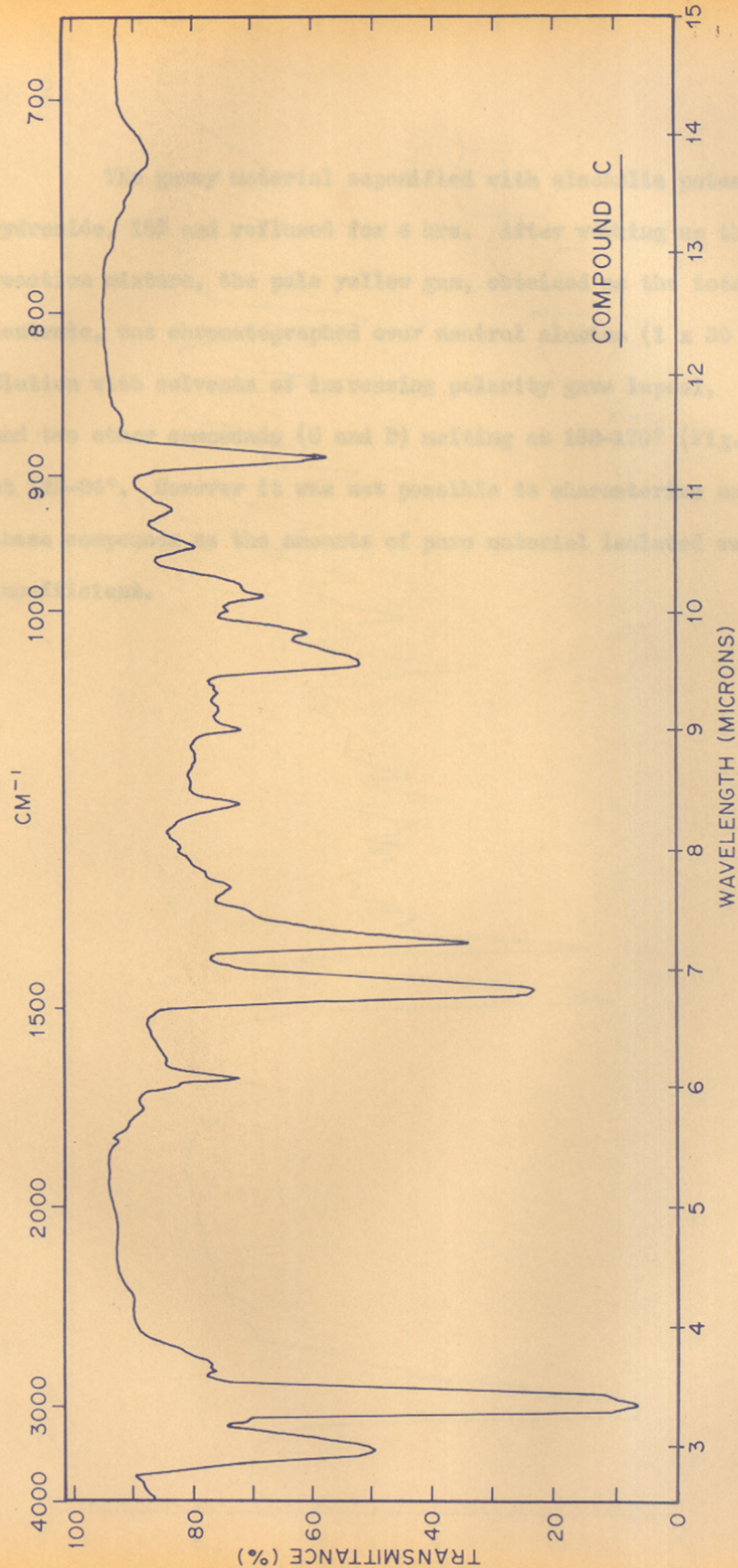


FIG. 3. IR SPECTRUM (NUJOL) OF COMPOUND C.

The gummy material saponified with alcoholic potassium hydroxide, 10% and refluxed for 4 hrs. After working up the reaction mixture, the pale yellow gum, obtained as the total concentrate, was chromatographed over neutral alumina (1 x 30 times). Elution with solvents of increasing polarity gave lupeol, sitosterol and two other compounds (C and D) melting at 168-170° (Fig.3), and at 122-24°. However it was not possible to characterize any of these compounds as the amounts of pure material isolated were insufficient.

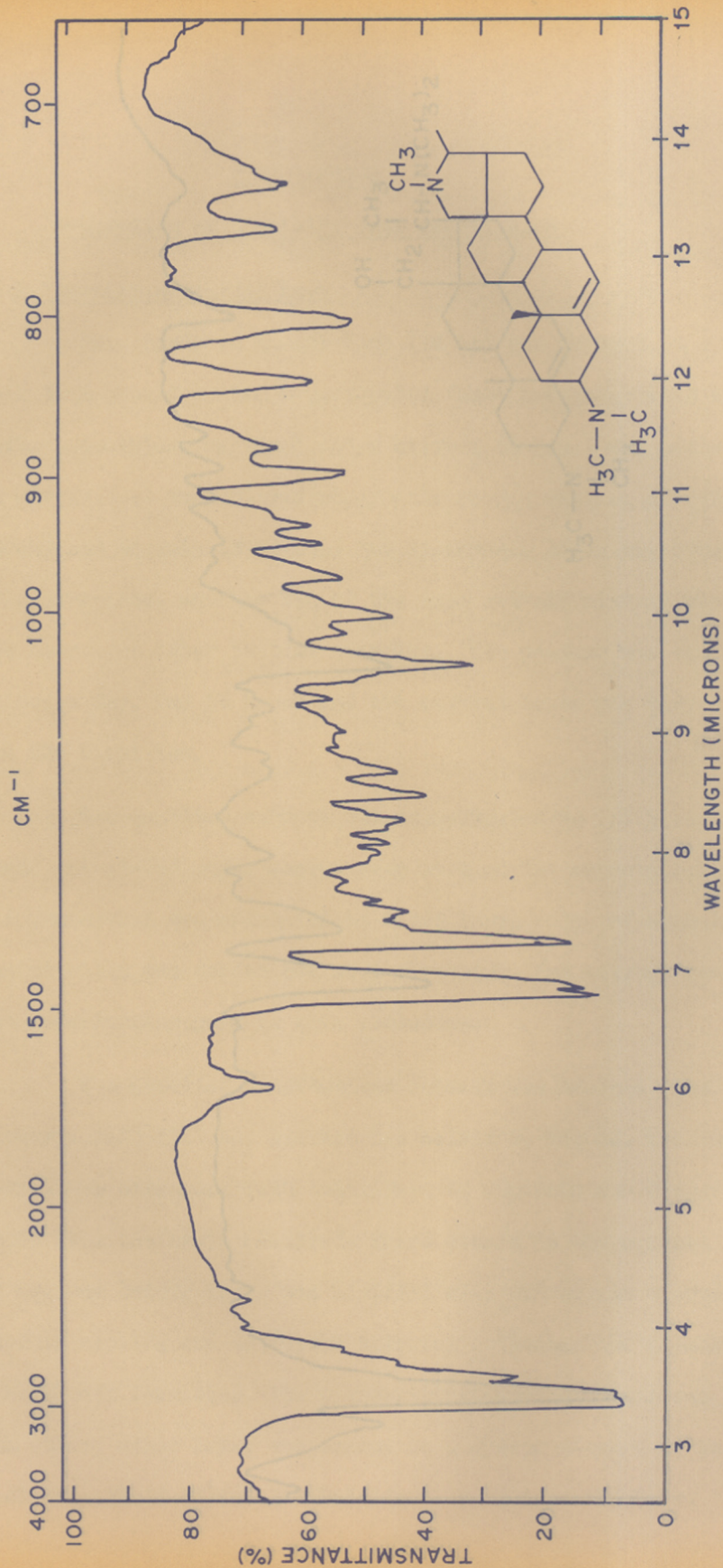


FIG. 4. IR SPECTRUM (NUJOL) OF CONESSINE.

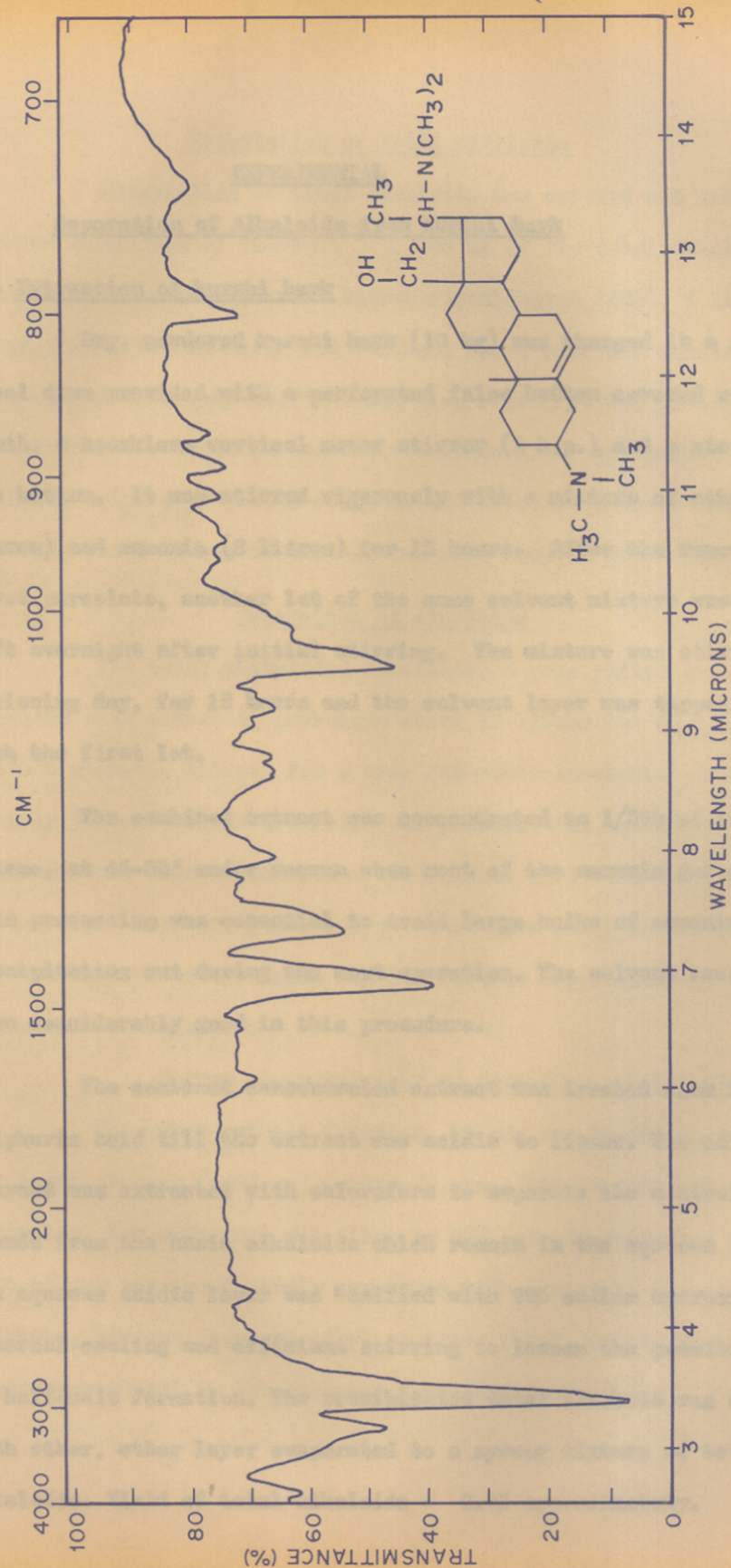


FIG. 5. IR SPECTRUM (NUJOL) OF TETRAMETHYL HOLARRHIMINE.

Methylation of Total Alkaloids

EXPERIMENTAL

Methylation of total alkaloids was carried out according to

Separation of Alkaloids from Kurchi bark

(1) Extraction of kurchi bark

Dry, powdered kurchi bark (10 kg) was charged in a stainless steel drum provided with a perforated false bottom covered with muslin cloth, a sparkless vertical motor stirrer (1 h.p.) and a stop-cock at the bottom. It was stirred vigorously with a mixture of ethanol (10 litres) and ammonia (8 litres) for 12 hours. After the removal of the first percolate, another lot of the same solvent mixture was added and left overnight after initial stirring. The mixture was stirred the following day, for 12 hours and the solvent layer was tapped and mixed with the first lot. The combined extract was concentrated to 1/5th of its original volume, at 45-50° under vacuum when most of the ammonia gas was expelled. This processing was essential to avoid large bulks of ammonium sulphate precipitating out during the next operation. The solvent recovery was also considerably good in this procedure.

The combined concentrated extract was treated with 20% sulphuric acid till the extract was acidic to litmus. The acidic extract was extracted with chloroform to separate the neutral compounds from the basic alkaloids which remain in the aqueous layer. The aqueous acidic layer was basified with 20% sodium hydroxide with external cooling and efficient stirring to lessen the possibility of half-salt formation. The precipitated total alkaloid was extracted with ether, ether layer evaporated to a syrupy mixture of total alkaloids. Yield of total alkaloids - 2.6% approximately.

Methylation of Total alkaloids

Methylation of total alkaloids was carried out according to method described by Siddiqui^{31 B}. 100 gm of the total alkaloids were treated with a mixture of 4 litres formaldehyde (40%), 4 litres formic acid (80%), 500 ml water and refluxed for 4 hrs. The reaction mixture was cooled thoroughly and basified with stirring. The precipitated alkaloids were taken in benzene. Benzene layer was washed free of alkali, dried over sodium sulphate and evaporated under vacuum below 55-60°. Final traces of the solvent were removed under high vacuum.

Separation of alkaloids

The total methylated alkaloids, a pale yellow syrup, was treated with excess of petroleum ether (2 litres for 80 gm) and stirred with a magnetic stirrer for 8 hrs. Pet-ether insoluble part consisting mainly tetramethyl holarrhinine was precipitated out which was filtered over Buchner funnel. The pet-ether soluble part contained mainly conessine. However, conessine obtained here still contained some traces of tetramethyl holarrhinine and its further separation was effected by column chromatography.

Column chromatography of pet-ether soluble fraction

Pet-ether soluble fraction, 68 gms was loaded on to a column of basic alumina (1400 gm), grade II, in a 120 cm tall column with 9 cm diameter. The substance was taken in 50% pet-ether-benzene mixture and the column was successively developed with a mixture of pet-ether and benzene, benzene, benzene-ethyl acetate, ethyl acetate and ethyl acetate-methanol in the sequence described in Table-I.

Fractions 1 to 6 were pooled together, concentrated from a mixture

of benzene and ether, after passing it over a short column of basic alumina

and eluting with benzene. The compound melted at 130-131°C. For

TABLE I

COLUMN CHROMATOGRAPHY OF PET-ETHER SOLUBLE FRACTION

Wt. of the total alkaloids = 68 gm

Basic alumina, (grade II), = 4400 gm

Fraction No.	Eluent	Volume ml	Weight gm	Nature of the residue
1	50% Pet-ether-benzene	250	-	-
2	-do-	250	3.5	crystalline
3	-do-	500	32.1	crystalline
4	-do-	500	11.9	crystalline
5	Pet-ether-benzene 80%	250	4.17	crystalline
6	-do-	250	1.82	crystalline
7	Benzene	250	0.75	semi crystalline
8	Benzene	250	0.68	semi crystalline
9	Benzene-ethyl acetate 10%	250	0.41	gummy
10	Benzene-ethyl acetate 50%	250	0.17	gummy
11	Ethyl acetate	250	0.175	oily traces
12	Ethyl acetate-methanol 5%	250	4.80	crystalline
13	Ethyl acetate-methanol 10%	250	1.2	crystalline
14	Ethyl acetate-methanol 25%	250	0.45	oily
15	Methanol	1000	2.05	colored residue, oily
			Total recovery	64 gm

Fractions 1 to 6 were pooled together, crystallized from a mixture of acetone-ether, after passing it over a short column of basic alumina and eluting with benzene. The compound melted at 124-25°, lit. for conessine^{31A} 126°. $[\alpha]_D = +121^\circ$ (c=0.018 in ethanol).

Analysis: C 80.58, H 11.01, N 7.54, N-CH₃ 12.31%.

Conessine C₂₄H₄₀N₂ requires: C 80.89, H 11.24, N 7.87, N-CH₃ 12.64%.

IR (Fig.4). Band at 2820 cm⁻¹ indicating the presence of N-CH₃ grouping.

Band at 1667 cm⁻¹ for 5:6 double bond.

Pet-ether insoluble part

Pet-ether insoluble part was dissolved in boiling ethanol. On cooling light crystals separated out. Fractions 12 and 13 from the above column chromatography were combined with this crystalline compound and recrystallized from methanol. The identity of the compound as tetramethyl holarrhimine was confirmed from its physical constants. m.p. 220-226°. lit.^{31A} 224-226°.

$[\alpha]_D = -45.5^\circ$ (c=0.01 in absolute alcohol).

Analysis: Found C 76.91, H 11.39, N 7.25%. Tetramethyl holarrhimine C₂₅H₄₄ON₂ requires: C 77.02, H 11.34, N 7.21%.

IR (Fig.5) showed a band at 2820 cm⁻¹ accounting for N-CH₃ group.

Absorption at 3320 cm⁻¹ and at 1667 cm⁻¹ representing hydroxyl group and 5:6 double bond respectively.

Acetate of tetramethyl holarrhimine was prepared by acetic anhydride and pyridine m.p. 136-37°.

Kurchi stem

Isolation and separation of alkaloids from stem

Kurchi stems, dried at 50° for 8 hrs under vacuum were powdered and charged in a stainless steel vessel provided with perforated false bottom.

TABLE II (contd.)

covered with muslin cloth. Method of extraction was essentially the same as that used for bark. The total alkaloids (7 gm from 2 kg) were put over basic alumina column (20 times the weight of alkaloids) and eluted successively with solvents of increasing polarity. The results are tabulated in Table II.

TABLE II

COLUMN CHROMATOGRAPHY OF TOTAL ALKALOIDS FROM STEM

Wt. of crude alkaloidal mixture 7 gm.

Wt. of alumina basic (grade II) 140 gm.

Fraction No.	Eluent	Volume ml	Weight gm	Residue (nature)
1	Pet-ether-benzene 50%	15	.006	oily
2	-do-	15	.014	oily
3	-do-	50	.070	oily
4	-do-	50	.030	oily
5	-do-	50	.008	oily
6	Pet-ether-benzene 80%	50	.750	crystalline
7	-do-	100	1.500	crystalline
8	-do-	50	.230	crystalline
9	-do-	50	.080	crystalline
10	Benzene	50	.021	semi cryst.
11	Benzene	50	.210	crystalline
12	Benzene	50	.410	crystalline
13	Benzene	50	.031	semi cryst.
14	Benzene	50	.020	semi cryst.
15	Benzene-ethyl acetate 10%	50	.020	semi cryst.
16	-do-	50	.114	semi cryst.

contd.

TABLE II (contd.)

Fraction No.	Eluent	Volume ml	Weight gm	Residue nature
17	Benzene-ethyl acetate 10%	50	.300	semi cryst.
18	do	50	.037	semi cryst.
19	do	50	.017	semi cryst.
20	Ethyl acetate 20%-Benzene	50	.020	semi cryst.
21	do	50	.038	semi cryst.
22	do	50	.029	semi cryst.
23	do	50	.020	semi cryst.
24	Benzene-ethyl acetate 50%	50	.035	gum
25	do	50	.030	gum
26	Ethyl acetate	50	.037	gum
27	Ethyl acetate	50	.105	gum
28	Ethyl acetate	50	.105	semi cryst.
29	Ethyl acetate	50	.075	semi cryst.
30	Ethyl acetate	50	.031	gum
31	Ethyl acetate	50	.025	gum
32	Ethyl acetate	50	.017	gum
33	Methanol	200	2.03	gum
			Total recovery	6.46 gm

of authentic leucosaccharin.

Fractions 1 to 5 were pooled together. A gummy compound insoluble in most of the common polar solvents, but soluble in pet-ether was isolated. As the compound gave a negative test Dragendorff's reagent it was concluded

that the compound was not an alkaloid but a fatty acid ester. IR spectra showed a strong band at 1750 cm^{-1} , the characteristic of an ester.

Fractions 6 to 13 were pooled together, passed over a short alumina column. The crystalline compound obtained gave a precipitate with Dragendorff's reagent. The alkaloid was identified as conessimine, from its physical constants. Fractions 15 to 19 were pooled together and rechromatographed over a small (basic) alumina column. Fractions eluted by 5% ethyl acetate in benzene gave an alkaloid, identified as conessimine from its TLC behaviour. Mixed melting point with authentic sample of conessimine showed no depression.

$$[\alpha]_D = -22.6^\circ (c = 0.017).$$

Analysis: Found- C 80.32, H 11.20, N 8.14, N-CH₃ 8.44%.

Conessimine, C₂₃H₃₈N₂ requires C 80.70, H 11.10, N 8.20, N-CH₃ 8.86%.

IR showed an absorption at 3260 cm^{-1} for NH.

Fractions 20 to 23 were treated with excess ether. Ether soluble part on the basis of its behaviour on TLC was combined with the fractions (10% ethyl acetate-benzene) obtained in the above rechromatography. A careful crystallization from ethyl acetate gave isoconessimine melting at 90-92°. Mixed melting point 90°. $[\alpha]_D = +31^\circ$.

Analysis: Found- C 80.45, H 10.96, N 7.92, N-CH₃ 8.50%.

Isoconessimine, C₂₃H₃₈N₂ requires: C 80.70, H 11.10, N 8.19, N-CH₃ 8.80%.

IR showed a band at 3260 cm^{-1} for NH (spectrum identical with that of authentic isoconessimine).

The ether-insoluble part from fractions 20 to 23 was dissolved in acetone. Crystallization from the same solvent yielded a compound different from conessimine and isoconessimine. The compound gave a precipitate with Dragendorff's reagent.

Analysis: Found C 77.82, H 11.41, N 7.94%.

U.V. $\lambda_{\max}$ 241 m μ . I.R. band 3330 cm^{-1} (OH or NH), 1668 cm^{-1} (carbonyl group) (Fig. 1, Compound A).

Fractions 24 and 25 were pooled and rechromatographed. It gave a positive test with Dragendorff's reagent. TLC showed that it is a mixture of three compounds and further separation was not possible.

Fractions 26 to 30 when treated with ether, gave an ether insoluble precipitate which was recrystallized from acetone. The compound gave a precipitate with Dragendorff's reagent. m.p. 236-240°. U.V. $\lambda_{\max}$ 240 m μ . I.R. (Fig. 2, Compound B) 3226 cm^{-1} (NH or OH), 1667 cm^{-1} , 1639 cm^{-1} (α - β -unsaturated ketone).

However, due to very low yield of this compound from stem, further experiments for determining its structure were not undertaken. Helarrhimine group of compounds were found to be absent in the stems. Final elution of the column with methanol gave a gummy material. This mixture of highly polar compounds was not studied further.

Examination of the neutral non-saponifiable material from the stem

Neutral fraction of the stem extracted (in chloroform) was freed from excess of alcohol by adding water and separating chloroform layer. Chloroform layer was concentrated under vacuo at 50° and the gummy material (8 gm) was treated with 1.5 litres of alcoholic KOH (10%) and refluxed for 4 hrs. After 4 hrs, the reaction mixture was diluted with excess of water. The gummy precipitate obtained was taken with depression, $[\alpha]_D^{25} = +27^\circ (\c = 0.010)$.

ether, washed free of alkali and evaporated to get a residue (about 8 gm) which was chromatographed over neutral alumina column (grade II).

TABLE III

COLUMN CHROMATOGRAPHY OF NEUTRAL EXTRACT OF KURCHI STEM

Wt. of substance 8 gm.

Wt. of neutral alumina (grade II) 160 gm.

Fract. No.	Eluent	Volume ml	Weight gm	Nature of residue
1	Pet-ether	50	.130	oily
2	-do-	50	.060	
3	Pet-ether-benzene 50%	50	.030	
4	-do-	50	.024	
5	-do-	50	.020	-
6	-do-	50	.020	-
7	Pet-ether-benzene 80%	50	.780	Semi cryst.
8	-do-	50	1.817	Same behaviour on TLC
		50	.532	
9	-do-	100	.125	Pooled together
10	-do-	50		
11	Benzene	50	.632	Cryst.
12	Benzene	50	.421	Pooled together
13	Benzene	50	.215	
14	Benzene	50	.075	
15	Benzene-methanol 2.5%	50	.275	Cryst. pooled
			.183	
16	Benzene-methanol 2.5%	50	.162	
17	Benzene-methanol 2.5%	50	.042	
18	Benzene-methanol 2.5%	50		
19	Benzene-methanol 2.5%	50		
20	Benzene-methanol 5%	50	.062	Cryst. pooled
21	Benzene-methanol 10%	50	.187	
22	Benzene-methanol 10%	50	.285	
23	Benzene-methanol 25%	250	1.035	gummy

Total recovery 5.973

Fractions 7 to 10 having the same mobility in TLC were

pooled together and recrystallized from methanol as clusters of

needles. m.p. 210-212°. Mixed melting point with lupeol showed no

depression. $[\alpha]_D = +27^\circ (c = 0.019)$.

28

Analysis: Found- C 84.47, H 11.63%. Lupeol $C_{30}H_{50}O$ requires C 84.90, H 11.83%. Acetate of lupeol m.p. 213-15°. I.R. spectrum identical with that of lupeol.

Fractions 11 to 15 were pooled together and recrystallized from methanol after filtration through a small column of neutral alumina. m.p. 138-139.5°. Mixed melting point with β -sitosterol 138-40°. $[\alpha]_D = -37^\circ$ (c = .02).

Analysis: Found- C 83.35, H 11.96%. β -sitosterol requires ($C_{29}H_{50}O$) C 83.99, H 12.15%.

Fractions 15 to 18 were pooled together. On TLC the pooled fraction showed two spots. On repeated crystallization from acetone, the material was resolved into lower melting (163-168°) which appeared first and a higher melting one (210-212°). On repeated crystallization the lower melting component yielded pure crystalline compound with m.p. 170-71° (Compound C). $[\alpha]_D = +33^\circ$ (c = 0.016).

Analysis: Found- C 84.3, H 11.25%. I.R. spectra (Figure 3, Compound C) showed a hydroxyl band at 3333 cm^{-1} .

The higher melting compound was confirmed as lupeol.

Fractions 19 and 20 pooled together and recrystallized from acetone. m.p. 122-24° (Compound D). $[\alpha]_D = -32^\circ$ (c = 0.018).

Analysis: Found- C 76.50, H 11.10%. I.R. spectrum showed an absorption at 3260 and 1052 cm^{-1} . Further investigations on this compound were not carried out owing to its low yields from stem. On TLC (solvent - benzene) lupeol, β -sitosterol, Compound C and Compound D showed the R_f values as 0.625, 0.5, 0.375 and 0.25 respectively. Further elution of the column yielded a mixture of polar compounds, difficult to separate.

TRANSFORMATIONS OF CONESSINE

Discussion

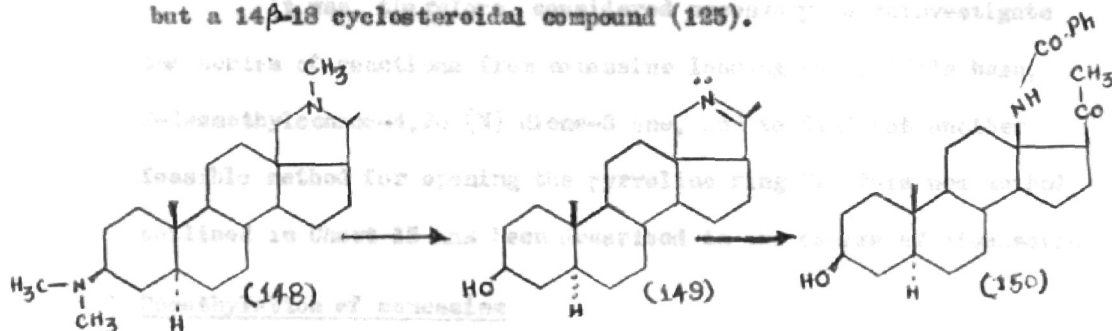
Conessine has been converted into steroidal intermediates by different groups of workers. Cerny and Sorm⁶⁰ reported the degradation of dihydroconessine (148) to 18-benzoylamino-5-pregnan,3 β -ol-20-one (150). Jeger *et al.*⁶¹ and Kulkarni⁶² synthesized 18-hydroxyprogesterone (68) from conessine, independently. Although the synthesis of N-desmethyleonan-4,(20) N diene-3-one (124), Δ Schiff's base, and 18-hydroxyprogesterone (68) has been reported by Jeger, no experimental details or yields were reported. In 1964, Dauben *et al.*⁶⁴ restudied the synthesis of Schiff's base (124) from conessine (113), describing experimental details.

All the four pathways, although reported independently, involved in principle, the degradation of tertiary amino groups of the alkaloid by cyanogen bromide to secondary amino groups which subsequently was degraded via chloramines to the Schiff's base.

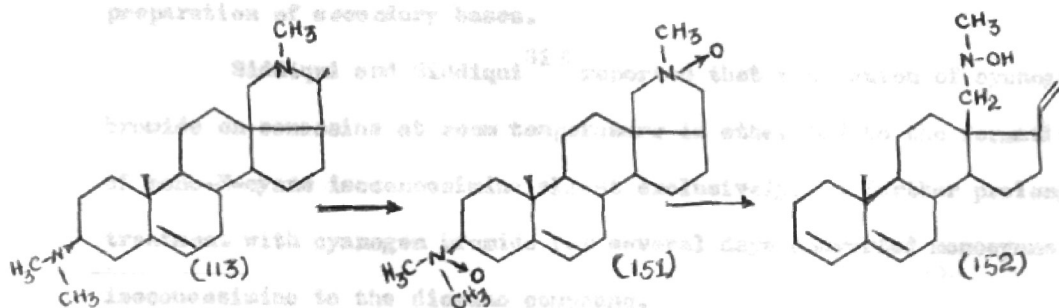
Opening of the pyrroline ring of Schiff's base type of compounds e.g., (124) and (149) was achieved in diverse ways by different workers. Cerny and Sorm⁶⁰ opened the pyrroline ring (E) in compound (149) by benzylation with benzoyl chloride in aqueous alkaline medium with the formation of 18-benzoylamino-5 pregnan,3 β -ol-20-one (150). The imino ring E of conessine (113) was opened by Haworth and co-workers⁴⁴, by Hofmann degradation. The same object was achieved by Bhattacharyya *et al.*³⁶ by preparing a di-N-oxide of

conessine (151) and subjecting it to pyrolysis which resulted in compound (152).

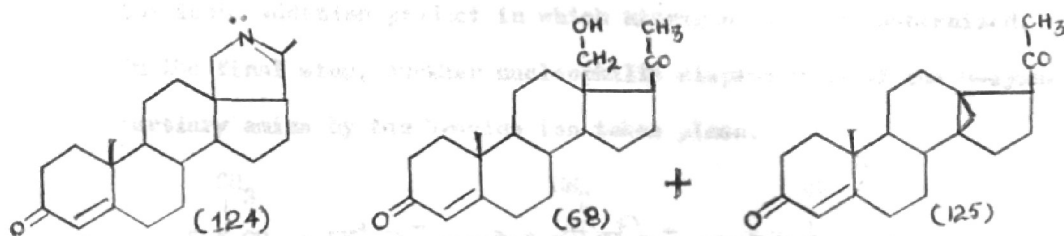
Dauben⁶⁴ showed that the action of nitrous acid on N-desmethyl-conane-4,20 (N)diene-3-one (124), the Schiff's base, for opening of ring E mostly gave rearranged product. Earlier Jeger *et al.*⁶¹ and Kulkarni⁶² reported the formation of 18-hydroxy progesterone (68) as a major product when Schiff's base (124) was treated with nitrous acid - in unspecified yields, whereas Hora and Cerny⁶³ reported the yield of (68) as only 10.5%. Dauben concluded that the major deamination product for the reaction of nitrous acid on pyrroline compound (124) is not the 18-hydroxyprogesterone (68) but a 14 β -18 cyclosteroidal compound (125).



For fully substituted tertiary amines, the reaction of cyanogen bromide reaction of demethylation was a useful for the preparation of secondary bases.



The initial reaction of cyanogen bromide on a basic steroid



The yield of Schiff's base (124) from conessine was poor, mainly because of the number of side products possible during the dehydrodehalogenation stage.

It was, therefore, considered necessary to reinvestigate the series of reactions from conessine leading to Schiff's base, N-desmethylconane-4,20 (N) diene-3 one, and to find out another feasible method for opening the pyrroline ring E. This new method, outlined in Chart-15 has been described in the course of discussion.

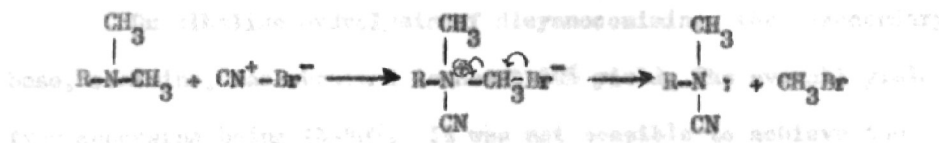
Demethylation of conessine

For fully substituted tertiary amines, the von Braun⁸⁹ cyanogen bromide reaction of demethylation was applied, for the preparation of secondary bases.

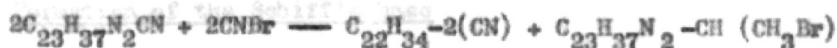
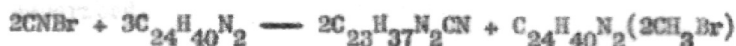
Siddiqui and Siddiqui^{31 D} reported that the action of cyanogen bromide on conessine at room temperature in ether led to the formation of mono-N-cyano isoeconessimine almost exclusively. A further prolonged treatment with cyanogen bromide for several days converted monocyano isoeconessimine to the dicyano compound.

The initial reaction of cyanogen bromide on a basic nitrogen appears to involve the nucleophilic displacement of the bromide in

cyanogen bromide by the nitrogen of the base with the formation of the ionic addition product in which nitrogen becomes quaternized. In the final step, another nucleophilic displacement of the N-cyano tertiary amine by the bromide ion takes place.



The stoichiometry of the reaction sequence can be represented as:



From the above equations it is apparent that the limit of the yield of dicyanoconimine can never exceed 35 per cent, since a third of conessine and half of the monocyanoisecoconessimine are utilized to trap the methylbromide 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 enhance the rate of demethylation, particularly at the relatively inert bridge nitrogen and (ii) it would allow the volatile methyl bromide to escape from the reaction mixture before it had a chance to react with conessine.

On the other hand, 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 improved considerably. Thus under the

experimental conditions employed, as described in Experimental Section, the yield of dicyanocompound from conessine was improved to 65%.

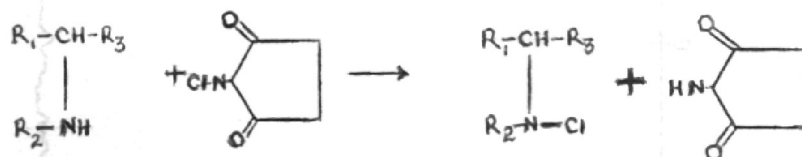
Hydrolysis of dicyanoconimine

On alkaline hydrolysis of dicyanoconimine, the dissecondary base, conimine, was obtained in about 60% yield. The overall yield from conessine being 45-50%. It was not possible to achieve the 100% yield reported by Jegar *et al.*⁶¹ under the experimental conditions described.

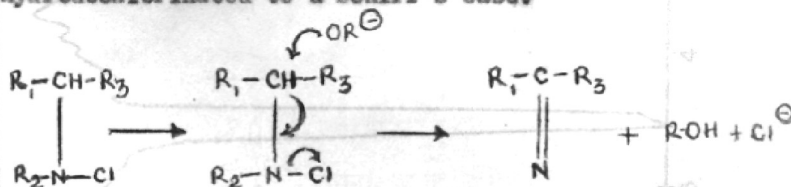
Formation of the Schiff's base

One of the procedures employed in the removal of partially substituted nitrogen atoms from the alkaloids is the Ruschig's procedure⁶⁵ of N-chlorination, dehydrochlorination and subsequent acid hydrolysis.

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



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



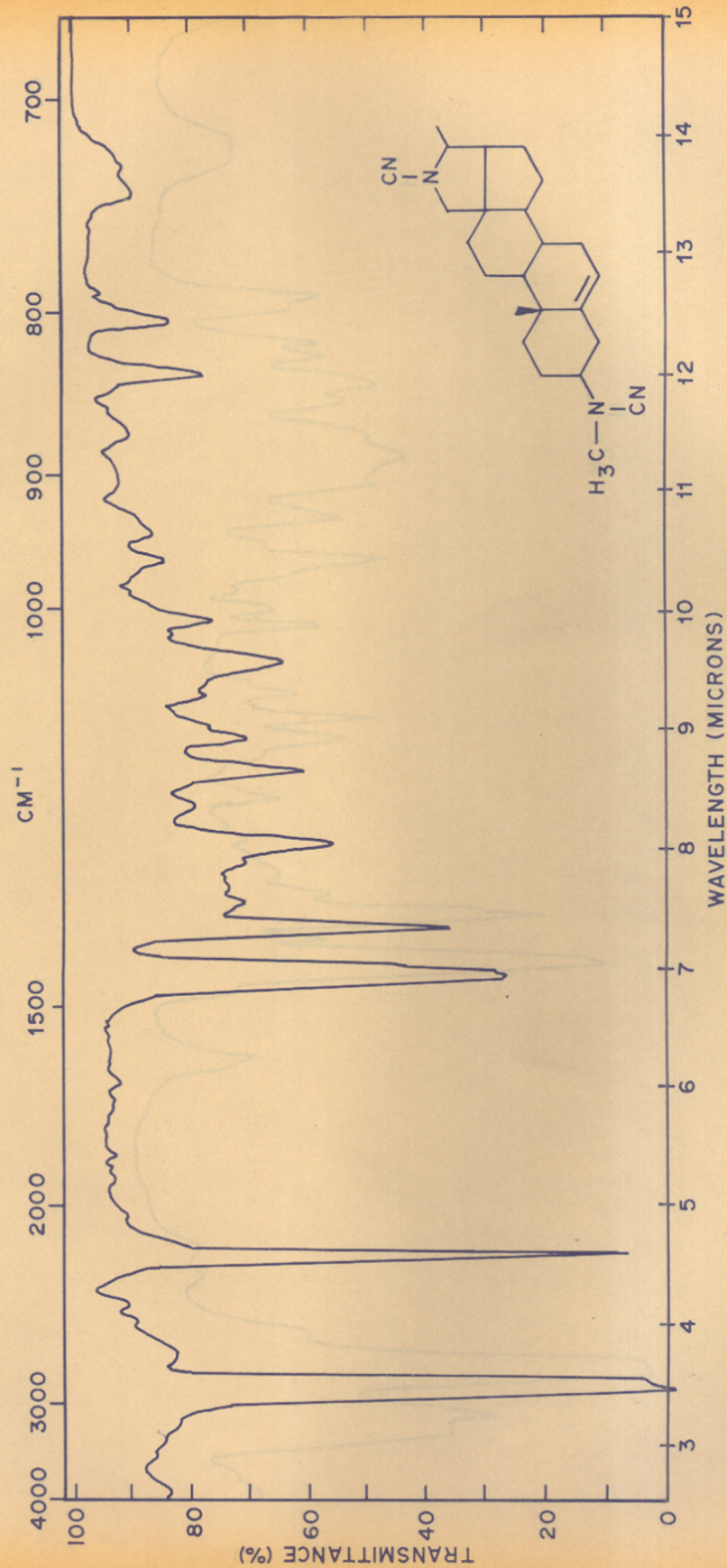


FIG. 7. IR SPECTRUM (NUJOL) OF DICYANOCONIMINE.

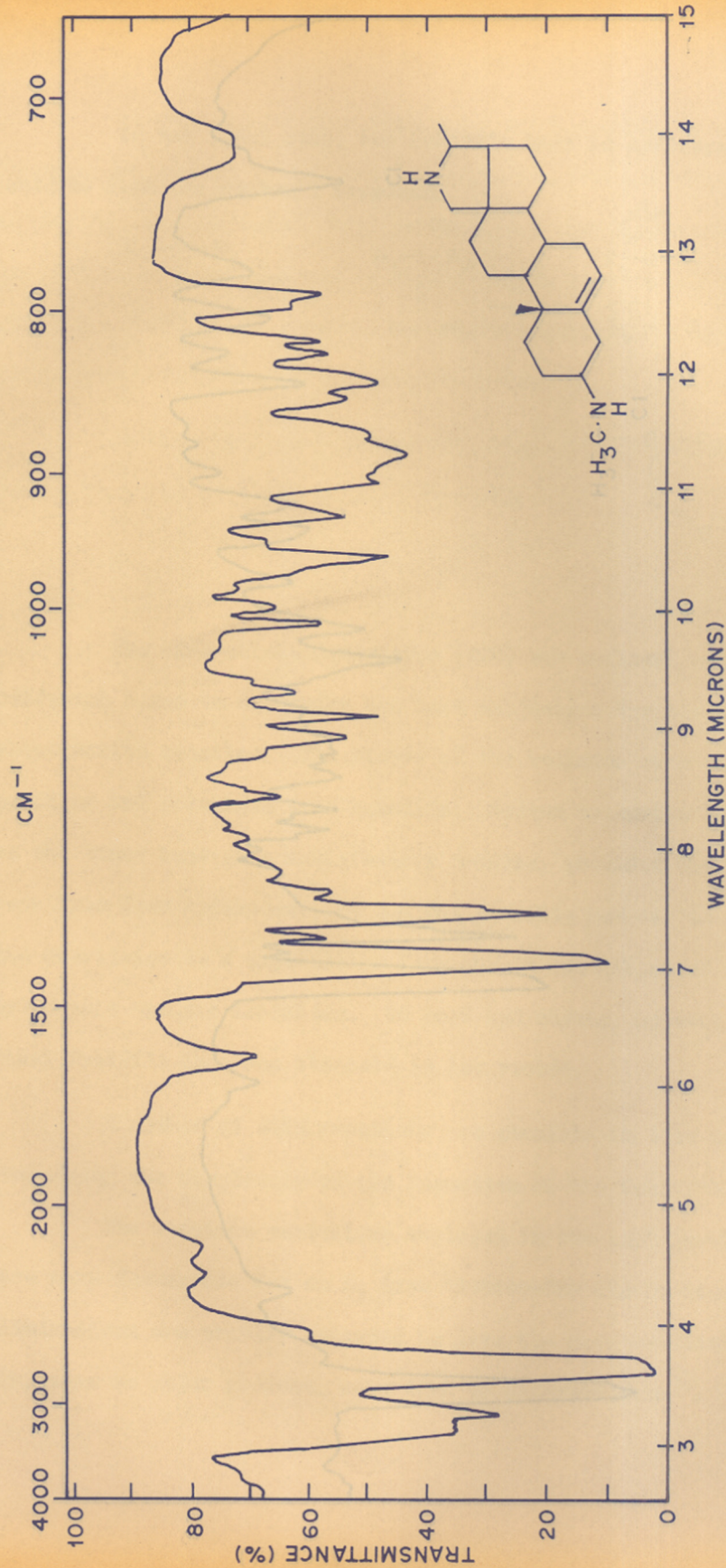


FIG. 9. IR SPECTRUM (NUJOL) OF CONIMINE.

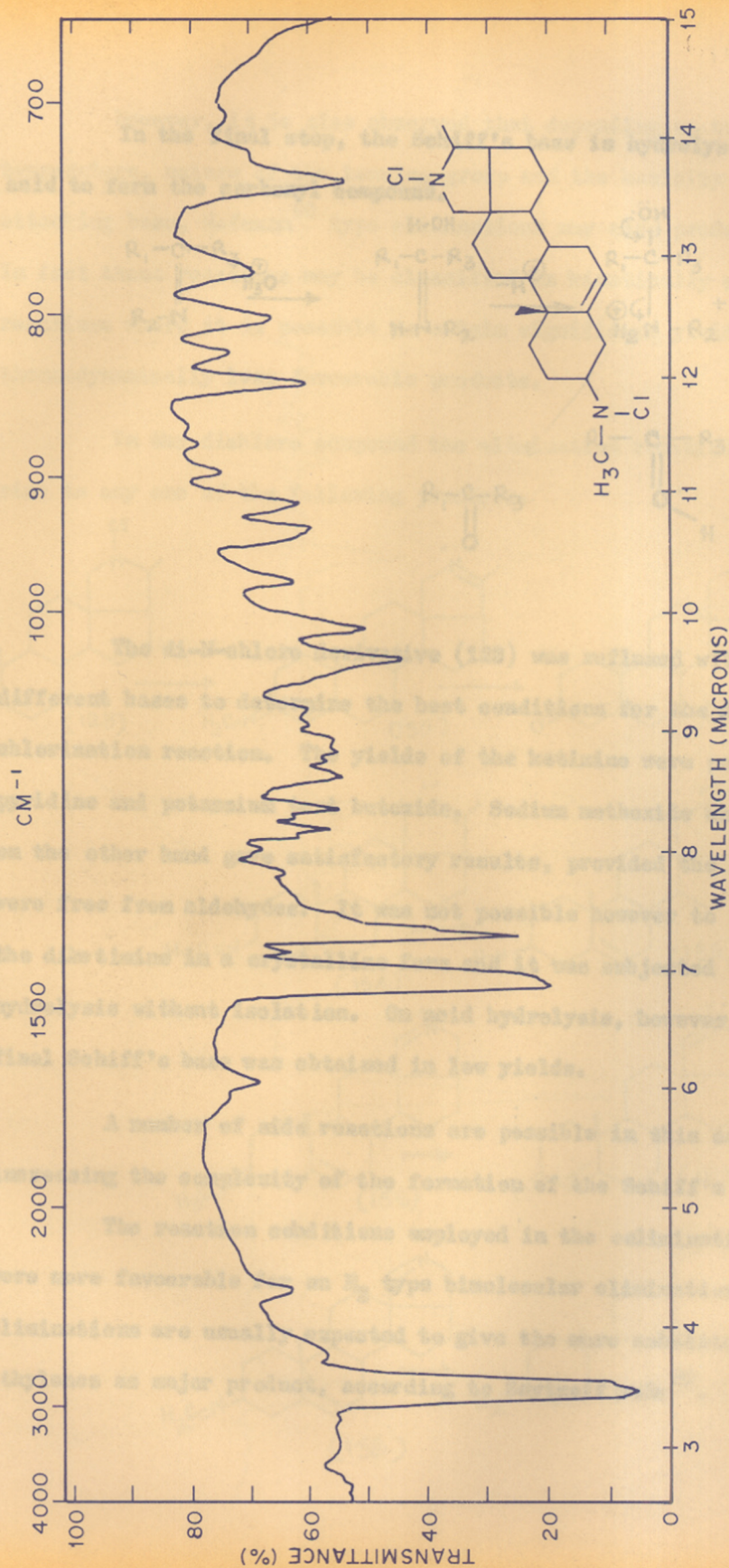
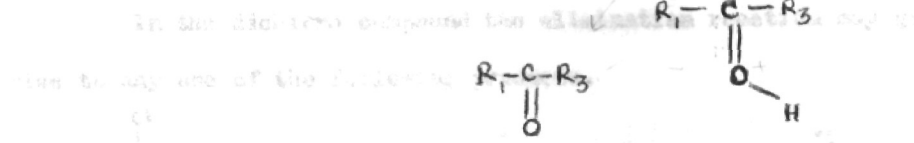
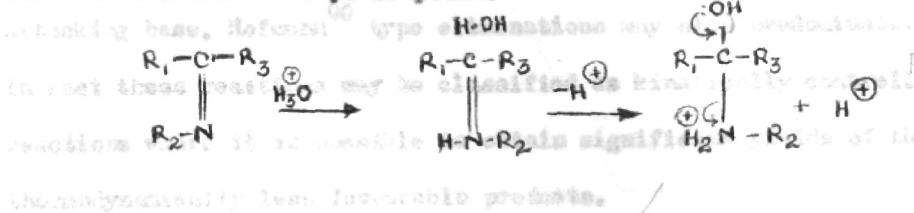


FIG. 11. IR SPECTRUM (NUJOL) OF DICHLOROCONIMINE.

however, it is also observed that depending on the solvent, temperature, nature of the leaving group and the acidity of the acid to form the carbonyl compound.



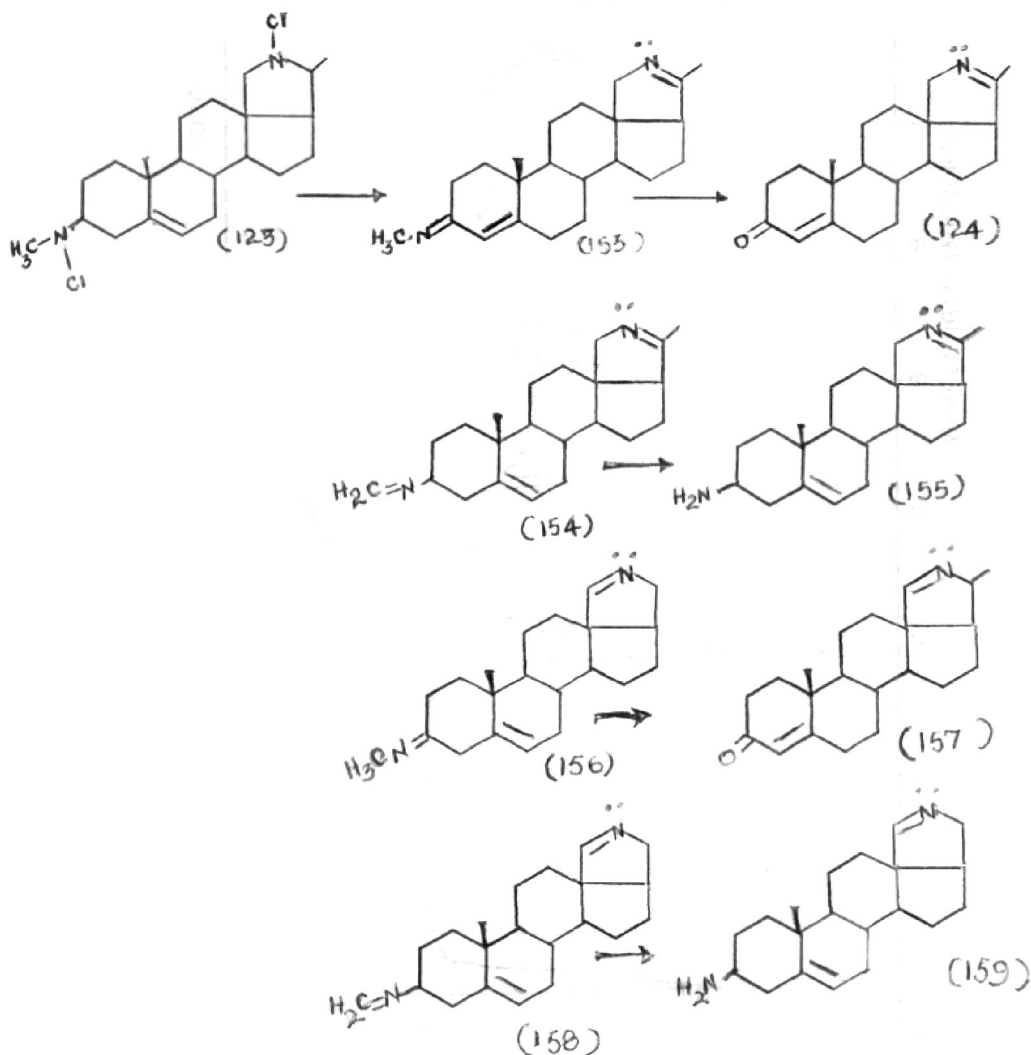
The di-N-chloro derivative (123) was refluxed with different bases to determine the best conditions for the dehydrochlorination reaction. The yields of the ketimine were poor with pyridine and potassium tert butoxide. Sodium methoxide and ethoxide on the other hand gave satisfactory results, provided the alcohols were free from aldehydes. It was not possible however to isolate the diketimine in a crystalline form and it was subjected to acid hydrolysis without isolation. On acid hydrolysis, however, the final Schiff's base was obtained in low yields.

A number of side reactions are possible in this context, increasing the complexity of the formation of the Schiff's base.

The reaction conditions employed in the elimination reaction were more favourable for an E₂ type bimolecular elimination. Such eliminations are usually expected to give the more substituted ethylenes as major product, according to Saytzeff rule⁹⁰.

However, it is also observed that depending on the solvent, temperature, nature of the leaving group and the basicity of the attacking base, Hofmann⁹⁰ type eliminations may also predominate. In fact these reactions may be classified as kinetically controlled reactions where it is possible to obtain significant yields of the thermodynamically less favourable products.

In the dichloro compound the elimination reaction may give rise to any one of the following products.



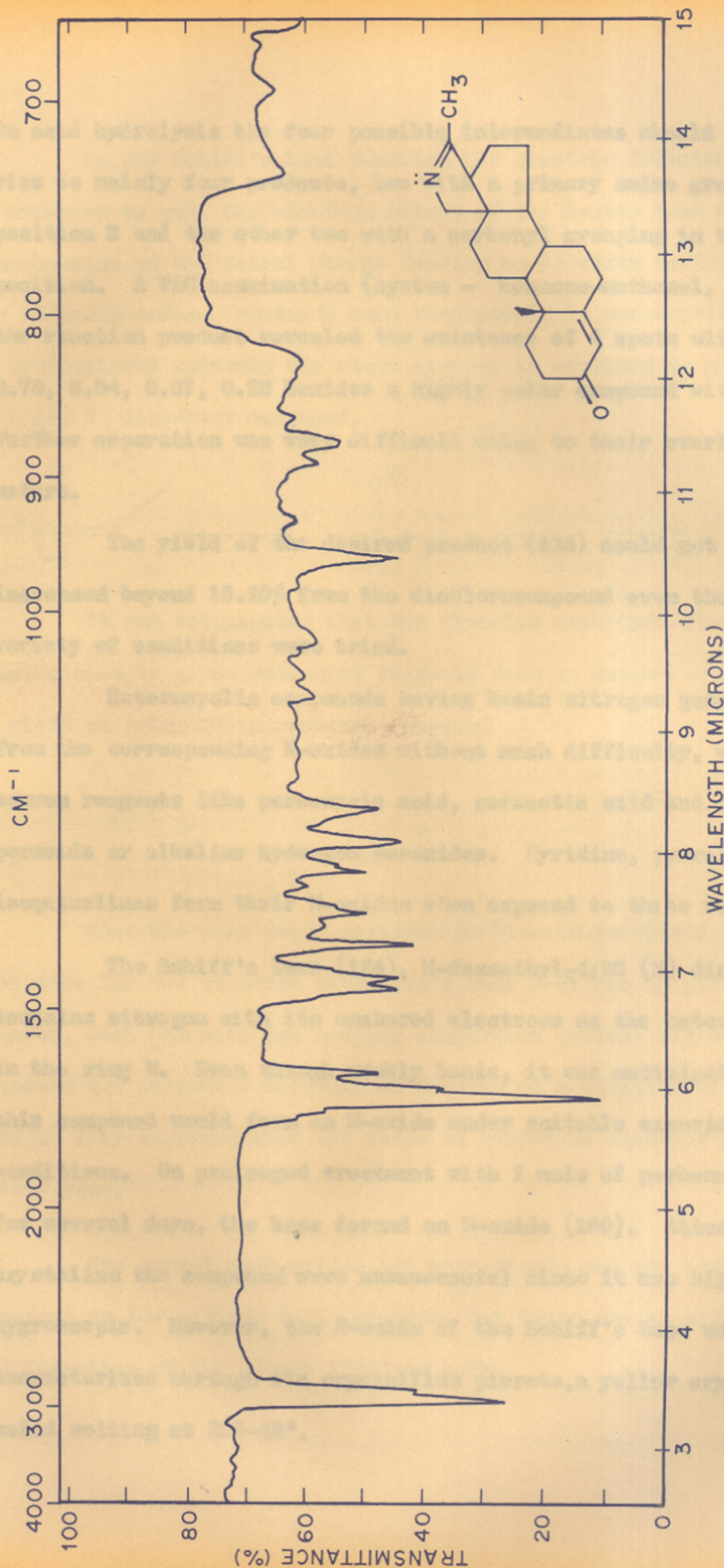


FIG. 13. IR SPECTRUM (CCl₄) OF N-DESMETHYL-CONAN,
4,20 (N)-DIENE-3-ONE (A SCHIFF'S BASE)

On acid hydrolysis the four possible intermediates should give

rise to mainly four products, two with a primary amino group at position 3 and the other two with a carbonyl grouping in the same position. A TLC examination (system - benzene-methanol, 6:1) of the reaction product revealed the existence of 4 spots with R_f s 0.78, 0.54, 0.37, 0.28 besides a highly polar compound with R_f 0.15.

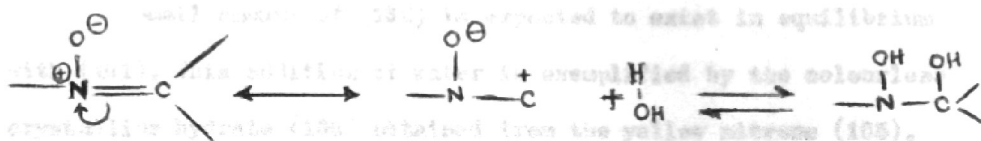
Further separation was very difficult owing to their overlapping nature.

The yield of the desired product (124) could not be increased beyond 15.20% from the dichlorocompound even though a variety of conditions were tried.

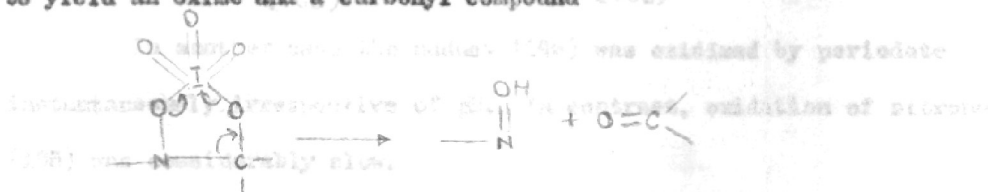
Heterocyclic compounds having basic nitrogen generally from the corresponding N-oxides without much difficulty, with common reagents like perbenzoic acid, paracetic acid and other peracids or alkaline hydrogen peroxides. Pyridine, pyrrolines, isoquinolines form their N-oxides when exposed to these reagents.

The Schiff's base (124), N-desmethyl-4;20 (N) diene-3-one contains nitrogen with its unshared electrons as the hetero atom in the ring E. Even though weakly basic, it was anticipated that this compound would form an N-oxide under suitable experimental conditions. On prolonged treatment with 1 mole of perbenzoic acid for several days, the base formed an N-oxide (160). Attempts to crystallize the compound were unsuccessful since it was highly hygroscopic. However, the N-oxide of the Schiff's base was characterized through its crystalline picrate, a yellow crystalline solid melting at 239-42°.

In the Schiff's base N-oxides the electron deficient nitrogen is expected to pull the electron clouds of the double bond for partial compensation of the formal charge leaving an electron deficiency in the adjacent carbon, making it more susceptible to nucleophilic attack. In hydroxylated solvents the above process is expected to give rise to a 41, 2 dihydroxy compound.



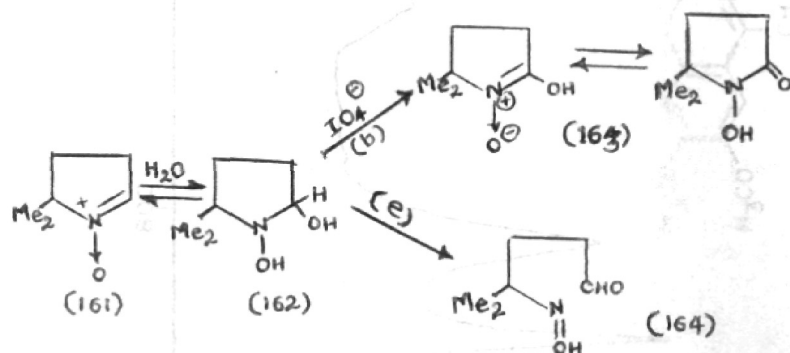
It was anticipated that the glycolic structure should be reacted upon by glycol-cleaving reagents such as sodium metaperiodate to yield an oxime and a carbonyl compound.



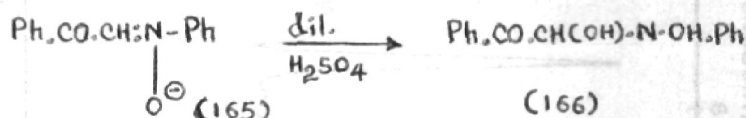
When the work was undertaken no literature reference was available for the cleavage of Schiff's base N-oxides by periodate. However, when the work was nearing completion, Qureshi and Sklarz⁹¹ reported the periodate cleavage of N-oxide of a substituted pyrroline (161). They observed that the rates of periodate cleavage were rather slow.

The pyrroline-1-oxides are hygroscopic and are occasionally obtained as low melting solid hydrate, but there was no evidence for the formation of the adduct (162) in bulk.

In the present investigation it was suggested that the sodium metaperiodate oxidation first on a small scale using



Small amount of (162) is expected to exist in equilibrium with (161). This addition of water is exemplified by the colourless crystalline hydrate (166) obtained from the yellow nitrene (165).



In another case the adduct (166) was oxidized by periodate instantaneously irrespective of pH. In contrast, oxidation of nitrene (165) was considerably slow.

If the oxidation of (166) is faster, then the reaction will take the following course:



as in step b. The alternative to this very fast oxidation would be a cleavage as in step (e) of the glycolysis type which would give the nitroso-aldehyde (164). Although Quereshi *et al.*⁹¹ could not show the accumulation of the aldehydic compound (164) in the experiments with limited amounts of periodate in the presence of chloroform, they could not rule out the possibility of its formation.

In the present investigation it was necessary to study the sodium metaperiodate oxidation first on a model compound, having

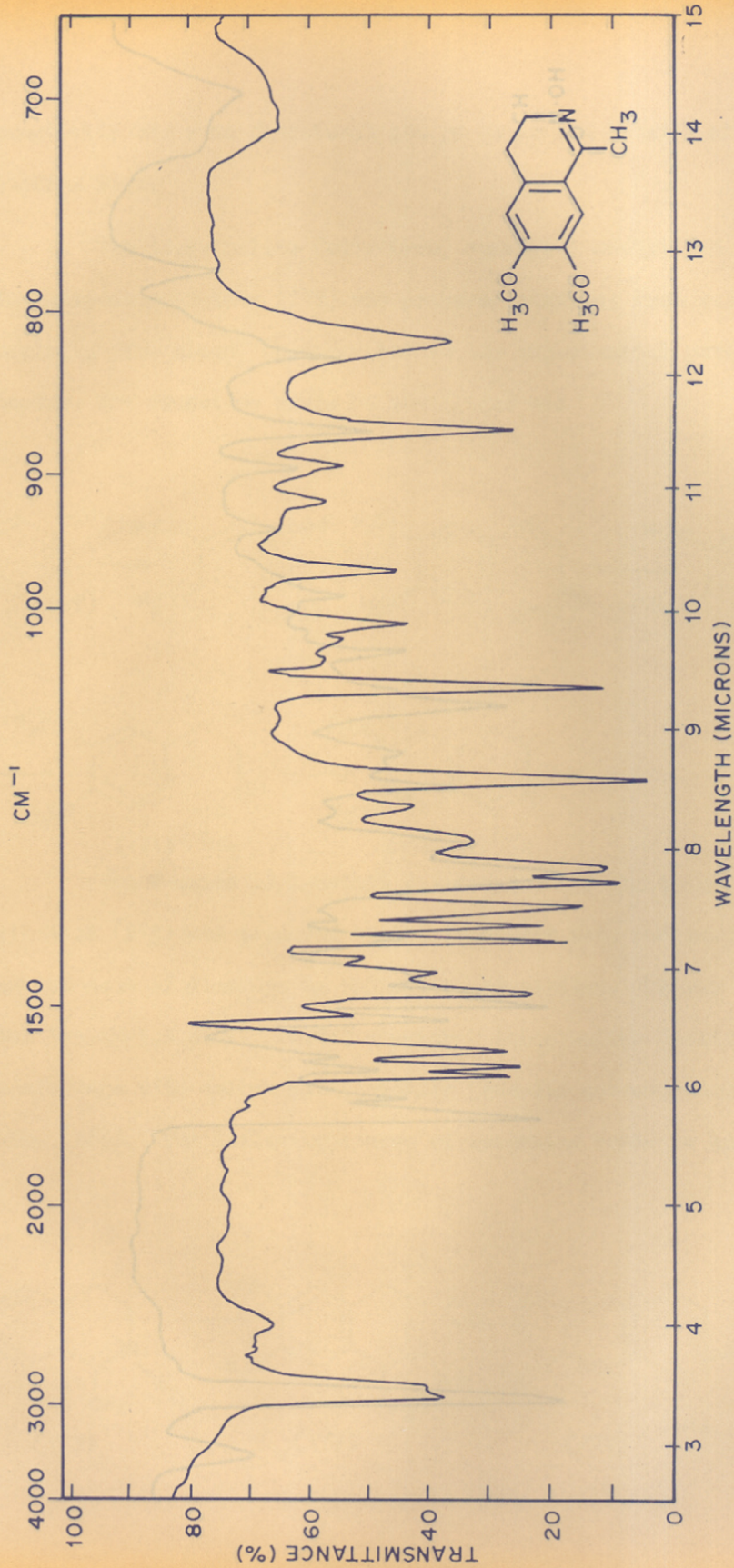
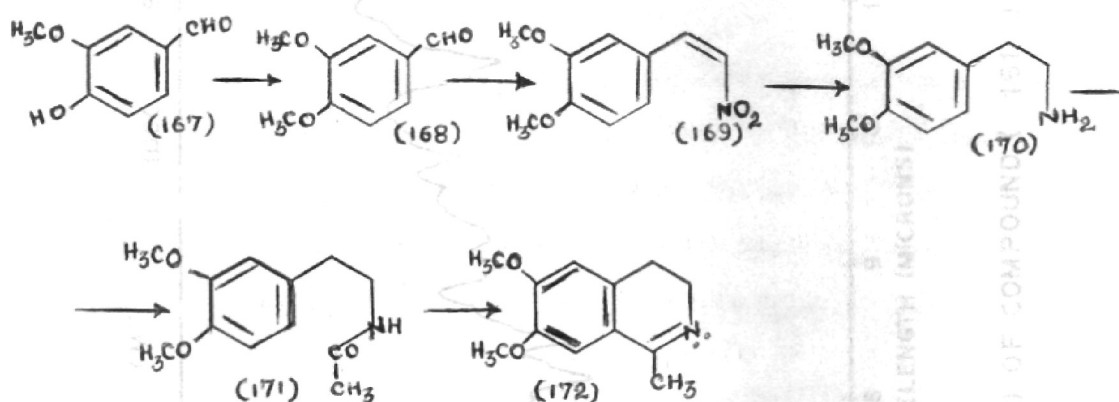


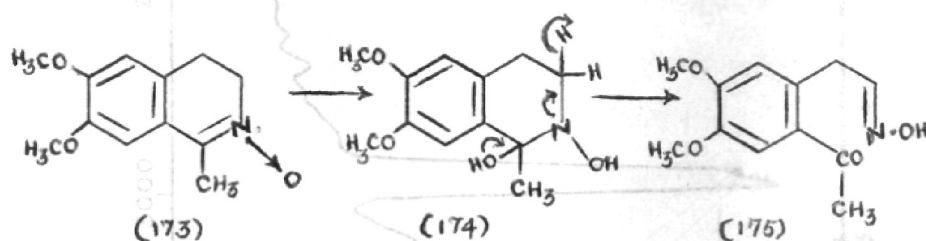
FIG. 17. IR SPECTRUM (CHCl_3) OF 1-METHYL,3:4 DIHYDRO-6:7 DIMETHOXY ISOQUINOLINE.

essentially the same functional groups as in the N-oxide of Schiff's base.

The isoquinoline derivative, namely, 1-methyl,3,4-dihydro,6,7-dimethoxy-isoquinoline (172) was prepared starting from vanillin (167) involving five steps, as described in the experimental part of the chapter. The reactions could be summarized as:



The N-oxide of 1-methyl 3,4-dihydro 6,7 dimethoxy isoquinoline (172) was prepared by the treatment with perbenzoic acid and its mode of cleavage by periodate was studied. It was found that 1-methyl,3,4-dihydro,6,7 dimethoxy isoquinoline N-oxide (173) on cleavage with sodium metaperiodate gave its corresponding oxime (175). The following course of mechanism could be suggested:



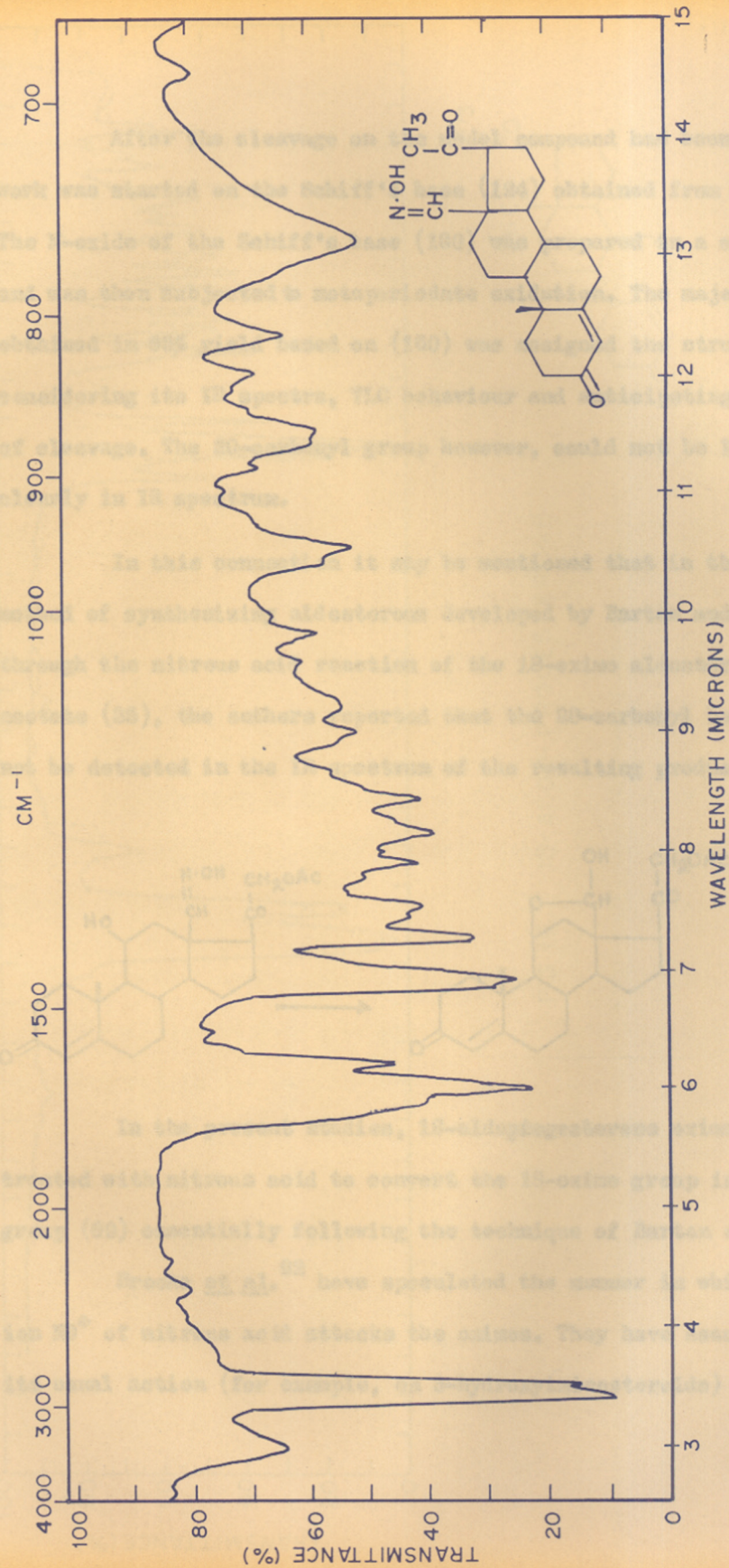
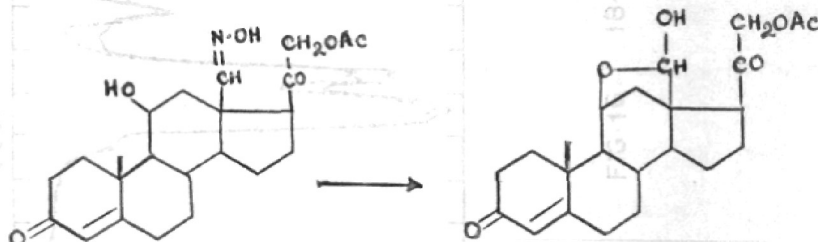


FIG. 15. IR SPECTRUM (NUJOL) OF COMPOUND (161) 18-OXIMO PROGESTERONE .

After the cleavage on the model compound had been successful, work was started on the Schiff's base (124) obtained from conessine. The N-oxide of the Schiff's base (160) was prepared in a similar way and was then subjected to metaperiodate oxidation. The major product obtained in 60% yield based on (160) was assigned the structure (161), considering its IR spectra, TLC behaviour and anticipating the mode of cleavage. The 20-carbonyl group however, could not be located very clearly in IR spectrum.

In this connection it may be mentioned that in the novel method of synthesizing aldosterone developed by Barton and Beaton¹³ through the nitrous acid reaction of the 18-oxime aldosterone-21 acetate (35), the authors reported that the 20-carbonyl band could not be detected in the IR spectrum of the resulting product.



In the present studies, 18-aldoptogesterone oxime (161) was treated with nitrous acid to convert the 18-oximo group into aldo group (69) essentially following the technique of Barton and Beaton¹³.

Brooks et al.⁹² have speculated the manner in which the ion NO^+ of nitrous acid attacks the oximes. They have assumed that its usual action (for example, on 3-hydroxyiminosteroids) can be

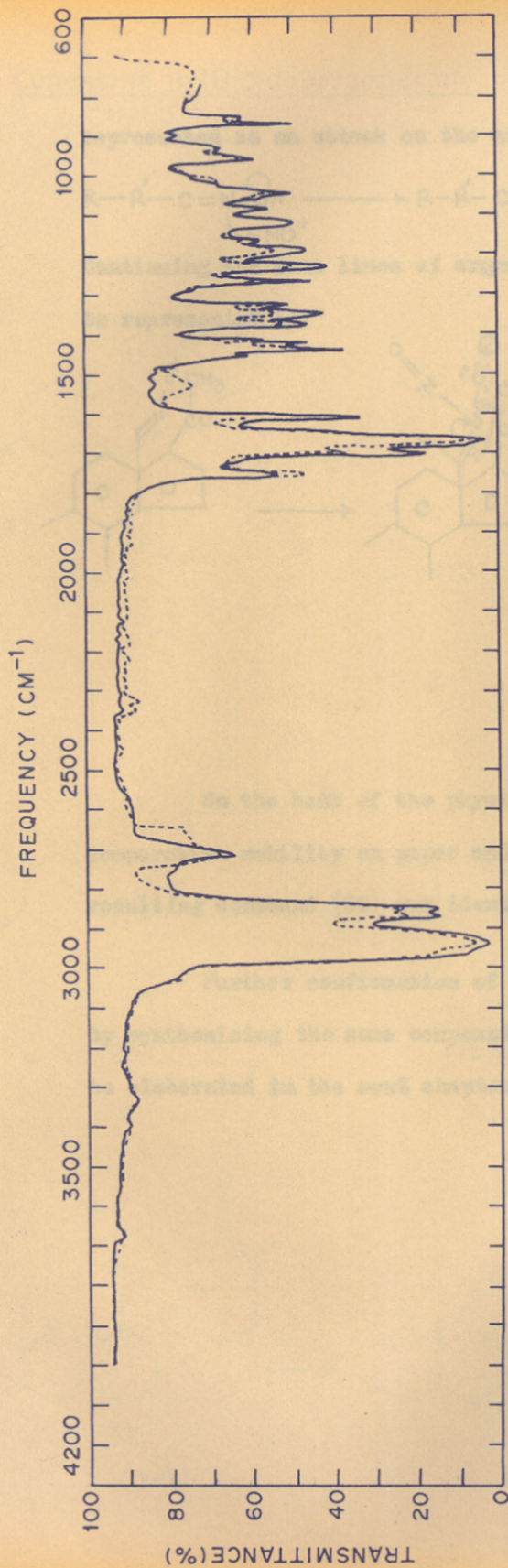
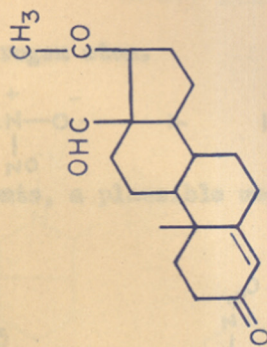


FIG.16. 18-ALDO-PROGESTERONE



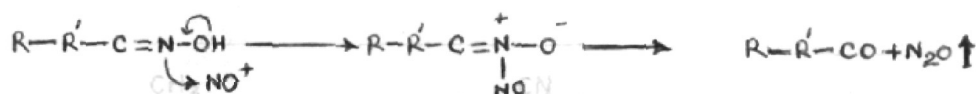
— COMPOUND OBTAINED FROM

CONESSINE TRANSFORMATIONS.

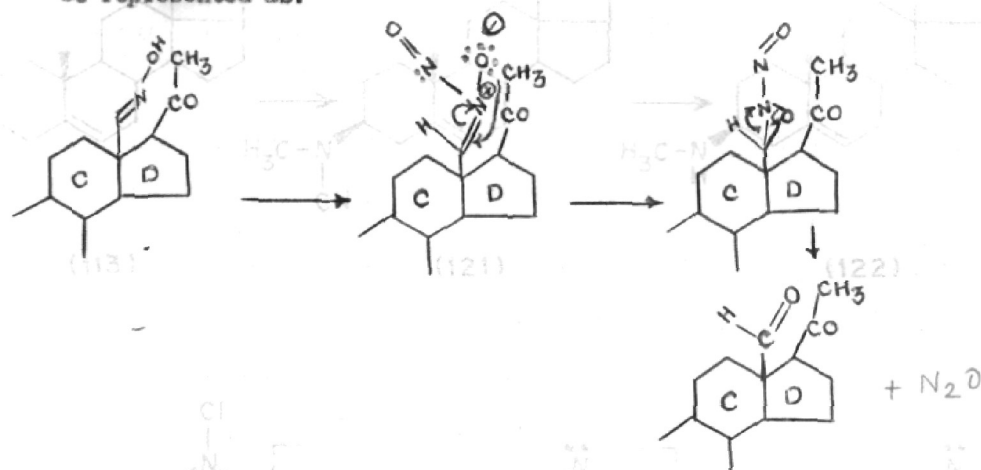
---- COMPOUND OBTAINED FOLLOWING

KALVODA'S PROCEDURE.

represented as an attack on the nitrogen atom.

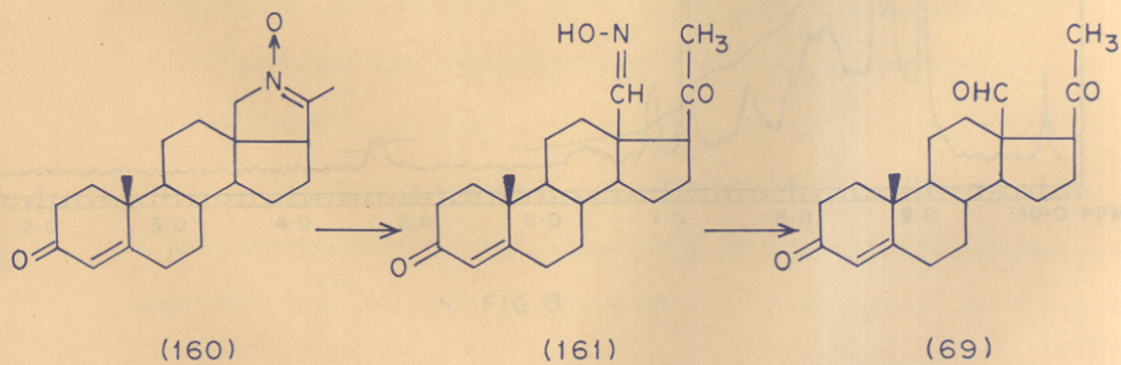
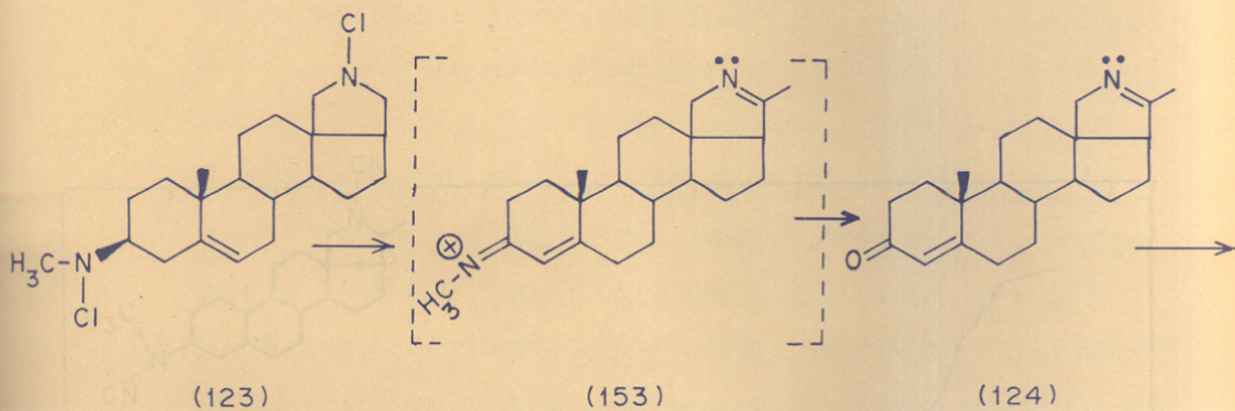
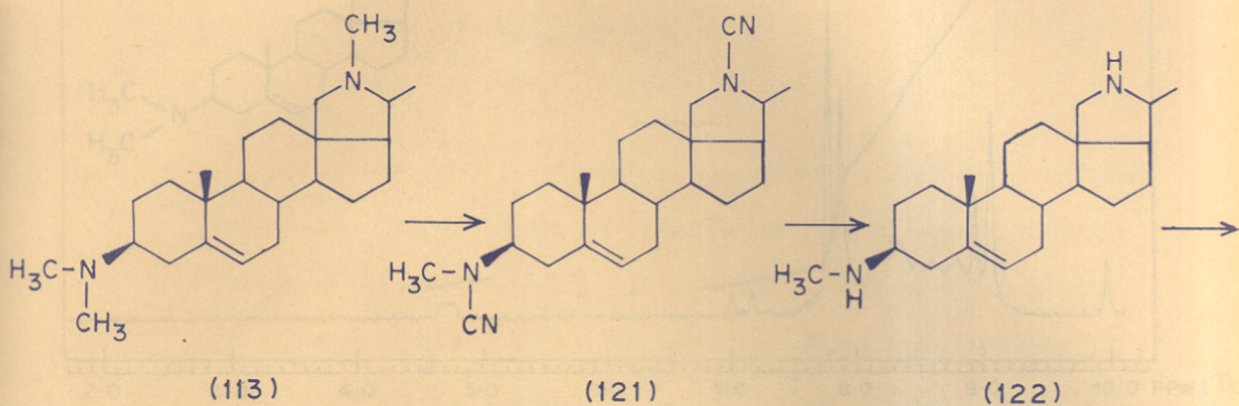


Continuing the same lines of arguments, a plausible mechanism could be represented as:



On the basis of the physical constants, IR spectrum and comparative mobility on paper and thin layer chromatograms, the resulting compound (69) was identified as 18-aldoprogesterone.

Further confirmation of the structure (69) was obtained by synthesizing the same compound in two different ways which will be elaborated in the next chapter.



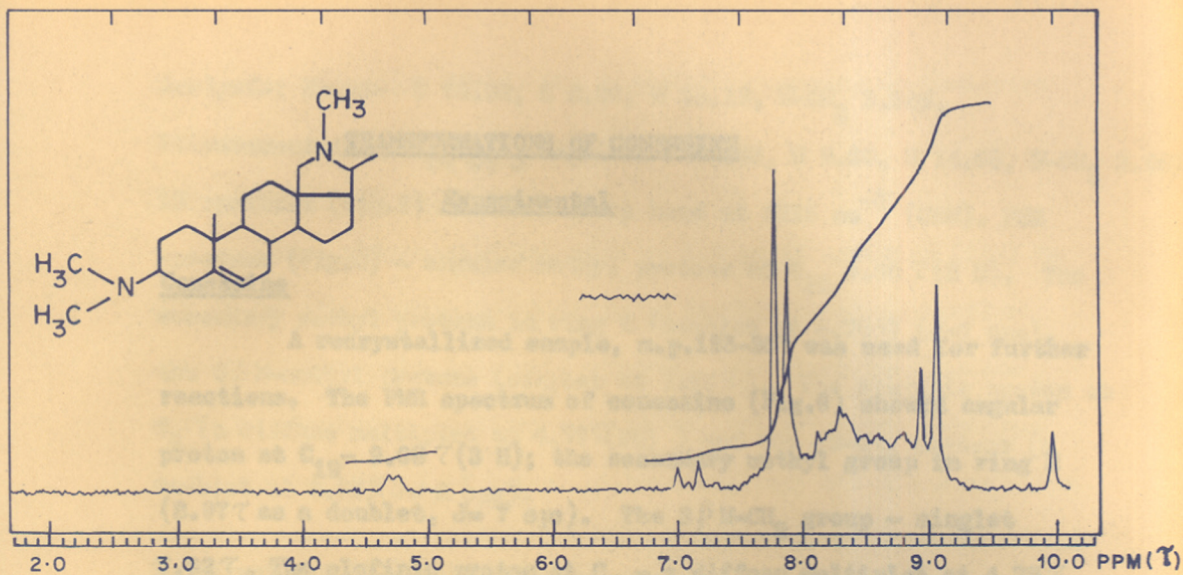


FIG. 6

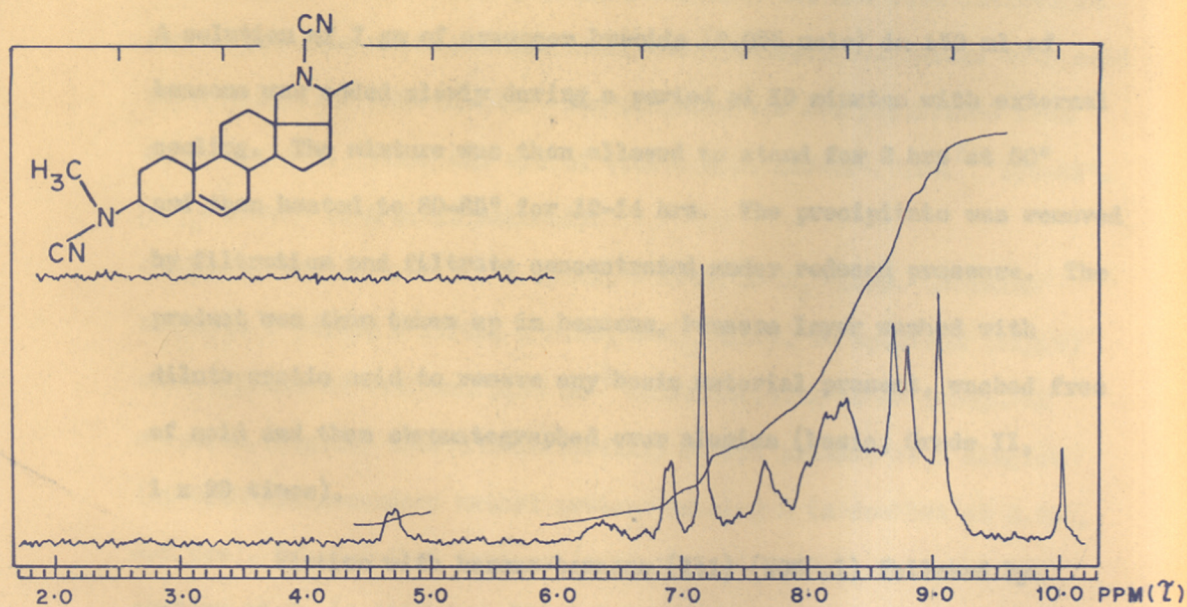


FIG. 8

analysis: Found: C 76.31, H 6.03, N 14.14, NH_2 2.30%.

Calc: C 76.31, H 6.03, N 14.66, NH_2 2.30%.

IR spectrum (Fig. 7) Experimental band at 2100 cm^{-1} (CN). PMR

spectrum (Fig. 8) - assigned methyl protons at δ_{H} 1.97 (1H). The

secondary methyl protons in ring E (doublet at δ_{H} 7.92).

Conessine
A recrystallized sample, m.p. 125-26° was used for further reactions. The PMR spectrum (Fig. 6) showed angular proton at C_{19} - 9.08 τ (3 H); the secondary methyl group in ring E (8.97 τ as a doublet, $J = 7\text{ cps}$). The $3\beta\text{-N-CH}_3$ group - singlet

7.92 τ . The olefinic proton at C_8 - a diffuse multiplet at 4.75 τ (1 proton intensity).

Action of cyanogen bromide on conessine

A solution of 10 gm of conessine (113) in 1 litre of dry benzene was dehydrated by removing 150 ml benzene by distillation. A solution of 7 gm of cyanogen bromide (0.066 mole) in 150 ml of benzene was added slowly during a period of 10 minutes with external cooling. The mixture was then allowed to stand for 2 hrs at 50° and then heated to 80-85° for 12-14 hrs. The precipitate was removed by filtration and filtrate concentrated under reduced pressure. The product was then taken up in benzene, benzene layer washed with dilute acetic acid to remove any basic material present, washed free of acid and then chromatographed over alumina (basic, Grade II, 1 x 20 times).

Elution with hexane-benzene (1:1) (200 ml) followed by elution with benzene (200 ml) gave 6.5 gm of dicyano compound (121).

m.p. 168°. lit. 168-170°. $[\alpha]_D^{25} = +63^\circ$ ($c = 0.019$).

Analysis: Found- C 76.02, H 8.83, N 14.19, N-CH₃ 3.80%.

Dicyanoeconimine C₂₄H₃₄N₄ requires C 76.25, H 9.05, N 14.66, N-CH₃ 3.93%.

IR spectrum (Fig.7) showed a sharp band at 2215 cm⁻¹ (C≡N). PMR spectrum (Fig.8) - angular methyl protons at C₁₉ 9.05 τ (3 H). The secondary methyl protons in ring E (doublet at 8.70 τ) (J=7 cps), the 3β-N-methyl protons (singlet at 7.17 τ). The olefinic proton at C₆ (a diffuse multiplet at 4.67 τ (1H)) and the -N-CH₂ protons (a doublet at 6.83 τ J= 3 cps).

Hydrolysis of dicyanoeconimine to conimine

A mixture of 6.5 gm of N-N' dicyano conimine (121), 120 gm of (2.4 moles) KOH, 500 ml methanol and 70 ml water was heated under reflux for 48 hours. A 170 ml portion of the solvent was removed by distillation, the resulting mixture filtered hot and then chilled in ice to get fine white needles which were filtered and washed with cold water till free from alkali. The compound was recrystallized from ethyl acetate. Yield 60% w/w of (122), m.p. 130-31°. lit.⁶² 130-32°. $[\alpha]_D^{25} = +28.5^\circ$ (c = 0.017 ethanol). $[\alpha]_D^{25} = +29^\circ$ (c = 0.001).

Analysis: Found- C 80.12, H 10.91, N 8.44, N-CH₃ 4.36%. Conimine requires C 80.43, H 11.03, N 8.50, N-CH₃ 4.50%. IR spectrum (Fig.9) showed bands at 3226 and 3125 cm⁻¹ characteristic of NH group. PMR spectrum (Fig.10) showed C-19 tertiary methyl protons as a singlet (at 9.08 τ), secondary methyl protons in ring E (a doublet at 8.90 τ, J=6 cps), 3β-N-methyl protons (a singlet at 7.63 τ), the olefinic protons at C₆ (a diffuse multiplet at 4.67 τ), the -N-CH₂ protons (a doublet at 7.35, J= 8 cps) and proton of secondary amino group of ring E (singlet at 9.17 τ). The heterocyclic ring NH resonated

at an unusually high field, the signal disappearing on deuterium exchange.

N-chlorination of conimine

To a well-cooled solution of 3.9 gm of conimine (122) in 400 ml dry ether was added 3.14 gm of freshly prepared N-chloro succinimide in small lots with stirring. N-chloro succinimide could be added even in ether suspension. Stirring was continued for a further period of 1 hour without cooling; the clear solution was then washed repeatedly with water to remove succinimide and any unreacted N-chlorosuccinimide. The ether solution was then evaporated slowly after drying over sodium sulphate. A part of ether layer was tested with starch iodide paper, which gave a characteristic test of N-chloro compounds. During evaporation of ethereal layer, the temperature was maintained strictly between 32° and 36°, as the N-halo compounds are unstable towards heat. The colourless crystalline residue was recrystallized from benzene methanol when white needles of compound (123) were observed. Yield 90%.

m.p. 110-220° with decomposition. $[\alpha]_D^{25} = +29^\circ$ (c = 0.021).

Analysis: Found- C 66.20, H 8.50, N 6.82, Cl 17.58%. Dichloroconimine $C_{22}H_{34}N_2Cl_2$ requires C 66.48, H 8.62, N 7.05, Cl 17.90%. IR spectrum (Fig. 11) showed a band at 735 cm^{-1} (N-Cl). PMR spectrum (Fig. 12) showed - tertiary C-19 methyl protons (9.10 τ) (3 H). The secondary methyl proton in ring E (doublet at 8.92 τ , J=6 cps), the 3 β -methyl protons (a singlet at 7.41 τ). The introduction of chlorine atom probably causes the shielding of the N-methyl signal.

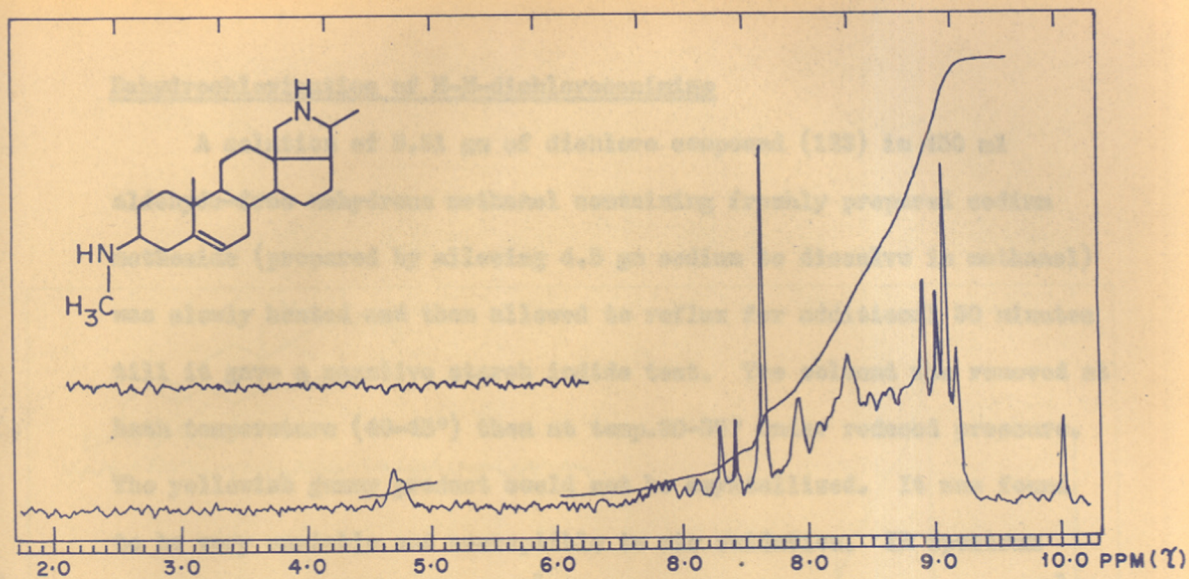


FIG. 10

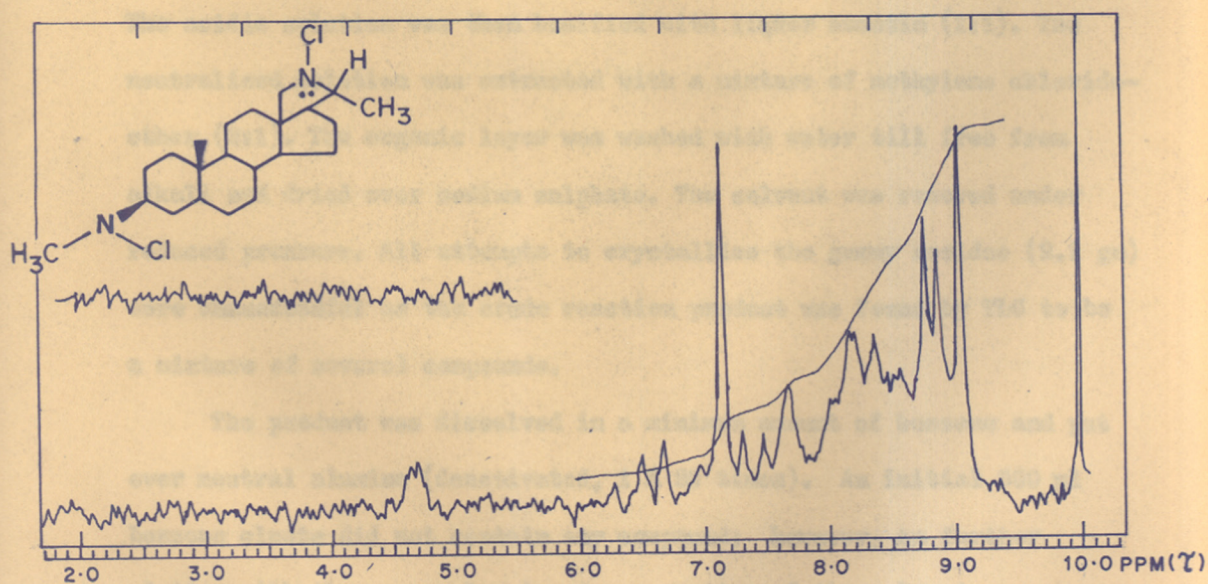


FIG. 12

Dehydrochlorination of N-N-dichloroconimine

A solution of 3.51 gm of dichloro compound (123) in 450 ml aldehyde-free anhydrous methanol containing freshly prepared sodium methoxide (prepared by allowing 4.5 gm sodium to dissolve in methanol) was slowly heated and then allowed to reflux for additional 30 minutes till it gave a negative starch iodide test. The solvent was removed at bath temperature (40-45°) then at temp. 30-35° under reduced pressure. The yellowish gummy product could not be crystallized. It was found to be very unstable and susceptible to air oxidation. IR spectrum showed bands at 1678, 1613 cm^{-1} (α - β unsaturated ketimine), 1650 cm^{-1} (C=N) and 838 cm^{-1} (trisubstituted double bond).

Hydrolysis of ketimine

The aliquot of ketimine (3 gm); (153) was dissolved in 250 ml of 2N H_2SO_4 and allowed to stand at room temperature for 12 hrs. The acidic solution was then basified with liquor ammonia (1:1). The neutralized solution was extracted with a mixture of methylene chloride-ether (2:1). The organic layer was washed with water till free from alkali and dried over sodium sulphate. The solvent was removed under reduced pressure. All attempts to crystallize the gummy residue (2.1 gm) were unsuccessful as the crude reaction product was found by TLC to be a mixture of several compounds.

The product was dissolved in a minimum amount of benzene and put over neutral alumina (deactivated, 1 X 20 times). An initial 500 ml benzene eluate did not contain any compound; however, on further elution with the same solvent and evaporation of the solvent from the eluate gave small amounts of the Schiff's base. A sample

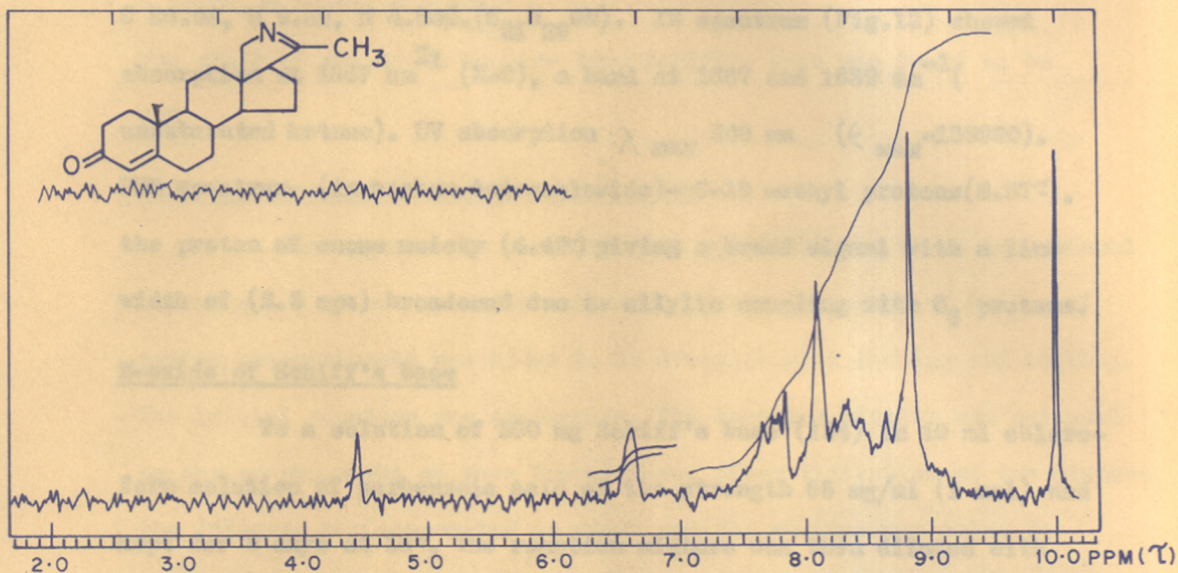


FIG. 14

crystallized from pet-ether and acetone gave clusters of needles of compound (124) in 15% yield based on (123). m.p. 183-185°. $[\alpha]_D^{+24} = +84^\circ$ (c=0.018). The compound formed a crystalline picrate, m.p. 190-192°.

Analysis: Found- C 80.78, H 9.21, N 4.35%. Schiff's base requires C 80.98, H 9.39, N 4.50%. ($C_{21}H_{29}ON$). IR spectrum (Fig.13) showed absorption at 1647 cm^{-1} (N=C), a band at 1667 and 1639 cm^{-1} (unsaturated ketone). UV absorption $\lambda_{\text{max}} 240\text{ m}\mu$ ($\epsilon_{\text{max}} = 159000$). PMR spectrum- (in carbon tetrachloride)- C-19 methyl protons (8.87 τ), the proton of enone moiety (4.42 τ) giving a broad signal with a line width of (2.5 cps) broadened due to allylic coupling with C_6 protons.

N-oxide of Schiff's base

To a solution of 500 mg Schiff's base (124) in 10 ml chloroform solution of perbenzoic acid of the strength 65 mg/ml (1 mol) and kept for 8 days at 15°. The reaction mixture was then diluted with excess of chloroform and treated with an aqueous paste of potassium carbonate (2 gm in 1 ml). The reaction mixture, on filtration and evaporation at 40-45° bath temperature under vacuum gave a semi solid frothing in high vacuum. The compound was found to be more polar than the Schiff's base (vide TLC) and was separated from the starting compound by a preparative thin layer chromatography, in the solvent system benzene-methanol (3:1). The product (320 mg) (a semi solid) however, failed to crystallize.

Analysis: Found- C 72.95, H 9.15, N 4.17%. Calculated for $C_{21}H_{29}O_2N$ C 77.02, H 8.93, N 4.28% and for $C_{21}H_{29}O_2N \cdot H_2O$ - required C 73.00, H 9.05, N 4.05%. IR spectrum showed bands at 960 and 943 cm^{-1} characteristic of N-O.

It could not be resolved by chromatography on alumina or silica gel column due to the highly reactive nature of its N-oxide. To a well-shielded solution of the IR-active compound (101) grouping. The compound formed a crystalline picrate m.p. 230-42°. (100 mg) in 5 ml acetic acid, an aqueous solution of sodium nitrite (5%, 2 ml) was added dropwise with shaking. The reaction mixture was C 58.26, H 10.07, N 5.80%.

The yield of the crude N-oxide from Schiff's base was found to be 65%.

Sodium metaperiodate reaction on N-oxide

The hygroscopic compound (320 mg) (160) was taken in methanol (10 ml and 2 ml water) and a saturated aqueous solution of 300 mg of sodium metaperiodate was added to it dropwise with shaking and cooling. The initial reaction was exothermic. The reaction mixture was agitated on shaker overnight at room temperature. After filtration of the mixture the filtrate was evaporated in vacuo and the residue was taken in by passing it over a short silica column (deactivated). The 2₂ value in chloroform, chloroform layer was washed with little water, dried over sodium sulphate and evaporated at 40°. The gummy product was taken in (system: nit-ether-ethylacetate, 5:4) it showed the 2₂ value between 0.30 to 0.32. The compound was crystallized from benzene-ethyl-ether. 10% ethyl acetate-benzene fraction gave 163 mg of (161), recrystallized from acetone (yield 60%). m.p. 140-42°. $[\alpha]_D^{25} = +51^\circ$ (c = 0.016).

Analysis: Found: C 73.24, H 8.55, N 4.01%. $C_{21}H_{28}O_3N$ requires C 73.44, H 8.59, N 4.08%. IR spectrum (carbon tetrachloride solution) showed bands at 3325, 1667 cm^{-1} with a shoulder at 1675 cm^{-1} (C-20 ketone). UV λ_{max} 242 mμ. A blank reaction on Schiff's base was carried out with sodium metaperiodate, under identical conditions and unreacted Schiff's base was recovered quantitatively (vide TLC and m.p.).

Nitrous acid reaction on oxime (161)

To a well-chilled solution of the 18-oxime compound (161) (100 mg) in 5 ml acetic acid, an aqueous solution of sodium nitrite (5%, 2 ml) was added dropwise with shaking. The reaction mixture was kept for 30 minutes in room temperature with mechanical agitation. It was extracted with excess of methylene chloride. The solvent layer was washed free of acid, dried over sodium sulphate and evaporated slowly at 40-45° (bath temperature). The crude reaction product was chromatographed over TLC along with the starting material in solvent system benzene-methanol (3:1). Visualization under U.V. indicated the presence of a single compound with R_f 0.60. The oxime had an R_f 0.1 in this system.

The reaction mixture contained alkaline. The reaction product (crude) 83 mg (80% yield) was purified by passing it over a short silica column (deactivated). The R_f values were rechecked in the same system which showed no change. On TLC (system: pet-ether-ethylacetate, 6:4) it showed the R_f value between 0.50 to 0.52. The compound was crystallized from benzene-pet-ether. m.p. 129-32°. $[\alpha]_D^{25} = +35^\circ$ (c=0.019).

Analysis: Found: C 75.99, H 8.48%. $C_{21}H_{28}O_3$ requires C 76.31, H 8.59%. IR spectrum (carbon tetrachloride solution) (Fig. 16) showed bands at 2720 cm^{-1} (CH), 1745, 1705, 1675 and 1615 cm^{-1} (H.C=O, CO.CH₃, unsaturated ketone, and double bond in conjugation to ketone).

R_f : Bush B₃ observed 0.65, reported 0.62; Bush B₈ observed 0.90, unreported; formamide-cyclohexane-benzene observed 0.68, reported 0.70.

Further confirmation of the structure was obtained by mixed melting point and comparative spectroscopy with progesterone 18-al prepared according to Kalvoda's procedure.

mp. 140°, b.p. 153°/8 mm, yield 91%.

Synthesis of Model Compound

The model compound, 1 methyl 3:4 dihydro 6:7 dimethoxy isoquinoline was prepared starting from vanillin.

Methylation of vanillin

In a 1-litre three necked flask fitted with a mechanical stirrer, a reflux condenser and a dropping funnel, 152 gm (1 mol) of vanillin (167) was melted by warming on a steam bath with vigorous stirring. A solution of 80 gm of pure KOH in 120 ml of water was added with stirring at rapid rate and 120 ml of purified dimethylsulphate was added dropwise by separating funnel over a period of 20 minutes. The reaction mixture remained alkaline. The stirring was continued for another 30 minutes after addition of dimethyl sulphate on the water bath. The reaction mixture was then cooled rapidly to 25° with continuous stirring and veratraldehyde (168) was extracted with 300 ml of ether. m.p. 43-44.5°, b.p. 153°/8 mm, yield 80%.

Veratraldehyde to ω -nitro 3:4 dimethoxy-styrene

It was prepared according to the procedure of Knoevenagel and Walter⁹⁵. Veratraldehyde (168) (16.6 gm, 0.1 molar) was dissolved in alcohol (20 ml) and treated with nitromethane (6.5 gm, 0.1 molar) and then with methylamine hydrochloride (0.5 gm) and anhydrous sodium carbonate. The mixture was shaken vigorously for five minutes and kept at 5°. After 30 minutes, the supernatant liquid was carefully decanted and the residue was washed with alcohol (5 ml) and the washings were mixed with the above liquid. The decanted liquid was allowed to stand for 3 days at 5°C and the crystals of 3:4 dimethoxy ω -nitrostyrene (169) were filtered, washed with little cold alcohol and

dried under vacuum, m.p. 140°, lit.140°, yield 91%.

ω-nitro3:4 dimethoxy styrene (169) to homoveratrylamine (170)

The procedure followed was that reported by Dutta *et al.*⁹⁶ Lithium aluminium hydride (9.0 gm) in tetrahydrofuran (100 ml) was taken in a 500 ml three-necked flask equipped with a mercury seal and stirrer, a reflux condenser and a dropping funnel. To this a solution of 3:4 dimethoxy -nitrostyrene (169) (20.9 gm) in 200 ml dry tetrahydrofuran was added in portions, with stirring. After the addition was over the reaction mixture was refluxed for one hour and cooled. The excess of lithium aluminium hydride was decomposed by adding moist tetrahydrofuran and finally water (just 5 ml). The reaction mixture was filtered and the residue was washed with little tetrahydrofuran. The washings and filtrate were pooled together and the solvent was removed under reduced pressure. The solution was made acidic with HCl and extracted with ether. Aqueous layer was basified and extracted with ether (3 x 100 ml). The ether extract was dried over anhydrous sodium sulphate and ether was removed. The residue - the amine (170) was distilled under vacuum 142°/2 mm. yield 80%. The homoveratryl amine is highly unstable, hence was acetylated immediately.

Acetylation of homoveratrylamine

Acetylation was carried out according to procedure of Dey *et al.*⁹⁷ Homoveratrylamine (5 gm) was dissolved in dry ether (100 ml); acetic anhydride (4 ml) was added dropwise and left overnight. Crystalline homoveratrylacetamide (171) which separated out was filtered and recrystallized from benzene. m.p.95°, lit.94-95°. yield 90%. IR spectrum the bands due to N-H were seen at 3300 and 3400

1. Methyl 3:4 dihydro, 6:7 dimethoxy isoquinoline from homoveratraldehyde-acetamide

The N-acide (171) (104 mg) was taken in dry toluene (25 ml) and treated with phosphorus oxychloride (10 ml). The reaction mixture was refluxed for two hours, cooled and poured into pet-ether and allowed to stand for few hours. The solid separated was dissolved in water and coloured material which separated, was taken in excess chloroform, extracted with ether to remove any unchanged amide. The aqueous portion was made alkaline and extracted with ether. The combined ether extract was dried over potassium carbonate. Removal of ether yielded 6:7 dimethoxy 1 methyl 3:4 dihydroisoquinoline (172) as pale yellow crystals, melting between 102-104°. Recrystallized from pet-ether gave crystal melting at 106-107°. lit. 108°. IR spectrum - Fig. (17).

The picrate was prepared by dissolving a little amount of compound in alcohol and adding few drops of a solution of picric acid in alcohol. Crystalline compound m.p. 205°, lit. 205°.

Perbenzoic acid reaction on isoquinoline (172)

To 150 mgm (of 172) in chloroform (2 ml), 2 ml of perbenzoic acid in chloroform (65 mg/ml) were added slowly with shaking. The reaction mixture was kept at 15° for 4 days. It was then diluted with more of chloroform. A paste of potassium carbonate and a drop of water were added. The reaction mixture was filtered after shaking well. The filtrate was evaporated under vacuum. Residue - oily, kept under vacuum froths up - settles to a hygroscopic mass from aqueous acetone. Recrystallized from aqueous methanol, m.p. 136-38°, yield 57%. A picrate of the above compound (173) was prepared. The greenish crystalline picrate had a m.p. 217-219° as against the picrate of compound (172) melting at 205°. In the IR spectrum the bands due to N-O were seen at 960 and 943 cm⁻¹.

Sodium metaperiodate reaction on N-oxide (173)

The N-oxide (173) (100 mgm) was taken in aqueous methanol (5 ml), treated with an aqueous solution of sodium metaperiodate (200 mg in 2 ml water) and then left overnight on a shaker. The reaction mixture was filtered and the filtrate was evaporated. Coloured material which separated, was taken in excess chloroform. The chloroform layer was washed with water and evaporated after drying. A practically colourless gum separated out which was taken in ethyl acetate. It crystallized out after leaving in cold for 10 days. m.p. 185-188°.

Analysis: Found- C 60.42, H 6.21, N 5.67%. Calculated for $C_{12}H_{15}NO_4$ C 60.30, H 6.15, N 5.50%. In IR spectra (Fig.18) the band at 3343 cm^{-1} represents OH grouping and at 1675 cm^{-1} carbonyl group absorption was observed.

Chapter V

Synthesis of 18-Aldoprogesterone from Progesterone

Partial synthesis of 18-aldeprogesterone
from progesterone

Kalvoda et al.⁹³ reported the synthesis of 18-aldeprogesterone from progesterone involving 11 steps. A careful observation of the scheme revealed that this synthesis could be brought about in 7 steps with possible improvements in the yields of the final compound.

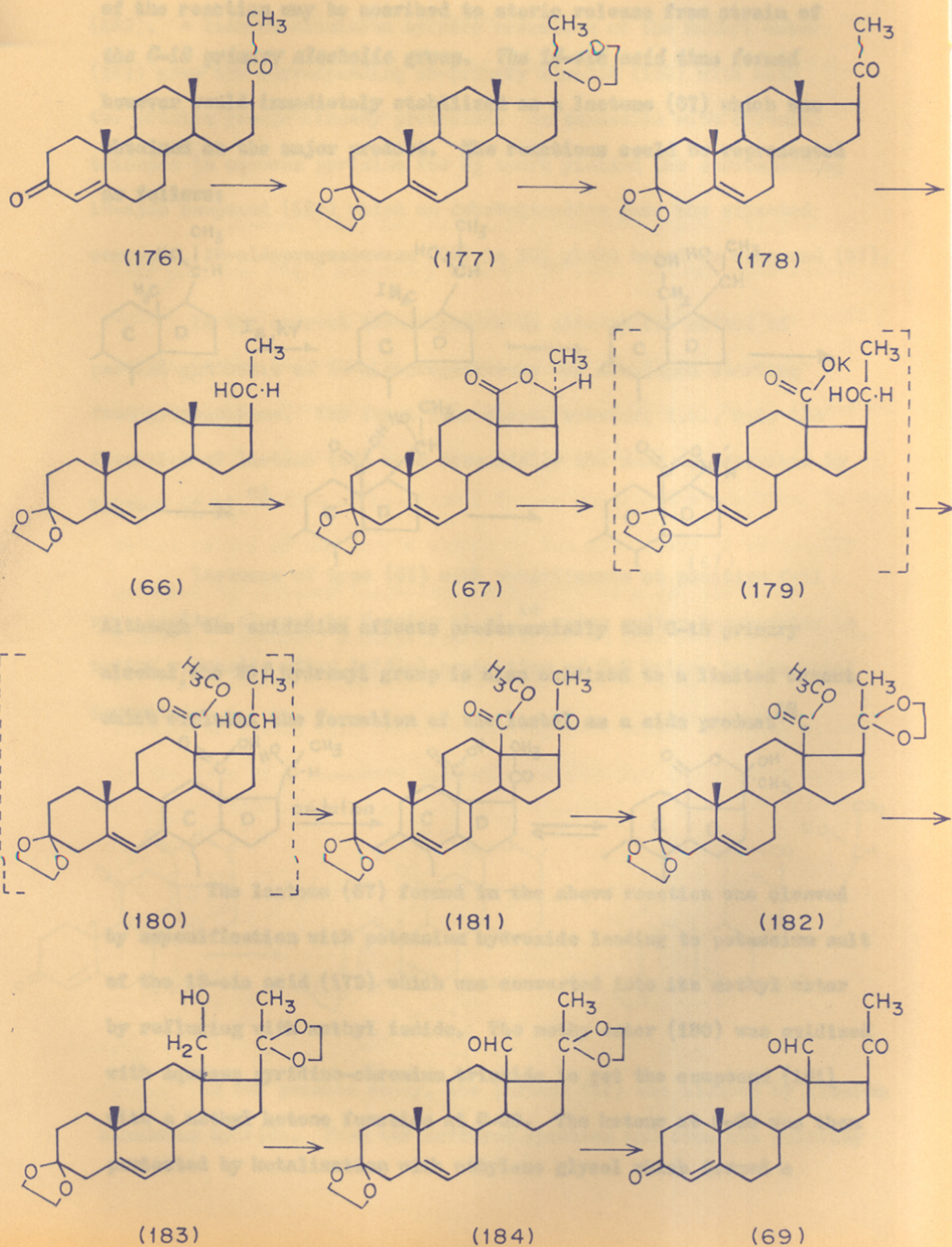
The steps in the partial synthesis of 18-aldeprogesterone according to Kalvoda's procedure, represented in Chart (XVI) can be outlined as follows:-

Progesterone (176) on treatment with ethylene glycol in toluene, in the presence of catalytic amount of p-toluenesulfonic acid gave a $\Delta^{5,3:20}$ diethylene ketal (177) as described by Williams et al.⁷⁰ On selective deketalization of C-20 carbonyl group, the diketal yielded the $\Delta^{5,3}$ -ethylene dioxy-ketal (178), earlier obtained by Ringold et al.⁷² by a different method. Lithium aluminium hydride reduction of (178) in dioxane gave 3-ethylene dioxy-20 β -ol Δ^5 pregnene (66).

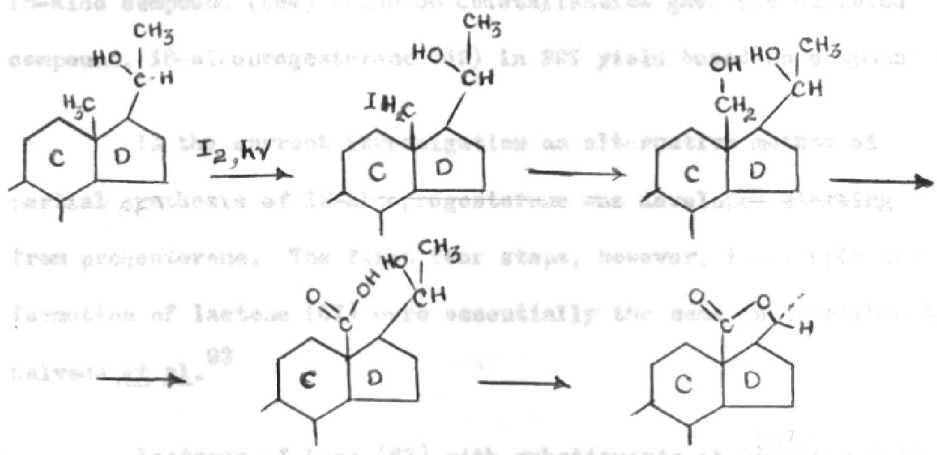
Wettstein et al.¹⁸ developed a method for conversion of (66) to the corresponding 18- α lactone (67) involving intramolecular oxidation. Iodination in cyclohexane in presence of calcium carbonate and lead tetraacetate results probably in a selective iodination at C-18. Irradiation was necessary to accelerate the substitution at C-18 axial methyl group. The 18-iodo compound, on treatment with lead tetraacetate was transformed into 18-hydroxy compound which was selectively oxidized with silver chromate-chromium trioxide in

Progesterone to 18-aldoprogesterone

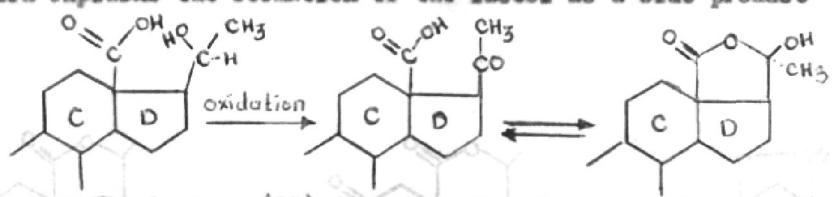
Chart XVI



pyridine in preference to the 20 β hydroxyl group. The selectivity of the reaction may be ascribed to steric release from strain of the C-18 primary alcoholic group. The 18-oic acid thus formed however would immediately be stabilized as a lactone (67) which was obtained as the major product. The reactions could be represented as follows:



Although the oxidation affects preferentially the C-18 primary alcohol, the 20 β hydroxyl group is also oxidized to a limited extent which explains the formation of the lactol as a side product¹⁸.

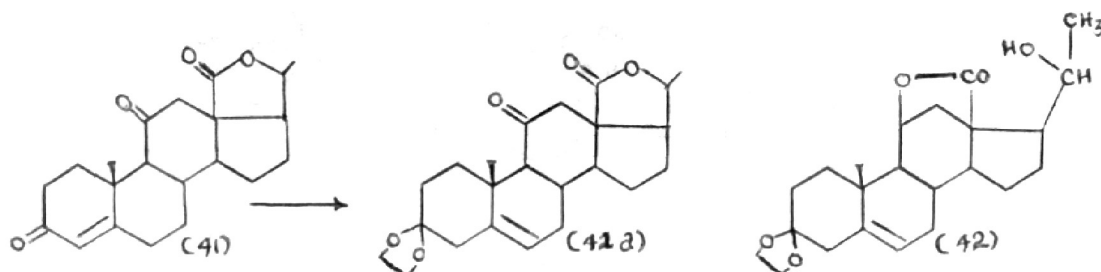


The lactone (67) formed in the above reaction was cleaved by saponification with potassium hydroxide leading to potassium salt of the 18-oic acid (179) which was converted into its methyl ester by refluxing with methyl iodide. The methyl ester (180) was oxidized with aqueous pyridine-chromium trioxide to get the compound (181) in the present study, the lactone (67) was cleaved by lithium aluminium hydride. From the infrared spectrum and from the polarity protected by ketalization with ethylene glycol which formed a

3;20-diethylene ketal-18 methyl ester of progesterone 18-oic acid (182). A lithium aluminium hydride reduction of the methyl ester (182) gave the corresponding 18-primary alcohol (183) with both the ketonic groups already protected. An oxidation with chromium trioxide in aqueous pyridine for $3\frac{1}{2}$ hours yielded the corresponding 18-aldo compound (184) which on deketalization gave the expected compound, 18-aldeprogesterone (69) in 30% yield based on compound (67).

In the current investigation an alternative method of partial synthesis of 18-aldeprogesterone was developed starting from progesterone. The first four steps, however, i.e., upto the formation of lactone (67) were essentially the same as described by Kalveda *et al.*⁹³

Lactones of type (67) with substituents at position C-11 were earlier cleaved by Heusler *et al.*¹⁴ using sodium borohydride in sodium hydroxide after initial protection of C-3 ketone by the ketal formation.

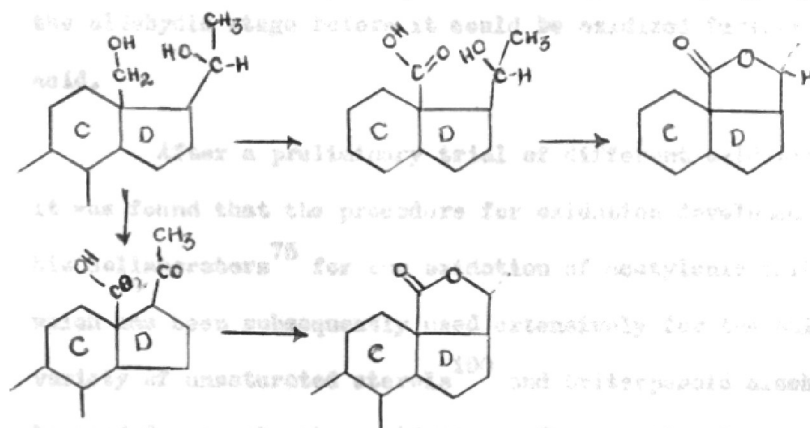


In the present study, the lactone (67) was cleaved by lithium aluminium hydride. From the infrared spectrum and from the polarity

behaviour the reduced compound was assigned the structure (185)

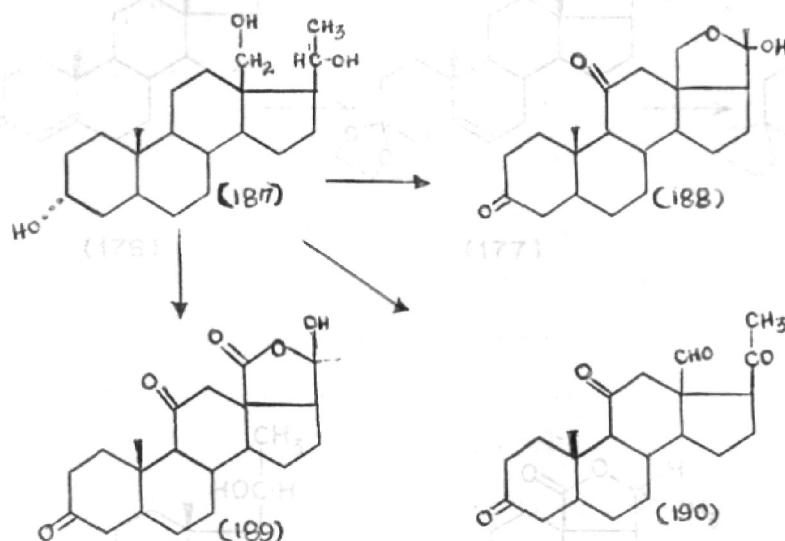
3-ethylene dioxy-18,20 dihydroxy-5-pregnene. The compound (185) was further converted to (186) by mild hydrolysis. The melting point, infrared etc. agreed with the known compound (186) reported by Sorm et al.⁹⁸

Compound (185) has an axially oriented primary alcohol at C-18 position and a secondary alcohol at C-20. It was anticipated that a controlled oxidation of primary and secondary alcohols would result in the formation of corresponding aldehyde and a ketone respectively. However, in this case the oxidation of axial 18-alcohol takes place much faster than the quasi equatorial 20 β -ol. It is quite probable that an additional factor helping the reaction is due to the relief of the steric strain by the conversion of the axially oriented Sp^3 primary alcoholic group first to a Sp^2 aldehydic function. However, a complicating factor in this reaction would be the formation of 18-carboxylic acid which would immediately form a lactone or lactol with the neighbouring C-20 oxygen function either in the form of a secondary alcohol or a carbonyl group (shown below).



be used for a selective oxidation. The reaction is usually of a short time duration.

Wettstein et al.⁹⁹ have reported that 11-substituted 18:20 (α or β) ols of the type (187) on oxidation with chromium trioxide for 15 hrs in pyridine resulted in the formation of (188), (189) and (190) in the yields of 22%, 3.4% and 3.8% respectively.



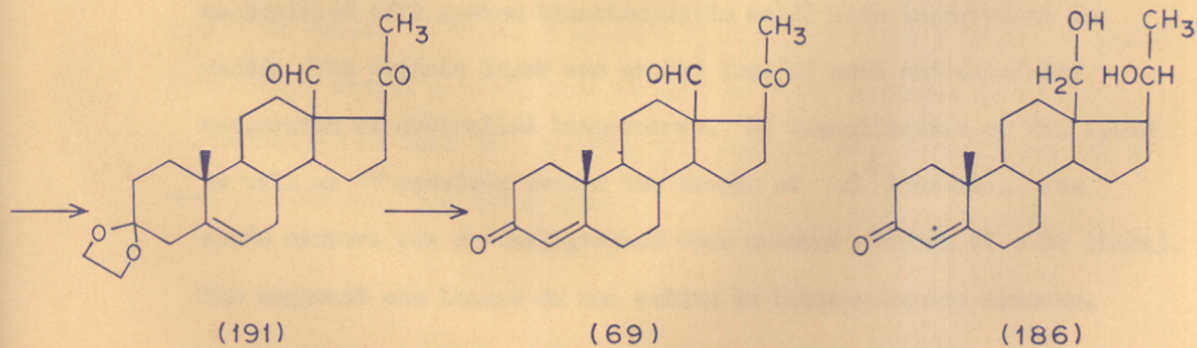
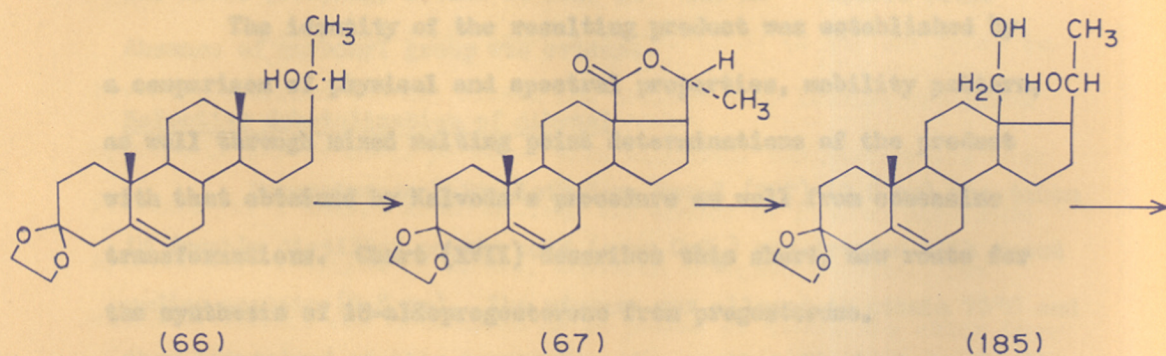
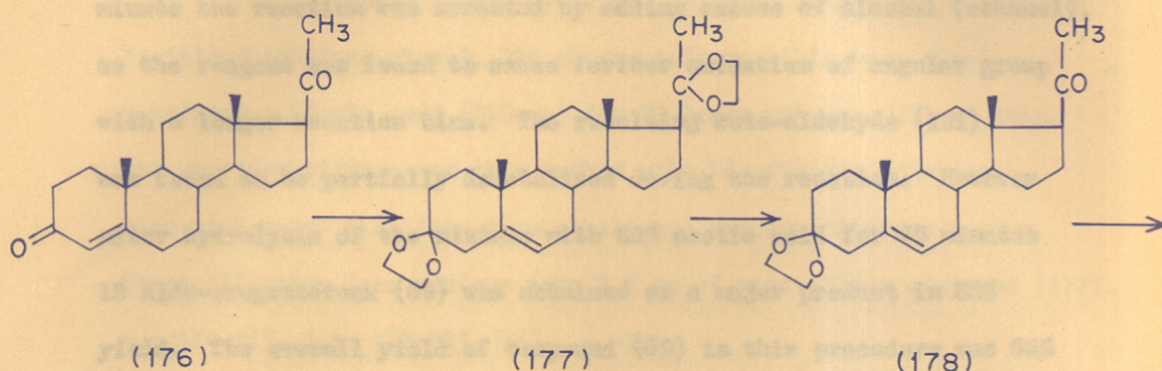
To improve the yields of 18 alde compound, it was necessary to explore some means of arresting the oxidation of primary alcohol at the aldehydic stage before it could be oxidized further to 18-oic acid.

After a preliminary trial of different oxidizing systems, it was found that the procedure for oxidation developed by Jones and his collaborators⁷⁵ for the oxidation of acetylenic carbinols and which has been subsequently used extensively for the oxidation of a variety of unsaturated sterols¹⁰⁰ and triterpenoid alcohols¹⁰¹ could be used for a selective oxidation. The reaction is usually of a short time duration.

Progesterone to 18 aldoprogestosterone

Chart XVII

by a new route



The diol (185) was treated with Jones's reagent and the reaction was carried out at room temperature. After the 8th minute the reaction was arrested by adding excess of alcohol (ethanol), as the reagent was found to cause further oxidation of angular group with a longer reaction time. The resulting keto-aldehyde (191) was found to be partially deketalized during the reaction. However after hydrolysis of the mixture with 80% acetic acid for 45 minutes 18-aldo-progesterone (69) was obtained as a major product in 85% yield. The overall yield of compound (69) in this procedure was 65% starting from the lactone (67) compared to 30% reported in the earlier procedure by Kalvoda et al.⁹³

IR spectrum showed absorption at 1000-1100 cm^{-1} for ketal grouping, a band at 1687 cm^{-1} for $\text{C}=\text{O}$ bond.

The identity of the resulting product was established by a comparison of physical and spectral properties, mobility pattern, as well through mixed melting point determinations of the product with that obtained by Kalvoda's procedure as well from conessine taken transformations. Chart (XVII) describes this short, new route for the synthesis of 18-aldo-progesterone from progesterone.

The mixture was then maintained at this temperature for another 10 minutes. Acetic acid was then removed by azeotropic distillation with benzene under vacuum below 50°. In an alternative procedure the acid was directly neutralized with sodium bicarbonate (in solid) with improved yield. The organic layer was washed free of acid and solvent was evaporated at controlled temperature. IR visualization of the crude mixture as well as UV spectrum showed the traces of Δ^4 -pregnen-20-one.

The crude mixture was chromatographed over neutral alumina. The compound was loaded on the column in benzene-hexane mixture.

EXPERIMENTAL

Progesterone (5 gm) (176) was refluxed with 40 ml of freshly distilled ethylene glycol, 220 ml dry toluene (thiophene free), p-toluene sulfonic acid (210 mg) for 24 hours using a Claisen trap.

The reaction mixture was then washed with bicarbonate solution, then with water till free from alkali and evaporated under vacuum.

Crystallization from acetone gave 3.5 gm of crystalline compound (177). m.p. 179-81°. lit. 178-82°. $[\alpha]_D^{25} = -29^\circ$.

Analysis: Found- C 74.53, H 9.83%. Calculated for $C_{25}H_{38}O_4$

requires C 74.59, H 9.51%. IR spectrum showed absorption at 1085-90 cm^{-1} for ketal grouping, a band at 1657 cm^{-1} for $\Delta^{5,6}$ double bond.

Absence of carbonyl group was evident.

Selective deketalization of diketal

Progesterone 3:20 diethylene ketal (177) (3.5 gm) was taken in freshly distilled acetic acid (over CrO_3) and treated with fused sodium acetate (3.5 gm). The mixture was allowed to attain 75°C and then maintained at this temperature for another 20 minutes. Acetic acid was then removed by azeotropic distillation with benzene under vacuum below 55°. In an alternate procedure the acid was rapidly neutralized with sodium bicarbonate (in cold) with improvement in yield. The organic layer was washed free of acid and carefully evaporated at controlled temperature. UV visualization of TLC spots as well as UV spectrum showed the traces of Δ^4 3-ketone. The crude mixture was chromatographed over neutral alumina (1 x 30 times).

The compound was loaded on the column in benzene-hexane mixture.

Elution of the column with 20% benzene in hexane (100 ml) yielded 70% of the compound loaded (Fraction I). Next elution with 30% benzene in hexane yielded another 15% of the compound (Fraction II). Elution with 40% benzene in hexane gave a mixture of the monoketal and progesterone (2%) and further elution with 50% benzene in hexane gave progesterone (8%).

Fractions I and II showing same R_f value on TLC were pooled together and crystallized from benzene-hexane to a constant m.p. 178-80°. $[\alpha]_D^{25} = +47^\circ$. Yield - above 80%. IR spectrum showed a band at 1695 cm^{-1} (C-20 methyl carbonyl) and 1085 cm^{-1} (ketal group).

Lithium aluminium hydride reduction of the monoketal (178)

Progesterone-3 ethylenedioxyketal (178) (3 gm) was taken in water (30 ml) for 3 hours at room temperature. Water for freshly distilled (85 ml) dioxan and treated with lithium aluminium hydride (3 gm) in small portions with stirring. The mixture was then with ice. The mixture was then heated at 60° with stirring for refluxed for 1 hour. After cooling the excess of lithium aluminium hydride was destroyed by careful addition of aqueous ether. The of carbon salt (10 gm) filtered through celite. The reaction mixture was then taken up in excess of ether. The ether layer was washed with toluene repeatedly. The organic phase was washed with water till free from alkali and evaporated after drying with common salt solution, dried and evaporated at 40° m.p. The residue was taken in benzene-ethylacetate mixture (1:1) and washed over sodium sulfate. Crystallization from acetone-ether gave compound (66) (2.7 gm) m.p. 192-94°. yield 90%. $[\alpha]_D^{25} = -54^\circ$ (c = 0.016). Analysis: Found- C 76.48, H 10.10%. $\text{C}_{23}\text{H}_{36}\text{O}_5$ requires C 76.62, H 10.07%. IR spectrum showed a broad band at 3333 cm^{-1} for hydroxyl group and an absorption at 1667 cm^{-1} showing the presence of a double bond and a peak at 1085 cm^{-1} for ketal group.

(1 x 10 gms) and mixing with diethylpropanone (100%) pure

Lactone formation

To a boiling mixture of 18 gm of lead tetraacetate, 6 gm calcium carbonate and 550 ml cyclohexane, 5 gm of iodine was added and the mixture was refluxed for 1 hour. A 3 gm portion of carbinol (66) i.e., 3-ethylenedioxy 20 β -hydroxy Δ^{5-6} pregnene was added to the reaction mixture. After refluxing the stirring under irradiation with a 1000 watts lamp for another 2 hours, the reaction mixture was cooled, filtered and washed with cyclohexane. The filtrate was washed with sodium thiosulfate solution and then with water. It was then treated with 5 ml pyridine and evaporated under vacuum at 55-60°. The resulting oil was taken up in pyridine (10 ml) and was stirred with a mixture of silver chromate (3 gm), chromium trioxide (3 gm) and water (30 ml) for 3 hours at room temperature. Another lot of 30 ml of pyridine was added to the reaction mixture after cooling with ice. The mixture was then heated at 60° with stirring for 16 hours. The reaction mixture was cooled and after the addition of common salt (10 gm) filtered through celite. The celite cake was washed with toluene repeatedly. The organic phase was washed with common salt solution, dried and evaporated at water pump. The residue was taken in benzene-ethylacetate mixture (9:1) and passed over deactivated silica column. After evaporating the eluent, the residue was dissolved in methylene chloride-ether mixture, when 1.3 gm of crystalline compound was obtained, showing some impurities on TLC. On passing the compound over neutral deactivated alumina (1 x 15 times) and eluting with pet-ether-benzene (50:50) pure crystalline lactone (67) (1.2 gm) was obtained. m.p. 220-22°. lit. 222°-225°. (Yield 40%). $[\alpha]_D = -22^\circ$ (c = 1.079).

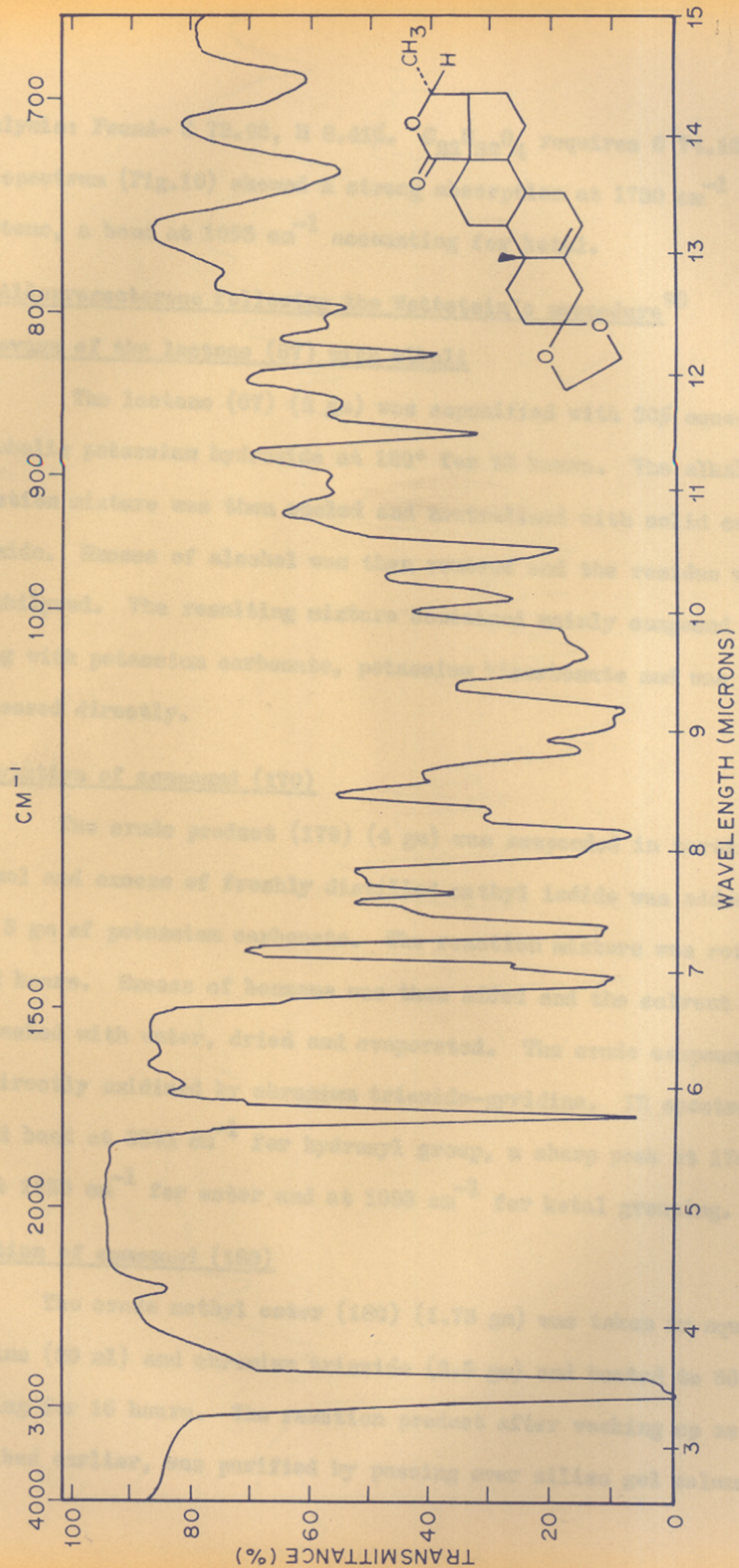


FIG. 19. IR SPECTRUM (NUJOL) OF LACTONE
3-3-ETHYLENEDIOXY-20 β -HYDROXY Δ^5 PREGNENE-18 ACID.

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Analysis: Found- C 73.92, H 8.41%. $C_{23}H_{32}O_4$ requires C 74.16, H 8.66%. IR spectrum (Fig.19) showed a strong absorption at 1730 cm^{-1} indicating lactone, a band at 1093 cm^{-1} accounting for ketal.

18-Aldoprogesterone following the Wettstein's procedure⁹³

Cleavage of the lactone (67) with alkali

The lactone (67) (2 gm) was saponified with 30% aqueous alcoholic potassium hydroxide at 130° for 12 hours. The alkaline reaction mixture was then cooled and neutralized with solid carbon dioxide. Excess of alcohol was then removed and the residue was lyophilized. The resulting mixture contained mainly compound (179) along with potassium carbonate, potassium bicarbonate and was processed directly.

Methylation of compound (179)

The crude product (179) (4 gm) was suspended in tertiary butanol and excess of freshly distilled methyl iodide was added together with 3 gm of potassium carbonate. The reaction mixture was refluxed for 2 hours. Excess of benzene was then added and the solvent layer was washed with water, dried and evaporated. The crude compound (180) was directly oxidized by chromium trioxide-pyridine. IR spectrum showed band at 3333 cm^{-1} for hydroxyl group, a sharp peak at 1740 cm^{-1} and at 1250 cm^{-1} for ester and at 1093 cm^{-1} for ketal grouping.

Oxidation of compound (180)

The crude methyl ester (180) (1.75 gm) was taken in aqueous pyridine (20 ml) and chromium trioxide (3.3 gm) and heated to 60° with stirring for 16 hours. The reaction product after working up as described earlier, was purified by passing over silica gel column

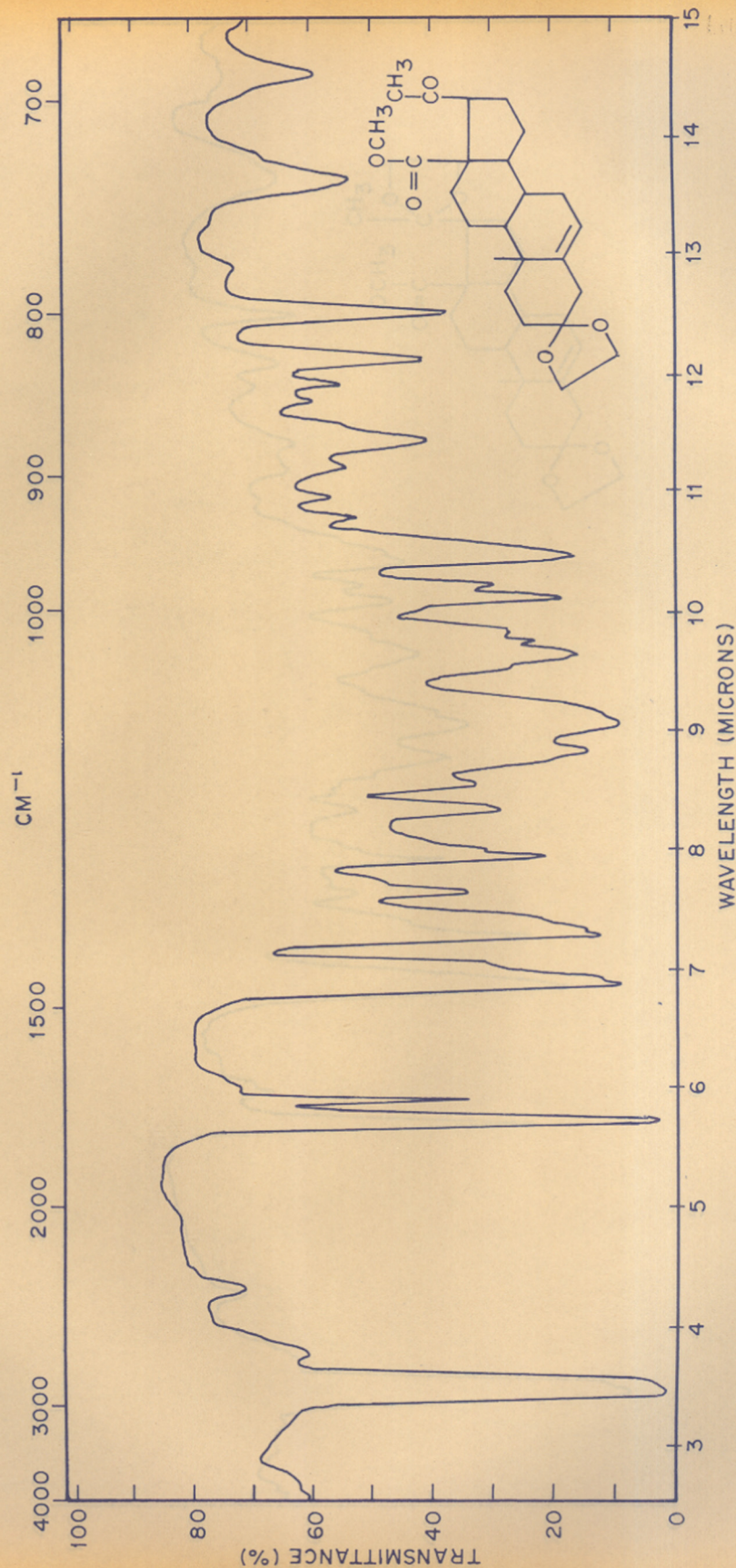


FIG. 20. IR SPECTRUM (NUJOL) OF 3-3 ETHYLENEDIOXY-20-OXO, Δ^5 -PREGNENE-18-OIC ACID METHYL ESTER.

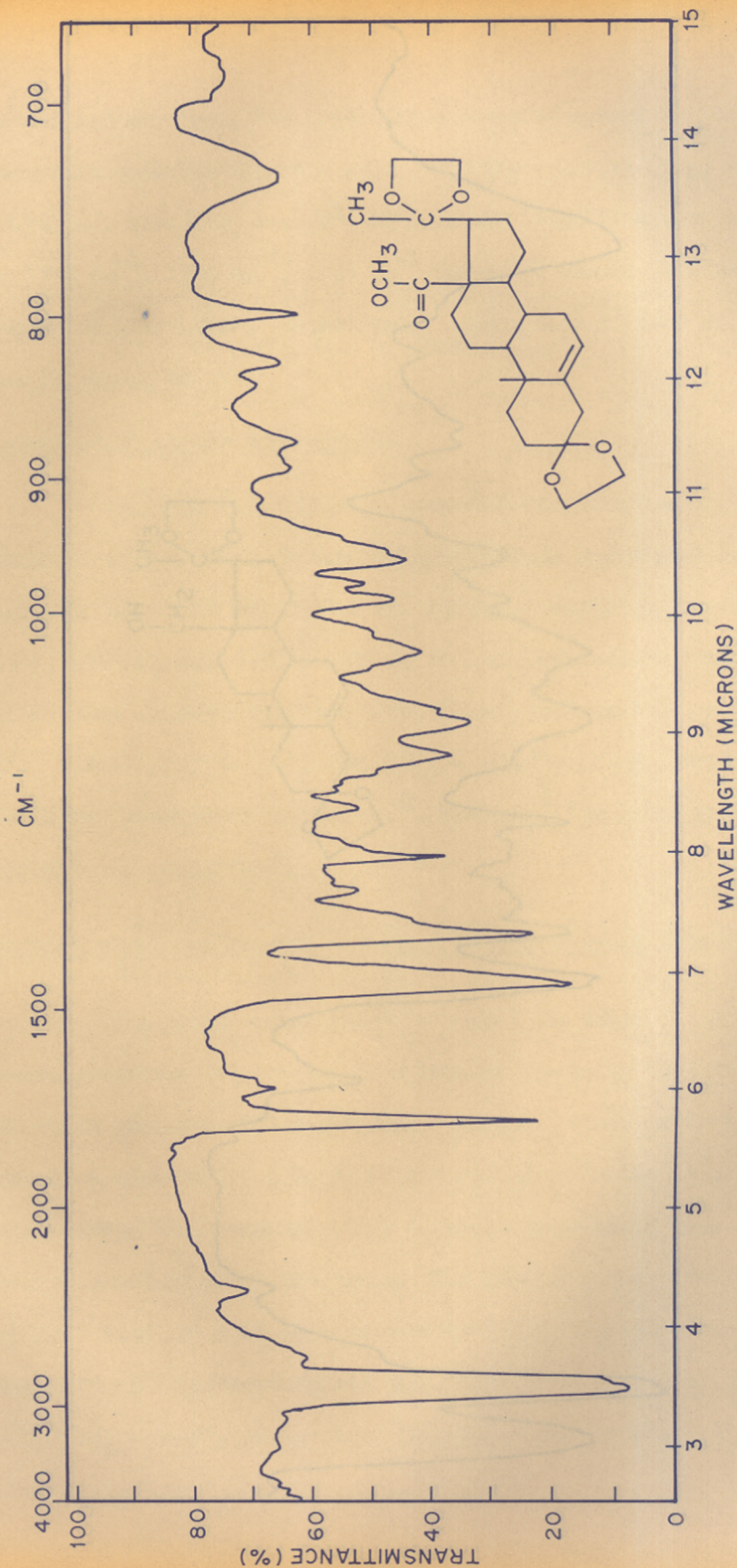


FIG. 21. IR SPECTRUM (NUJOL) OF 3-3, 20-20, DIETHYLENEDIOXY Δ^5 PREGNENE-18 OIC ACID METHYL ESTER.

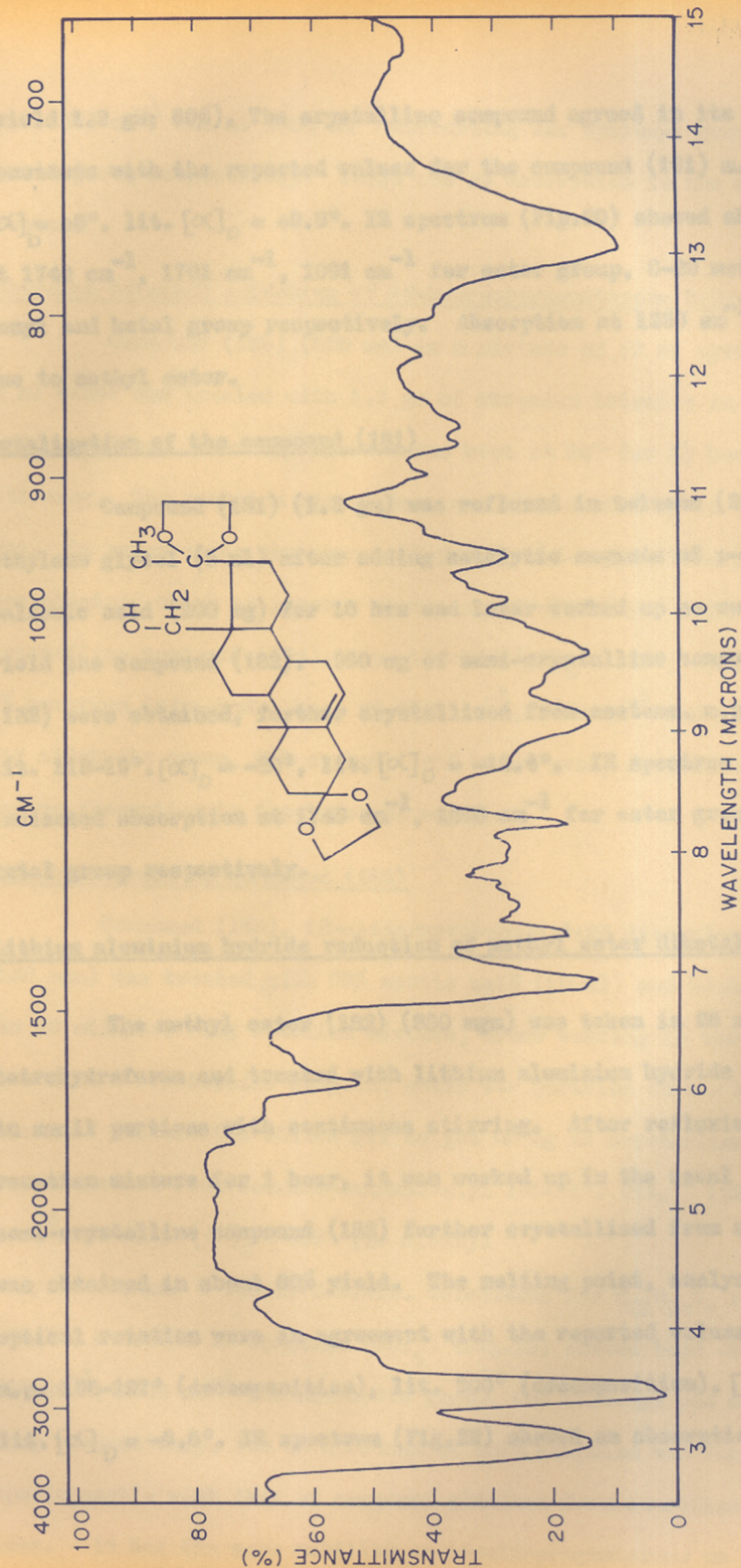


FIG. 22. IR SPECTRUM (NUJOL) OF 3:3, 20:20, DIETHYLENEDIOXY - 18-HYDROXY - Δ^5 - PREGNE

(yield 1.2 gm; 60%). The crystalline compound agreed in its physical constants with the reported values for the compound (181) m.p.173-74°. $[\alpha]_D = +8^\circ$. lit. $[\alpha]_D = +9.5^\circ$. IR spectrum (Fig.20) showed absorption at 1740 cm^{-1} , 1701 cm^{-1} , 1091 cm^{-1} for ester group, C-20 methyl carbonyl and ketal group respectively. Absorption at 1250 cm^{-1} was also due to methyl ester.

Ketalization of the compound (181)

Compound (181) (1.2 gm) was refluxed in toluene (25 ml), ethylene glycol (5 ml) after adding catalytic amounts of p-toluene sulfonic acid (200 mg) for 16 hrs and later worked up as usual to yield the compound (182). 950 mg of semi-crystalline compound of (182) were obtained, further crystallized from acetone. m.p.118-120°, lit. 118-19°. $[\alpha]_D = -20^\circ$, lit. $[\alpha]_D = -18.4^\circ$. IR spectrum (Fig.21) indicated absorption at 1740 cm^{-1} , 1090 cm^{-1} for ester group and ketal group respectively.

Lithium aluminium hydride reduction of methyl ester diketal

The methyl ester (182) (800 mgm) was taken in 25 ml dry tetrahydrofuran and treated with lithium aluminium hydride (800 mg) in small portions with continuous stirring. After refluxing the reaction mixture for 1 hour, it was worked up in the usual way. A semi-crystalline compound (183) further crystallized from acetone was obtained in about 80% yield. The melting point, analysis, optical rotation were in agreement with the reported values. m.p. 195-197° (decomposition), lit. 200° (decomposition). $[\alpha]_D = -8^\circ$, lit. $[\alpha]_D = -6.5^\circ$. IR spectrum (Fig.22) showed an absorption

at 3333 cm^{-1} and at 1093 cm^{-1} accounting for hydroxyl group and ketal group respectively. There was no absorption in the carbonyl region.

Chromium trioxide oxidation of 18-hydroxyprogesterone 3:20 diketal

Compound (183) (600 mg) in a mixture of 10 ml pyridine and 6 ml water was treated with 1.5 gm of chromium trioxide in small lots under cooling. Then the mixture was kept at 60° for $3\frac{1}{2}$ hours with stirring. The reaction mixture was then worked up in the usual way. On evaporating the organic layer a semi-crystalline mass (160 mg) was obtained which on passing over a small silica gel column gave a crystalline compound (184). m.p. $174-76^\circ$. $[\alpha]_D = +10^\circ$, lit. $[\alpha]_D = +11^\circ$. IR spectrum indicated a band at 2720 cm^{-1} , 1740 cm^{-1} and 1094 cm^{-1} for aldehydic group, CHO carbonyl and ketal group respectively. There was no absorption in the hydroxyl region.

Acid hydrolysis of compound (184)

Compound (184), 18-aldeprogesterone, 3:20 diethyleneketal (130 mg) was treated with 66% acetic acid (10 ml) and heated to 80° for 30 minutes. The reaction mixture, after working in the usual way, gave a semi-crystalline compound which was purified over a short column of deactivated silica gel to get 90 mg of crystalline compound. Recrystallized from benzene-hexane. m.p. $129-31^\circ$. lit.⁹³ $128-30^\circ$, lit.⁵⁵ $139-42^\circ$. $[\alpha]_D = +35^\circ$ ($c = 0.019$).

Analysis: Found- C 76.52, H 8.41%. Calculated for $C_{21}H_{28}O_3$ requires C 76.79, H 8.59%. IR spectrum (CCl_4) (Fig.16) showed band at 2720 cm^{-1} , 1745 cm^{-1} , 1705 cm^{-1} for aldehydic group, CHO carbonyl and C-20 methyl group respectively. The IR spectrum of this compound was found to be superimposable with that of compound obtained from concessine transformations. It had the same mobility as 18-aldeprogesterone on paper

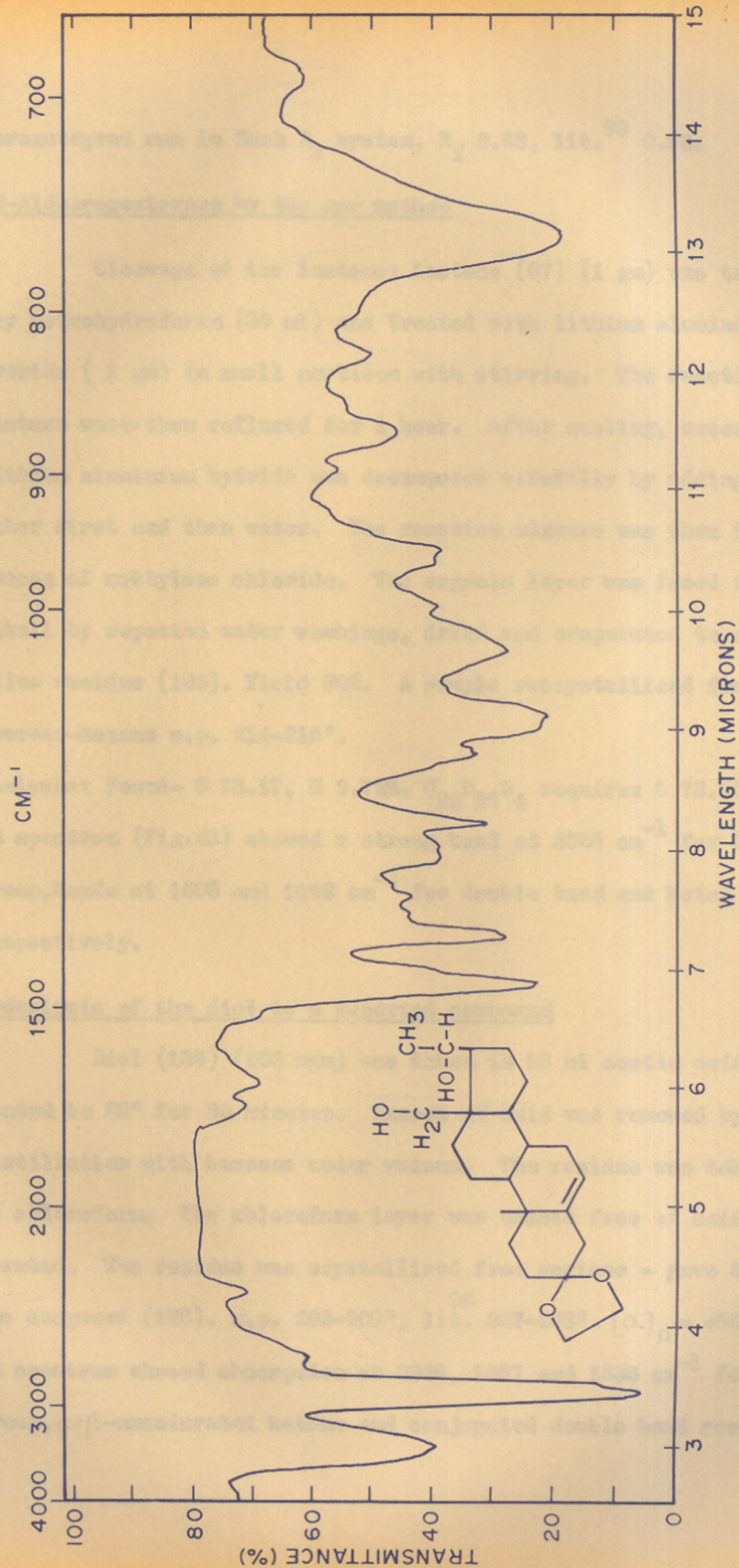


FIG. 23. IR SPECTRUM (NUJOL) OF 3:3 ETHYLENEDIOXY-, -18-20 DIHYDROXY- Δ^5 -PREGNENE.

chromatogram run in Bush B₃ system, R_f 0.63, lit.⁹³ 0.63.

18-Aldoprogesterone by the new method

Cleavage of the lactone: Lactone (67) (1 gm) was taken in dry tetrahydrofuran (30 ml) and treated with lithium aluminium hydride (1 gm) in small portions with stirring. The reaction mixture was then refluxed for 1 hour. After cooling, excess of lithium aluminium hydride was decomposed carefully by adding moist ether first and then water. The reaction mixture was then taken in excess of methylene chloride. The organic layer was freed from alkali by repeated water washings, dried and evaporated to a crystalline residue (185). Yield 82%. A sample recrystallized from benzene-hexane m.p. 214-216°.

Analysis: Found- C 73.17, H 9.72%. C₂₃H₃₆O₄ requires C 73.36, H 9.64%.

IR spectrum (Fig.23) showed a strong band at 3356 cm⁻¹ for hydroxyl group, bands at 1667 and 1093 cm⁻¹ for double bond and ketal group respectively.

Hydrolysis of the diol to a reported compound

Diol (185) (500 mgm) was taken in 20 ml acetic acid (60%) and heated to 80° for 30 minutes. Excess of acid was removed by azeotropic distillation with benzene under vacuum. The residue was taken in excess of chloroform. The chloroform layer was washed free of acid and evaporated. The residue was crystallized from acetone - gave 350 mg of the compound (186). m.p. 205-206°, lit.⁹⁸ 207-208°. $[\alpha]_D = +85^\circ$ (c=1.3). IR spectrum showed absorption at 3356, 1667 and 1635 cm⁻¹ for hydroxyl group, α - β -unsaturated ketone and conjugated double bond respectively.

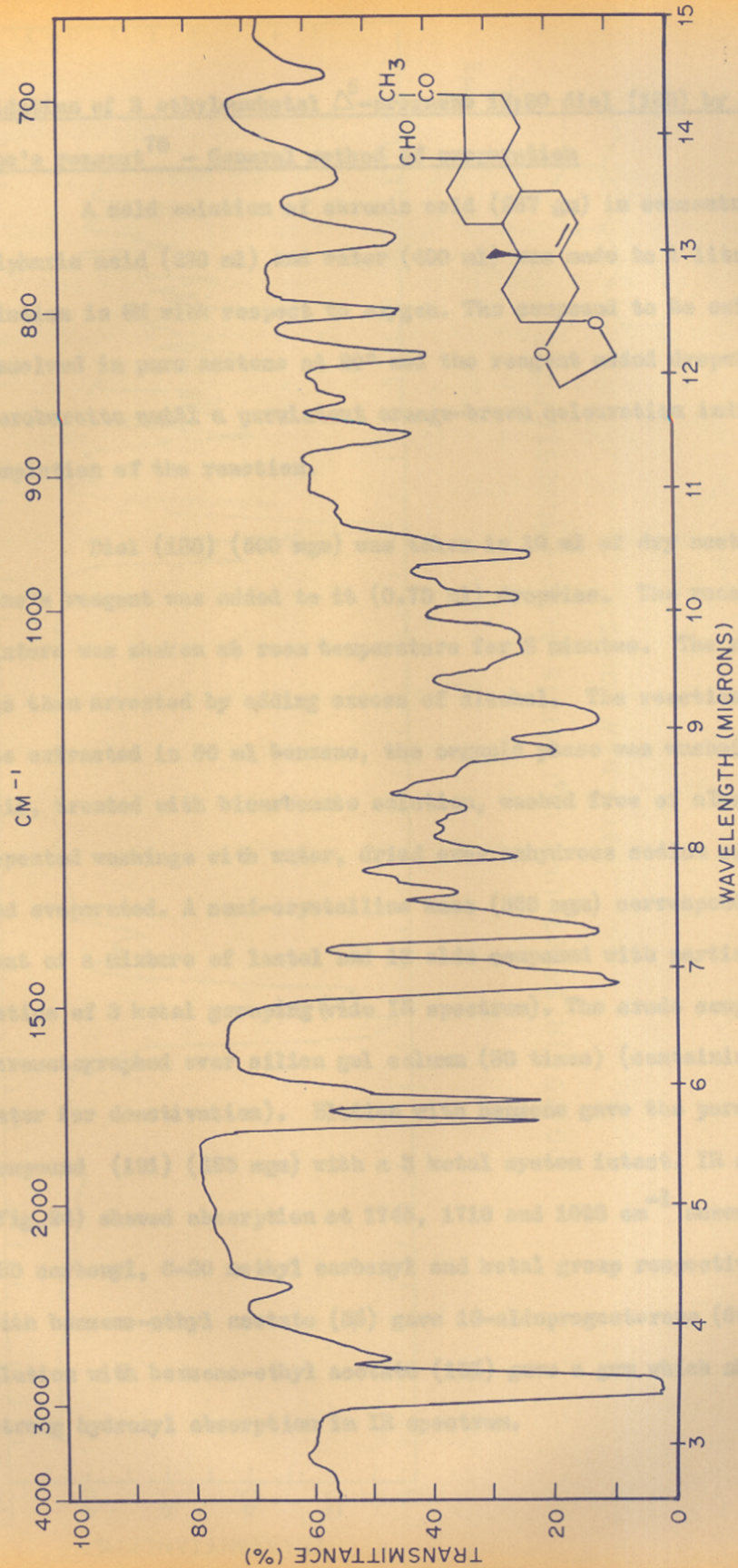


FIG. 24. IR SPECTRUM (NUJOL) OF 3-3 ETHYLENEDIOXY-18-OXO-PROGESTERONE.

Oxidation of 3 ethyleneketal Δ^5 -pregnene 18,20 diol (185) by Jones's reagent

Jones's reagent⁷⁵ - General method of preparation

A cold solution of chromic acid (267 gm) in concentrated sulphuric acid (230 ml) and water (400 ml) was made to 1 litre. This solution is 8N with respect to oxygen. The compound to be oxidized was dissolved in pure acetone at 20° and the reagent added dropwise from a microburette until a persistent orange-brown colouration indicated the completion of the reaction.

Diol (185) (500 mgm) was taken in 10 ml of dry acetone, and Jones's reagent was added to it (0.75 ml) dropwise. The reaction mixture was shaken at room temperature for 8 minutes. The reaction was then arrested by adding excess of alcohol. The reaction mixture was extracted in 50 ml benzene, the organic phase was washed free of acid, treated with bicarbonate solution, washed free of alkali by repeated washings with water, dried over anhydrous sodium sulphate and evaporated. A semi-crystalline mass (360 mgm) corresponded to that of a mixture of lactol and 18 aldo compound with partial deketalization of 3 ketal grouping (vide IR spectrum). The crude compound was chromatographed over silica gel column (30 times) (containing 15% water for deactivation). Elution with benzene gave the pure crystalline compound (191) (285 mgm) with a 3 ketal system intact. IR spectrum (Fig.24) showed absorption at 1745, 1710 and 1093 cm^{-1} accounting for CHO carbonyl, C-20 methyl carbonyl and ketal group respectively. Elution with benzene-ethyl acetate (5%) gave 18-aldeprogesterone (69) (37 mgm). Elution with benzene-ethyl acetate (15%) gave a gum which showed a strong hydroxyl absorption in IR spectrum.



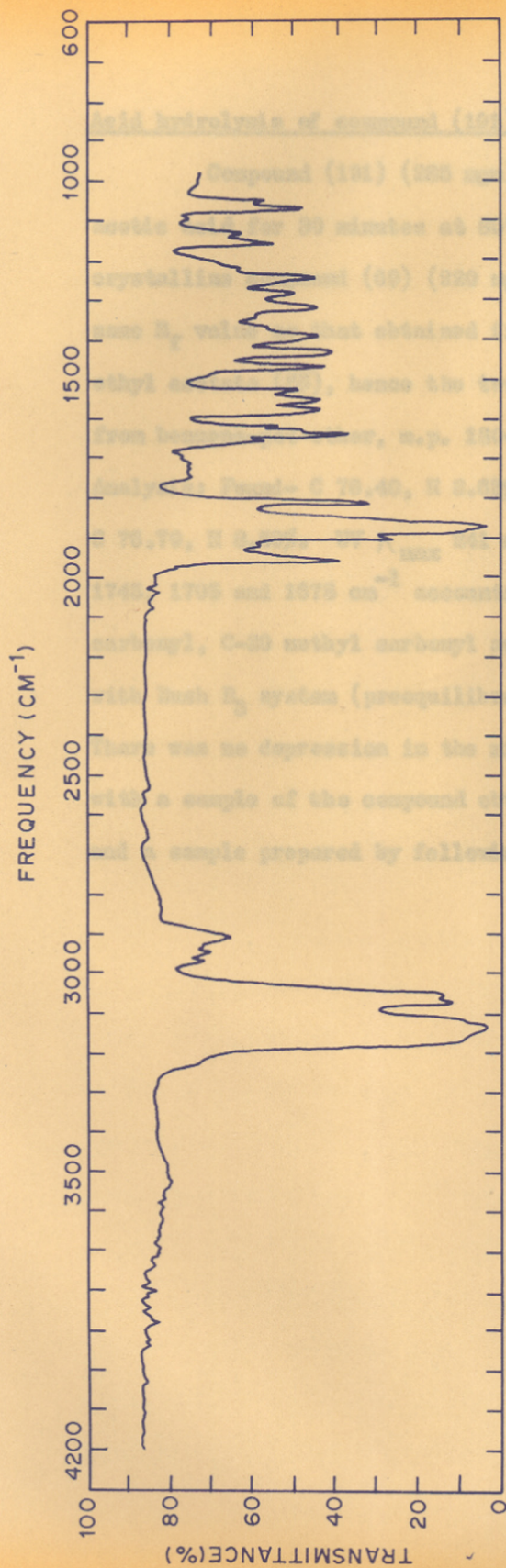
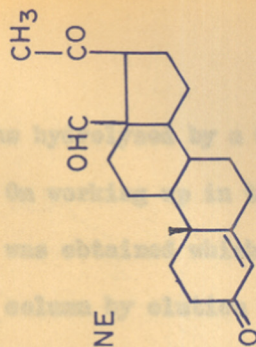


FIG. 25. IR SPECTRUM (CCl_4) OF 18-ALDO-PROGESTERONE
(BY A NEW ROUTE)



Acid hydrolysis of compound (191)

Compound (191) (285 mgm) was hydrolysed by a 60% aqueous acetic acid for 30 minutes at 80°. On working up in the usual way, crystalline compound (69) (220 mgm) was obtained which showed the same R_f value as that obtained from column by elution with benzene-ethyl acetate (5%), hence the two lots were combined and recrystallized from benzene-pet-ether, m.p. 130-132°, lit.⁹³ 128-30°, lit.⁵⁵ 139-42°. Analysis: Found- C 76.40, H 8.63%. Calculated for $C_{21}H_{28}O_3$ requires C 76.79, H 8.59%. UV λ_{max} 241 mμ. IR (CCl_4)- absorption at 2720, 1745, 1705 and 1675 cm^{-1} accounting for aldehydic group, aldehydic carbonyl, C-20 methyl carbonyl and unsaturated ketone. R_f value with Bush B_3 system (preequilibrated) 0.63, Bush B_5 system 0.90. There was no depression in the mixed melting point on admixture with a sample of the compound obtained from conessine transformations and a sample prepared by following Kelvoda's procedure.

in bacteria, yeasts, actinomycetes and even protozoa.

MICROBIOLOGICAL TRANSFORMATIONS OF STEROIDS

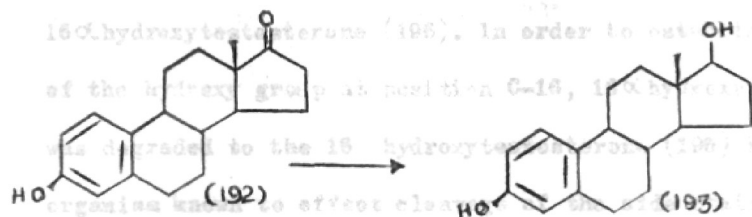
In the partial and total syntheses of adrenocortical hormones, the introduction of oxygen at position C-11 by purely chemical means had proved to be rather difficult, usually requiring a large number of steps. The discovery by Peterson and Murray¹⁰² in 1952 that a readily available substrate, progesterone, could be converted to 11 α -hydroxyprogesterone, in high yields by the mucorale fungi opened up a new pathway for the synthesis of 11-oxygenated steroids. This breakthrough was of great practical value for the chemist so that several attractive routes to the synthesis of cortisone, hydrocortisone and other adrenal steroids became readily available.

A series of excellent and comprehensive reviews are available on the subject notably by Peterson¹⁰³, Eppstein et al.¹⁰⁴ Vischer and Wettstein¹⁰⁵, Finch¹⁰⁶, Flerey¹⁰⁷, Fried¹⁰⁸, Shull¹⁰⁹ and by many others.

Chemical changes in steroids have been effected at various sites in many different ways by different microorganisms. These changes include the introduction of nuclear and side chain hydroxyl groups, reduction of ring double bonds, ring and side chain ketol groups, cleavage of carbon-carbon linkages in the side chain as well as rings A and D, dehydrogenation of ring hydroxyl groups as well as rings A and B, opening of oxide rings, hydrolysis of steroid esters and many more combinations of some of the above reactions. Although these changes are mainly carried out by the filamentous fungi, the same type of biochemical transformations take place to a lesser extent

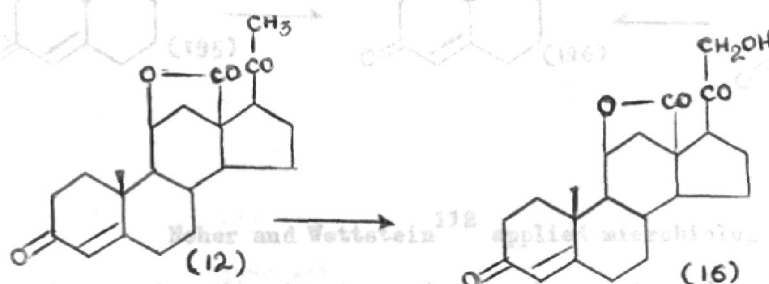
in bacteria, yeasts, actinomycetes and even protozoa.
by *Nidulomyces lycopersici*

In many instances the stereospecificity of enzymatic reactions in microorganisms have been exploited to achieve many highly selective transformations of steroid hormones. Wettstein *et al.*⁶ made ingenious use of this stereospecificity of the moulds for the resolution of racemic steroids. When synthetic (+)estrone (192) was fermented with yeast, (+)estradiol 17 β -ol (193) and unreacted (-)estrone were isolated.



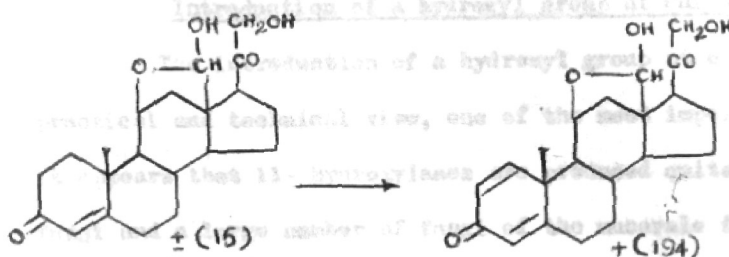
compound was thus established.

The Ciba group⁸ also demonstrated that only the (+)isomer of the totally synthetic lactone (12) underwent hydroxylation at C-21 by cultures of *Ophiobolus herpetrichus* whereas the enantiomorph remained unaffected.

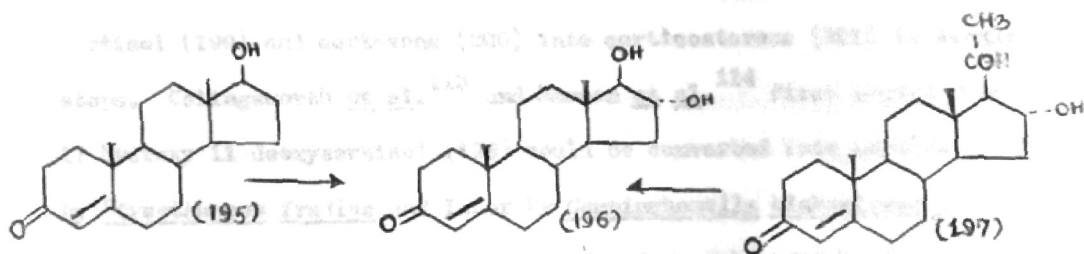


Utilizing the same principle, the naturally occurring enantiomorph of racemic aldosterone (15) was dehydrogenated at the 1,2 positions

by Didymella lycopersici¹¹⁰.



Microbiological transformations have also been used for proving the structures of new natural or synthetic steroids. For instance¹¹¹ using Streptomyces roseochromogenus, testosterone (195) was converted to 16 α -hydroxytestosterone (196). In order to establish the configuration of the hydroxy group at position C-16, 16 α -hydroxyprogesterone (197) was degraded to the 16 α -hydroxytestosterone (196) by S. lavandulae, an organism known to effect cleavage of the side chain and identity of the compound was thus established.

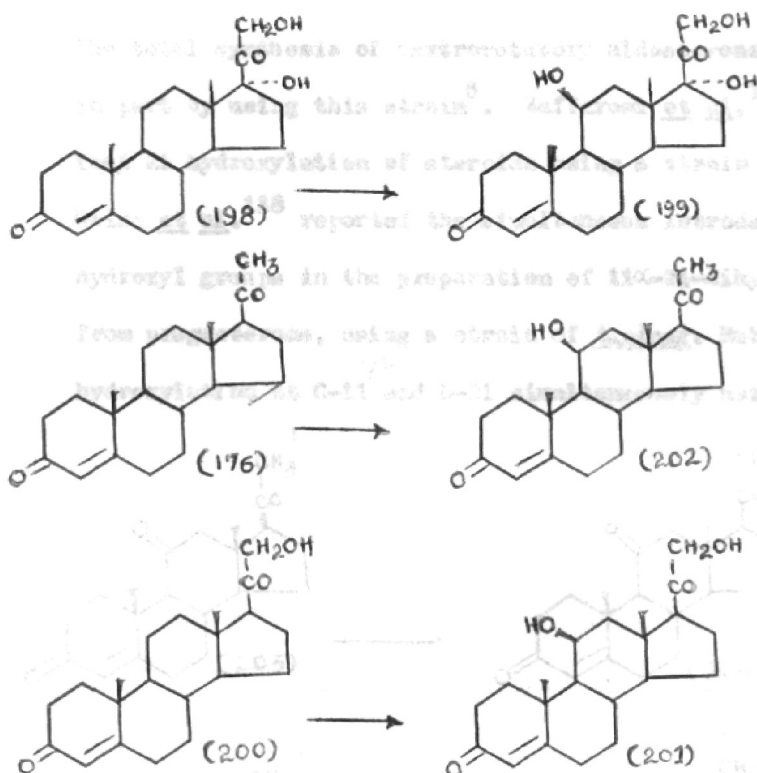


Neher and Wettstein¹¹² applied microbiological hydroxylation for proving the structure of several new steroids such as 6 β ,17 α -dihydroxy cortisone, 17 α -19-dihydrocortisone, 6 β -hydroxycorticosterone and 19-hydroxy corticosterone, isolated from the adrenals.

Introduction of a hydroxyl group at Carbon 11

The introduction of a hydroxyl group at carbon 11 is from a practical and technical view, one of the most important transformations. It appears that 11-hydroxylases are produced quite frequently by fungi and a large number of fungi of the *micorale* family and *Aspergillus*. However this hydroxylation is frequently accompanied by the introduction of an additional hydroxyl group, such as in the 6β , 7β , 17α and 21 positions. In some cases, 11α hydroxylations proceed uniformly and in excellent yields. 11α -hydroxyprogesterone for instance, is obtained from progesterone in almost quantitative amounts by the action of *Rhizopus nigricans*.

From a commercial viewpoint the microbiological 11 hydroxylation is the more important, as it leads directly to the syntheses of natural hormones if the proper substrates are chosen. 17 Hydroxy 11 deoxycortisol (198) for example can be transformed directly into cortisol (199) and cortexone (200) into corticosterone (201) in single steps. Colingsworth et al.¹¹³ and Hanson et al.¹¹⁴ first showed that 17 hydroxy 11 deoxycortisol (198) could be converted into cortisol (199) by *Streptomyces fradiae* and later by *Cunninghamella blakesleeana*. In the latter case cortisone was also produced as a by-product in this reaction. Shall et al.¹¹⁵ demonstrated that another organism *Curvularia lunata* could 11 β hydroxylate compound S i.e., 17α hydroxy 11-deoxycortisol (198), progesterone (176) and deoxycorticosterone (200) giving rise to cortisol (199), 11β -hydroxyprogesterone (202) and corticosterone (201) respectively.

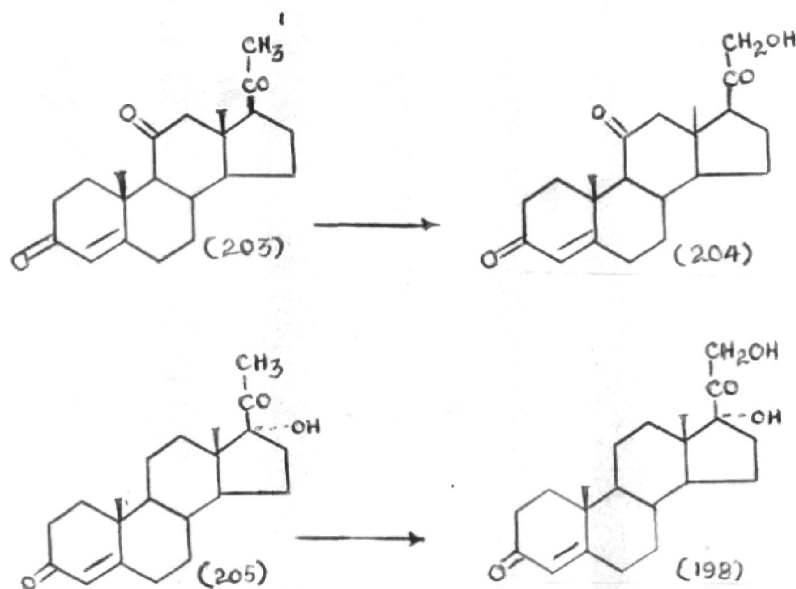


Other microorganisms such as Coniothyrium sp. have also been found to carry out this specific hydroxylation. The most effective 11β -hydroxylators reported, however, are Cunninghamella blakesleeana and Curvularia lunata which give higher yields with lesser amount of side products.

Hydroxylation at C-21

Meystre et al.¹¹⁶ were first to report hydroxylation on the methyl group of C-21 position using Ophiobolus herpetrichus. These workers were able to convert progesterone (176) to deoxycorticosterone (200), 11-ketoprogesterone (203) to 11-dehydrocorticosterone (204) and 17 α -hydroxyprogesterone (205) to 17 α -hydroxy 11-deoxycortisol (198). The 11β -hydroxyl compound (206) was separated from the

The total synthesis of dextrorotatory aldosterone was accomplished in part by using this strain⁶. Zaffaroni *et al.*¹¹⁷ also reported that 21 hydroxylation of steroids using a strain of *Aspergillus niger*. Weiss *et al.*¹¹⁸ reported the simultaneous introduction of 11 α and 21-hydroxyl groups in the preparation of 11 α -21-dihydroxyprogesterone from progesterone, using a strain of *A.niger*. Rubin *et al.*¹¹⁹ achieved hydroxylation at C-11^(β) and C-21 simultaneously using *Curvularia lunata*.



In the present investigations, a hydroxyl group at 11 β position was introduced in the substrate 18-aldoprogesterone by the strain *C.blakesleeani*. The substrate was incubated with a 36 hr- culture of *C.blakesleeani* on shake flasks for 36 hours. The steroidal residue was obtained from the broth by extraction with solvents and was made free of lipids by counter-current distribution between hexane and 70% ethanol. The 11 β -hydroxyl compound (206) was separated from the

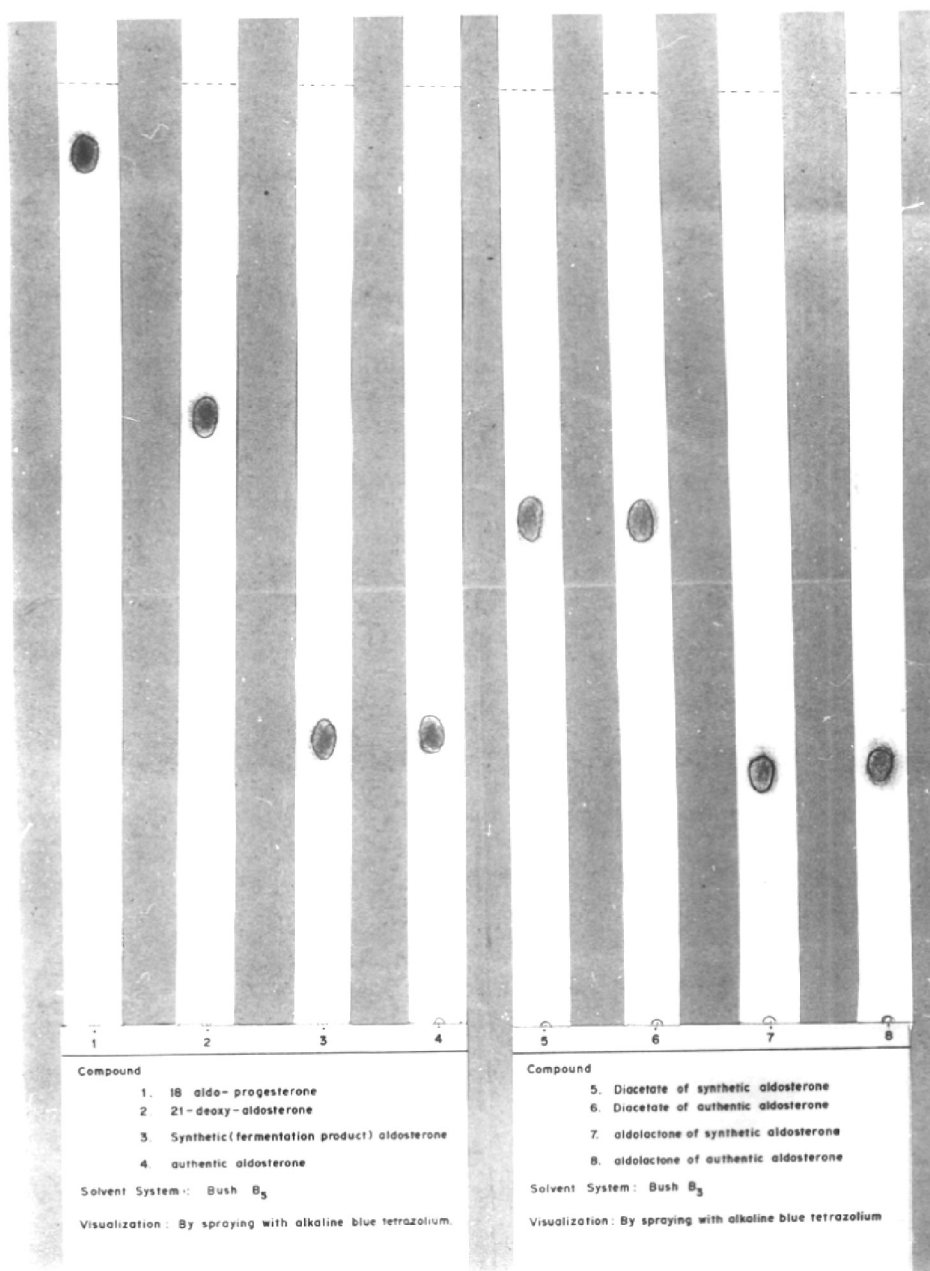
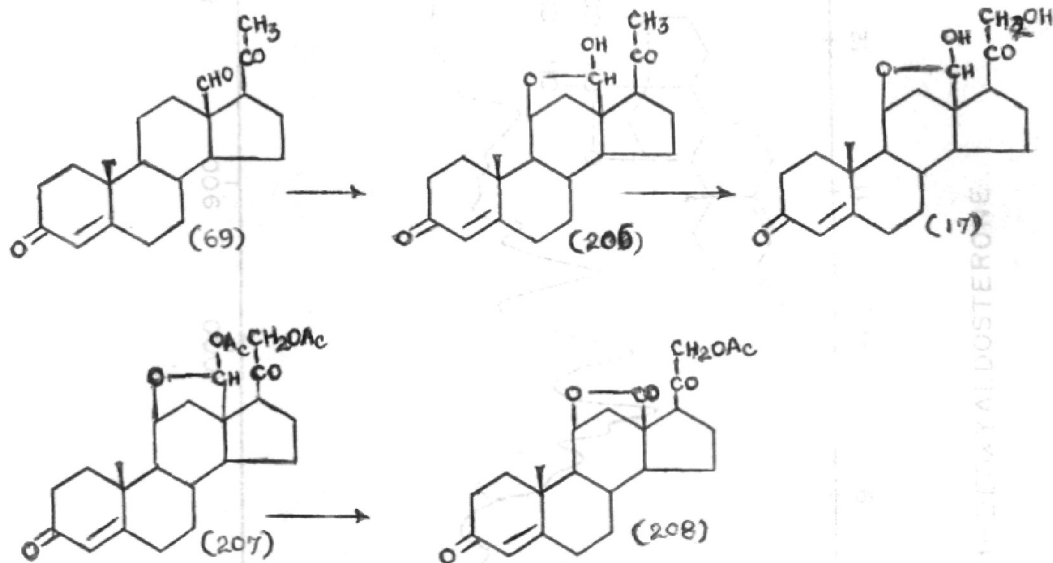


PLATE - 1

starting compound by two successive preparative thin layer chromatograms. The infrared spectra which showed a hydroxyl band at 3300 cm^{-1} , an ether linkage at 1100 cm^{-1} and the absence of aldehydic absorption indicating the hemiacetal formation between $11\beta\text{-OH}$ and 18-aldehyde groups (Fig. 26). The R_f value of the compound (206) on descending paper chromatogram (Bush B_g) was found to be 0.68 as against R_f 0.90 of starting compound (Plate-1).

The final step in this partial synthesis of aldosterone is the introduction of a hydroxyl group at C-21 position in 21-deoxyaldosterone to give the expected compound, aldosterone. This was achieved by incubating 21-deoxyaldosterone (206) with the strain Ophiobolus herpeticus. The organism was grown in an aerobic condition in submerged Czapek-Dox medium on a rotary shaker for 10 days. The substrate, 21-deoxyaldosterone was then added in ethanol-propylene glycol solvent mixture and was further incubated for a period of 7 days. The fermentation broth was then extracted in the usual manner and was separated from the lipid matter by counter-current distribution between hexane and 70% ethanol.

The residue was chromatographed on paper (Whatman 1) in Bush B_g system along with the authentic sample of d-aldosterone and developed with tetrazolium chloride. The fermentation mixture showed the presence of two clear-cut zones, one with the mobility (R_f 0.34) corresponding with that of authentic aldosterone (R_f 0.34) and the other with R_f 0.68 indicating the presence of the starting compound. The zone corresponding to aldosterone was eluted with methylene chloride and was rechromatographed on thin layer plate in



system- pet-ether-ethyl acetate (3:7). Its identity as aldosterone was further confirmed by converting it to its diacetate (207) and oxidizing the diacetate to lactone (208) as per the method of Raman *et al.*¹¹⁹ and comparing the mobility patterns both on paper and thin layer with the same derivatives prepared from authentic aldosterone. Plate-1 shows the mobility patterns of these compounds on paper chromatography. The yield of aldosterone was approximately 40% in this microbial step. The physical constants of synthetic aldosterone were in agreement with the reported values for dextrorotatory aldosterone.

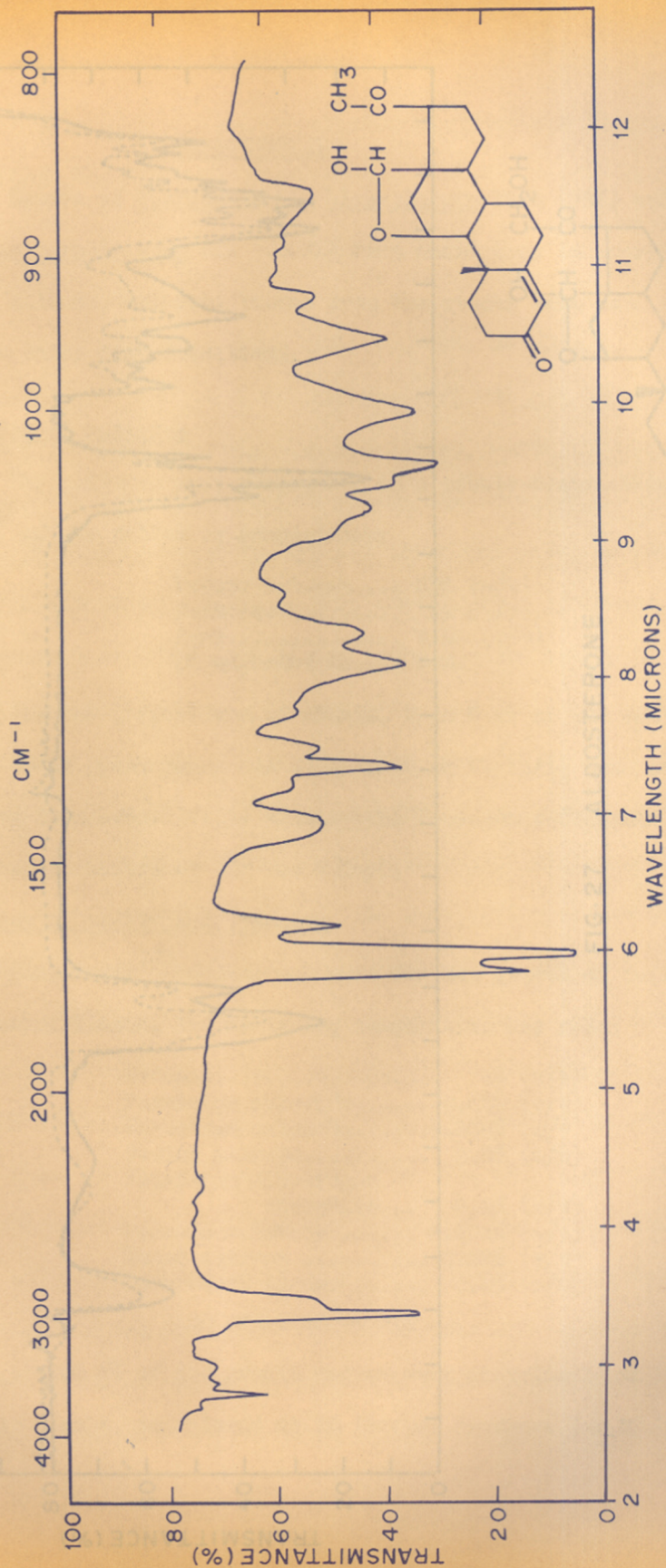


FIG. 26. IR SPECTRUM (CH₂Cl₂) OF 21-DEOXYALDOSTERONE.

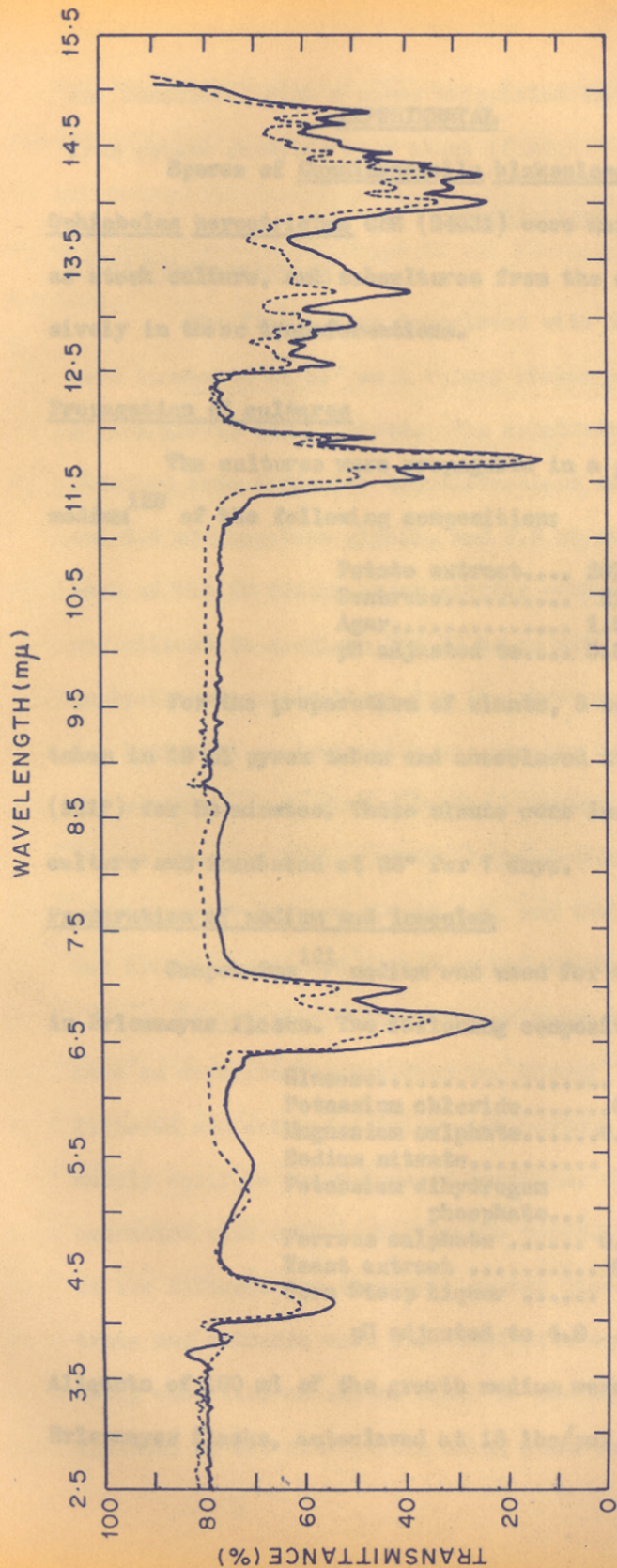
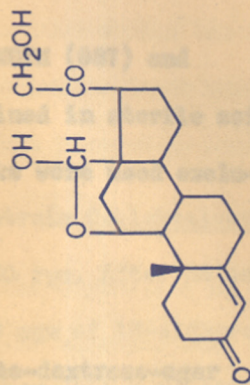


FIG. 27 ALDOSTERONE



— AUTHENTIC-d-ALDOSTERONE
---- SYNTHETIC-d-ALDOSTERONE

EXPERIMENTAL

Spores of Cunninghamella blakesleeana NCIM (687) and Ophiobolus herpotrichus CBS (24031) were maintained in sterile soil as stock culture, and subcultures from the stocks were used exclusively in these transformations.

Propagation of cultures

The cultures were propagated in a potato-dextrose-agar medium¹²⁰ of the following composition:

Potato extract....	20%	(w/v)
Dextrose.....	2%	(w/v)
Agar.....	1.5%	(w/v)
pH adjusted to....	5.8.	

For the preparation of slants, 5 to 6 ml of the medium were taken in 15 ml pyrex tubes and autoclaved at 15 lb/sq inch pressure (121°) for 20 minutes. These slants were inoculated from the stock culture and incubated at 28° for 7 days.

Preparation of medium and inoculum

Czapek-Dox¹²¹ medium was used for the growth of cultures, in Erlenmeyer flasks. The following composition was made:

Glucose.....	4%	(w/v)
Potassium chloride.....	0.05%	(w/v)
Magnesium sulphate.....	0.025%	(w/v)
Sodium nitrate.....	0.2%	(w/v)
Potassium dihydrogen phosphate...	0.1%	(w/v)
Ferrous sulphate	0.001%	(w/v)
Yeast extract	0.05%	(w/v)
Corn Steep Liquor	0.5%	(w/v)

pH adjusted to 4.8

Aliquots of 100 ml of the growth medium were distributed in 500 ml Erlenmeyer flasks, autoclaved at 15 lbs/psi pressure for 20 minutes

and inoculated with a spore suspension in sterile distilled water from potato dextrose agar slant (PDA).

Fermentations

a) 11 β Hydroxylation

The flasks were inoculated with the strain C.blakesleeana and were incubated at 28° on a rotary shaker at 220 rpm. After 36 hours, a good growth was obtained. The substrate, 50 mgm of 18-aldeprogesterone obtained from conissine transformations was taken in 1.5 ml ethanol and 0.5 ml propylene glycol, and 0.2 ml of the solution was added to each of the 10 flasks under aseptic conditions. The fermentation was allowed to continue for 36 hours with periodic sampling for analysis and examinations for the absence of bacterial contamination. Any bacterial contamination was usually detected by estimation of pH and staining broth smears by the standard Gram-staining procedure. It was observed that when the fermentation proceeded normally the pH was steady between 4.4 to 4.8, and that bacterial contamination was always associated with a marked increase in pH.

Extraction: The method of extraction was essentially the same as described by Peterson and Murray¹²². The mycelium was filtered and extracted twice with acetone, using an amount approximately equal to the volume of mycelium. The mycelium was also extracted with methylene chloride twice. The extracts were added to the filtrate and the mycelium was discarded. The combined filtrate and extracts were extracted with methylene chloride 4 times using each time an amount equal to one half the original filtrate, followed by two extractions using each time an amount equal to

one fourth the original filtrate. The combined methylene chloride extracts were washed twice with a 2% aqueous solution sodium bicarbonate and twice with distilled water. The methylene chloride layer was dried over anhydrous sodium sulphate and the solvent was removed by distillation.

R_f values: Bush R_f system. $R_f = 0.46$. (Plate-I).

Purification of the reaction mixture: The separation of the relatively non polar lipid and pigments from polar steroids was effected by partitioning between n-hexane and 70% aqueous ethanol.

A modified Craig-four transfer distribution where each of the hexane layers was equilibrated in succession with the four alcoholic layers ensured a clearcut and complete separation of the non polar lipid, pigments and polar steroidal fractions.

3 days on the shaker. The extraction and purification of the compound

Characterization of the fermentation product: The steroidal

residue was chromatographed over thin layer chromatogram in the system 80% ethyl acetate in pet-ether (60-80°) along with the authentic 18-aldeprogesterone. The same system of solvents was used on a preparative scale thin layer chromatogram as it showed a better separation between the starting compound and fermentation product. The polar zone in the two bands was eluted with methylene chloride and was rechromatographed on preparative thin layer chromatogram for further purification.

The compound was crystallized from tetrahydrofuran-ether.

m.p. 165-167°, lit.¹²³ 186.5-195°, lit.¹²⁴ 167-68°.

The compound obtained after working up the reaction mixture in the usual way was chromatographed both on paper and on thin layer in various solvent systems, against the sample of aldosterone diacetate prepared from authentic aldosterone in the same way. The R_f value of

$[\alpha]_D^{20} = +18^\circ$, lit. $[\alpha]_D^{20} = +20^\circ$. system was found to be 0.57. Sp. value

Analysis: Found: C 73.20, H 8.25%. $C_{21}H_{28}O_4$ requires C 73.17, H 8.22%.

IR spectrum (Fig. 26) showed absorption at 3330 cm^{-1} for hydroxyl group, at 1100 cm^{-1} for ether linkage and at 1710 cm^{-1} , 1690 cm^{-1} , 1650 cm^{-1} accounting for 3 ketone, 20 ketone and double bond respectively. (207)

R_f value: Bush R_g system. $R_f = 0.68$. (Plate-I).

C-21 Hydroxylation

b) Fermentation

The flasks were inoculated with the strain O. herpotrichus and were incubated at 28° on a rotary shaker at 220 rpm. After 7 days a good growth was obtained. The substrate 21 deoxyaldosterone (206) in ethanol-propylene glycol was added to the flask and left for another 3 days on the shaker. The extraction and purification of the compound was done essentially on the same lines as described in the fermentation a.

The physical constants of the synthetic aldosterone were found to be in agreement with the reported ones. The IR spectrum (Fig. 27) of the synthetic aldosterone was found to be superimposable with that of authentic aldosterone. The formation of aldosterone was further confirmed by preparing its derivatives and comparing their mobility pattern on TLC and paper chromatogram.

Acetylation of aldosterone: About 2 mg of the compound were acetylated with acetic anhydride (0.5 ml) in pyridine (0.5 ml) for 36 hrs. The diacetate obtained after working up the reaction mixture in the usual way was chromatographed both on paper and on thin layer in various solvent systems, against the sample of aldosterone diacetate prepared from authentic aldosterone in the same way. The R_f value of

both the compounds on Bush R_3 system was found to be 0.57. On thin layer chromatography both the compounds showed same R_f value.

Chromic acid oxidation of diacetates: In two glass-stoppered centrifuge tubes 0.5 ml each of the oxidizing reagent (0.5% chromic acid in glacial acetic acid) was added to aldosterone-diacetate (207) prepared from aldosterone obtained from fermentation as well as from authentic aldosterone and kept at room temperature for 20 minutes. One ml of 20% aqueous ethanol was added to each tube to arrest the reaction. The reaction mixture was extracted with 10 ml methylene chloride the methylene chloride layer was washed with water, dried over sodium sulphate and evaporated under nitrogen.

The R_f value the aldolactone (208) formed was determined on TLC as well on Bush R_3 system and was found to be 0.26 identical with each other as well as with reported values^{119A}. (Plate-I).

The yield of aldosterone by this microbiological step was about 40%.

Chapter VII

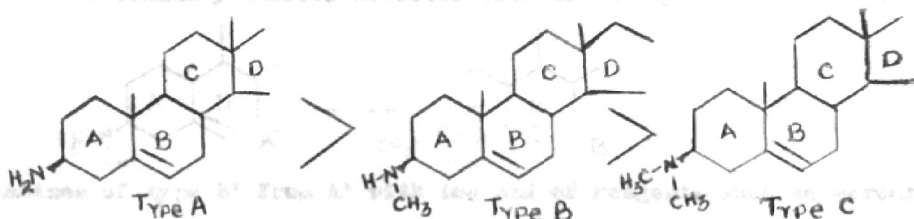
Transformations of Tetramethylholarrhimine

TRANSFORMATIONS OF TETRAMETHYLHOLARRHIMINE

Apolarrhimine - a possible intermediate for the synthetic of steroid hormones

Conversion of kurchi alkaloids into useful steroidal hormones centers around two main aspects, namely, i) elimination of both the nitrogens from the molecule and ii) introduction of carbonyl functions at positions 3 and 20.

Alkyl substitutions on a nitrogen atom increases the difficulty in its oxidative elimination. The order of reactivity of the ring A in kurchi alkaloids towards oxidative deamination to form the essential Δ^4 -3 keto structure of steroid hormones is:



Thus the kurchi alkaloids could be roughly divided into three types A, B and C.

The compounds belonging to type A are holarrhimine, conkurchine, conamine, conarrhimine, holarrhidine, having a primary amino group at position 3. Type B are the alkaloids with a secondary amino group at 3-position, viz: isoconessimine, conimine, conessidine, 3N-methylholarrhimine. Type C are the alkaloids with a tertiary amino group at position 3. Conessine, tetramethyl holarrhimine, trimethyl conkurchine, conessimine, holarrhenine, and holarrhetine, belong to this type.

The alkaloids of type A and B have been converted into type C which were obtained in increased yield by methylation^{31C}. The methylation resulted mostly in conessine and tetramethylholarrhimine. The inter-relationship of kurchi alkaloids has made it highly feasible to convert the total alkaloidal fraction into a few major alkaloids which may be available in larger quantities for further reactions.

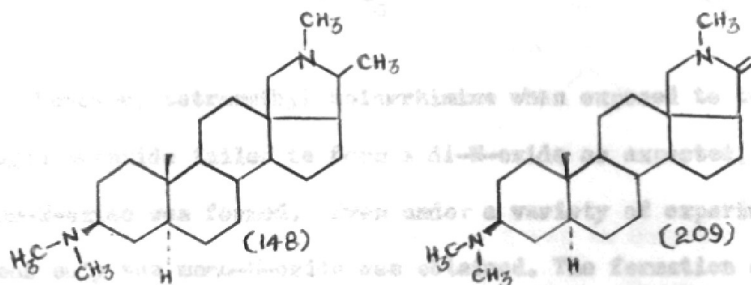
N-N,N'-N', Tetramethylholarrhimine, fully methylated derivative of holarrhimine, attracted the attention as the starting material for steroidal hormone synthesis since i) no degradation problems are involved such as opening up of the heterocyclic ring; ii) a primary alcoholic group at C-18 is already present in the molecule and iii) ease of isolation from natural source.

Preliminary studies directed towards the synthesis of conjugated enamines of type B' from A' with the aid of reagents such as mercuric acetate which has been widely used in the field of heterocyclic compounds by Leonard *et al.*¹²⁵ did not yield any fruitful results, either with conessine or with tetramethylholarrhimine.

In a subsequent experiment hydroxyl group at C-18 was acetylated and the tetramethyl holarrhimine-acetate thus formed was treated with mercuric acetate. However, this was also recovered unchanged.

Likewise tetramethylholarrhimine (216) was treated with 3 molar Favre and Marinier¹²⁶ used mercuric acetate for dehydrogenation of hydrogen peroxide with the presumption to obtain the di-N-oxide of dihydroconessine (148) and assigned on the basis of infrared spectrum, which on pyrolytic degradation would result in apoholarrhimine (149), the structure N-methylpyrroline to the isolated product. The infrared frequency is close to the region of $1632-1640\text{ cm}^{-1}$, which according to

Lukes *et al.*¹²⁷ is characteristic of Δ^2 pyrrolines. The isomeric 2-alkylidenepyrrolidines exhibit maxima between 1666-1676 cm^{-1} . The reaction was reinvestigated by Kasal *et al.*¹²⁸ and on the basis of infrared spectrum and chemical evidence the dehydrogenated product was assigned the structure (209), 1-methyl-2-methylenepyrrolidine of conessine base.



In the present work conessine when treated with mercuric acetate was recovered practically unchanged from the reaction mixture. Silver

oxide was also found inefficient for dehydrogenating any of these compounds. The formation of the N-oxide was evident from IR spectrum and other typical characteristics of N-oxides.

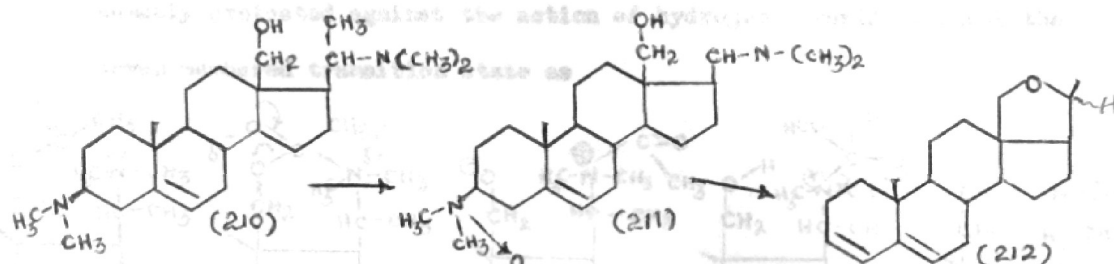
The next approach which was attempted was to eliminate both the nitrogens in tetramethylholarrhimine through Cope elimination reaction⁶⁹.

Bhattacharyya *et al.*³⁶ have reported that conessine (113) on treatment with 2 mols of hydrogen peroxide forms a di-N-oxide (151).

The pyrolytic degradation of the di-N-oxide results into an elimination product (152) characterized by UV spectra as containing a 3:5 diene system.

Likewise tetramethylholarrhimine (210) was treated with 2 mols of hydrogen peroxide with the presumption to obtain the di-N-oxide (211) which on pyrolytic degradations would result in apholarrhimine (212).

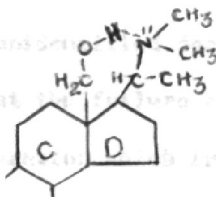
The mono-N-oxide of tetramethylholarrhimine was isolated as the only product.



However, tetramethyl holarrhimine when exposed to two mols of hydrogen peroxide failed to form a di-N-oxide as expected, instead, a mono-N-oxide was formed. Even under a variety of experimental conditions only the mono-N-oxide was obtained. The formation of the N-oxide was evident from IR spectrum and other typical characteristics of N-oxides.

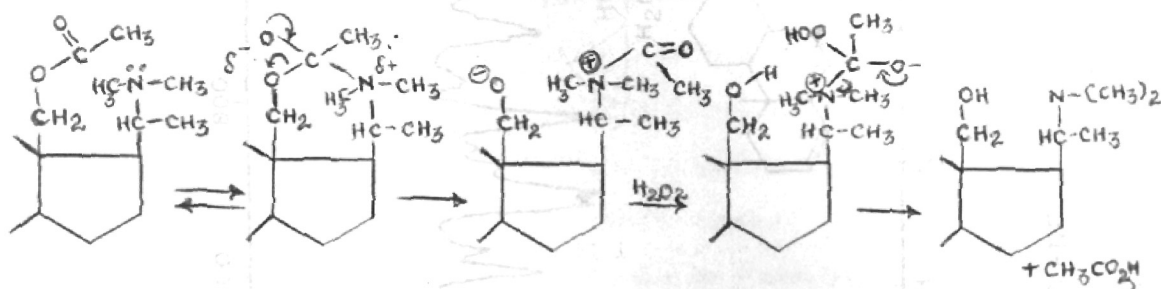
Pyrolysis of the mono-N-oxide described in the sequel revealed that the N-oxide is attached to the 3-position of the steroid nucleus.

Since the primary alcohol at C-18 is axial, it was thought that it would be intramolecularly hydrogen bonded with the nitrogen at C-20 as in the fig. thereby decreasing the basicity of nitrogen for the N-oxide formation.



With a view to get a di-N-oxide, namely, 3 and 20-N-oxide the neighbouring C-18 hydroxyl group was acetylated to avoid the possible interference. The tetramethylholarrhimine acetate thus formed was then treated with two mols of hydrogen peroxide. However in this case also 3-mono-N-oxide of tetramethyl holarrhimine was isolated as the only product.

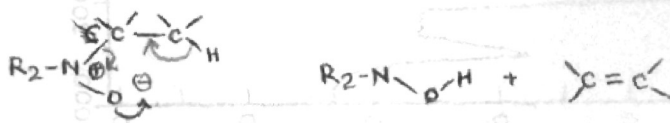
The tertiary nitrogen in the acetylated holarrhimine is presumably protected against the action of hydrogen peroxide through the seven membered transition state as



The solvolytic removal of the N-acetyl group in compound is facilitated by nucleophilic attack to hydroperoxide anion. Furthermore, in tetramethyl holarrhimine there is a possibility that the dimethylamine group at 20 (R) is protected from the action of hydrogen peroxide, through steric hindrance.

It is worthwhile to recall that Meisenheimer¹²⁹ reported that allyl methylamine and allyl diethylamine oxides did not rearrange on heating with aqueous sodium hydroxide and were recovered unchanged except for small amounts which were transformed into dimethylamine and diethylamine and to an unidentified decomposition product. Later Cope *et al.*⁶⁹ showed that the failure of rearrangement may be interpreted by probably a hydration which interferes with the rearrangement either sterically or through the influence on the dipole moment of the amine oxide or the dielectric constant of the solution.

Normally however, the reaction proceeds smoothly even at low temperatures with the elimination of dialkylhydroxylamine.



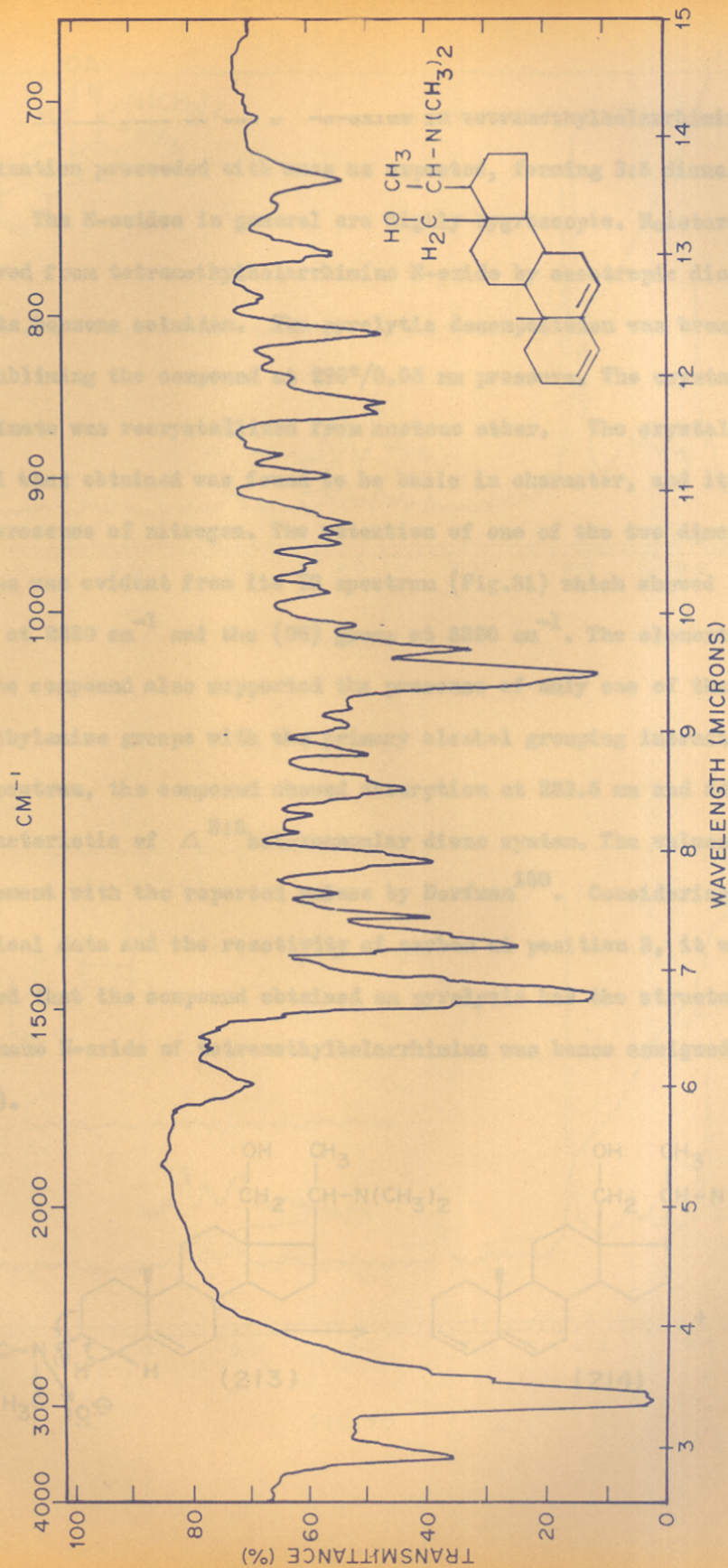


FIG. 31 . IR SPECTRUM (NUJOL) OF BASIC DIENE .

elimination proceeded with ease as expected, forming 3:5 diene.

The N-oxides in general are highly hygroscopic. Moisture was removed from tetramethylholarrhimine N-oxide by azeotropic distillation of its benzene solution. The pyrolytic decomposition was brought about by subliming the compound at $220^{\circ}/0.05$ mm pressure. The crystalline sublimate was recrystallized from acetone ether. The crystalline compound thus obtained was found to be basic in character, and it showed the presence of nitrogen. The retention of one of the two dimethylamino groups was evident from its IR spectrum (Fig.31) which showed the N-CH₃ band at 2820 cm^{-1} and the (OH) group at 3390 cm^{-1} . The elemental analysis of the compound also supported the presence of only one of the two dimethylamino groups with the primary alcohol grouping intact. In the UV spectrum, the compound showed absorption at 233.5 mμ and 242.5 mμ characteristic of $\Delta^{3:5}$ heteroannular diene system. The values are in agreement with the reported values by Dorfman¹³⁰. Considering the physical data and the reactivity of carbon at position 3, it was concluded that the compound obtained on pyrolysis has the structure (214). The mono N-oxide of tetramethylholarrhimine was hence assigned structure (213).

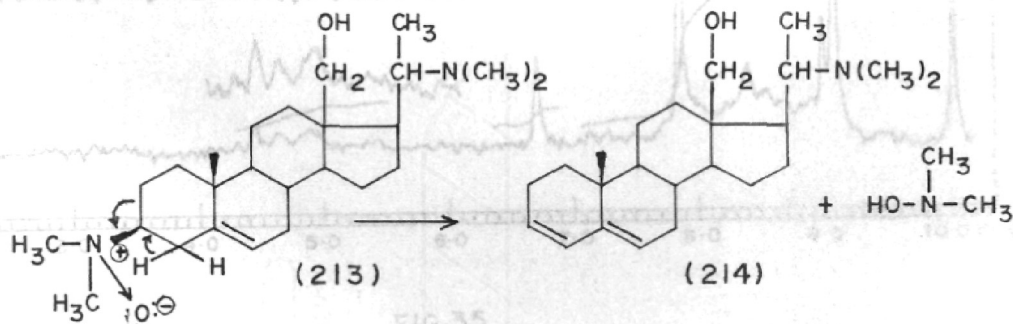


FIG.35

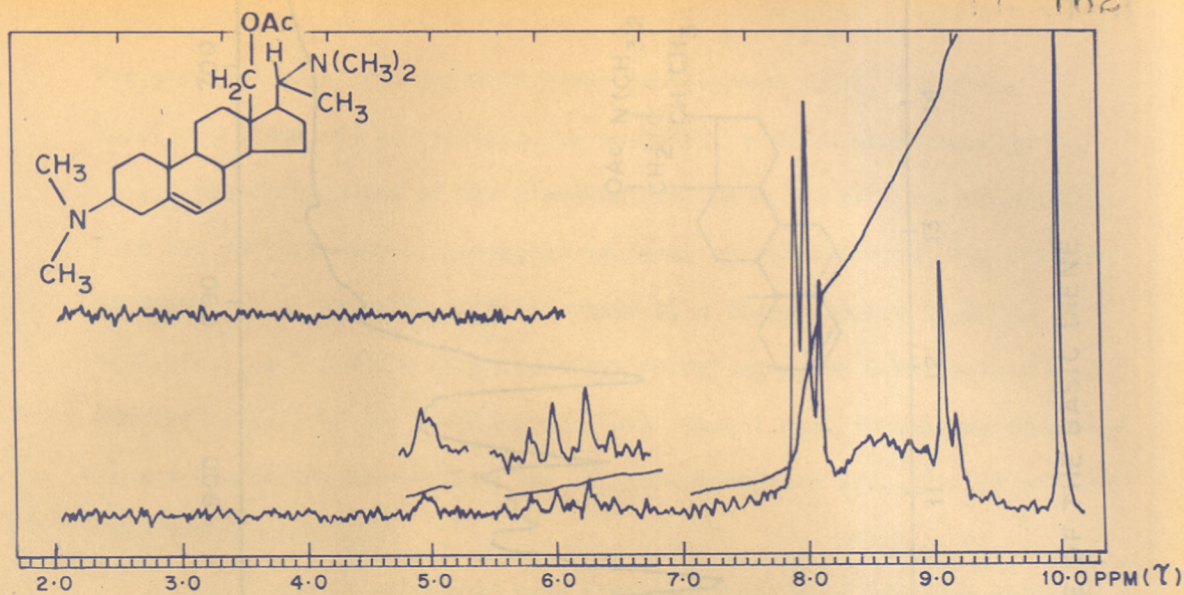


FIG. 34

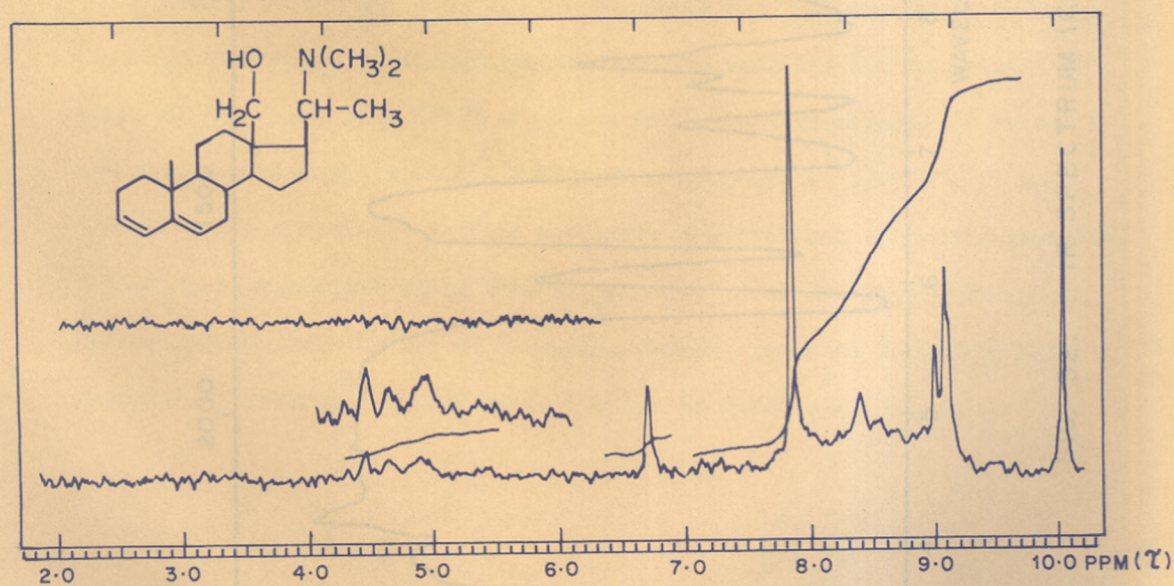
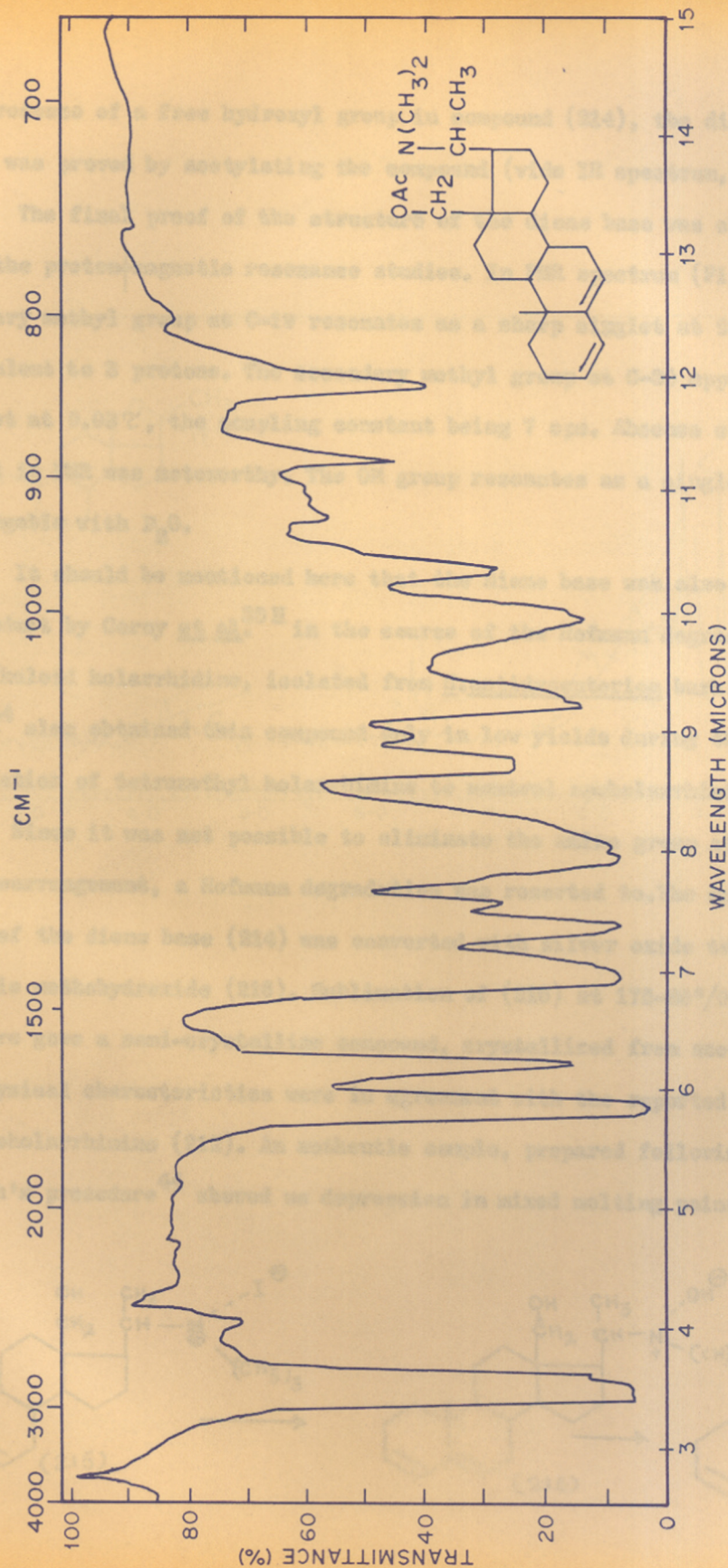


FIG. 35

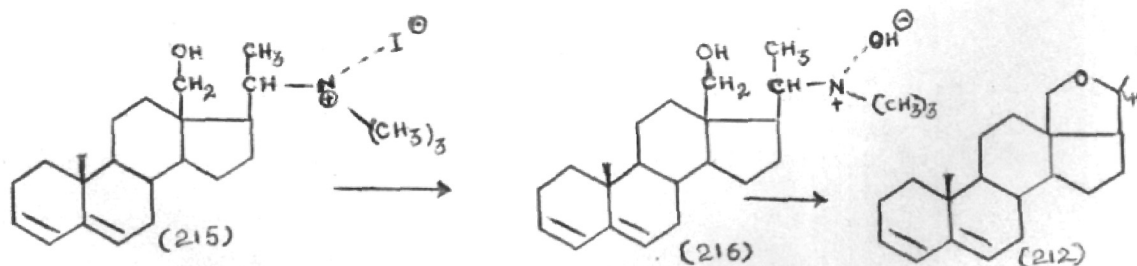
FIG. 32. IR SPECTRUM (CHCl_3) OF ACETATE OF THE BASIC DIENE.

The presence of a free hydroxyl group in compound (214), the diene base, was proved by acetylating the compound (vide IR spectrum, Fig. 32).

The final proof of the structure of the diene base was obtained from the proton-magnetic resonance studies. In PMR spectrum (Fig. 35) the tertiary methyl group at C-19 resonates as a sharp singlet at 9.05τ equivalent to 3 protons. The secondary methyl group at C-20 appears as a doublet at 9.03τ , the coupling constant being 7 cps. Absence of 3N-methyl signal in PMR was noteworthy. The OH group resonates as a singlet at 6.72τ exchangeable with D_2O .

It should be mentioned here that the diene base was also obtained as a by-product by Cerny *et al.*^{35H} in the course of the Hofmann degradations of the alkaloid holarrhidine, isolated from *H. antidysenterica* bark. McKenna *et al.*⁴⁴ also obtained this compound only in low yields during the Hofmann degradation of tetramethyl holarrhimine to neutral apoholarrhimine.

Since it was not possible to eliminate the amine group at C-20 by Cope rearrangement, a Hofmann degradation was resorted to. The methiodide (215) of the diene base (214) was converted with silver oxide to rather unstable methoxide (216). Sublimation of (216) at $175-80^\circ/0.1$ mm pressure gave a semi-crystalline compound, crystallized from acetone-ether. The physical characteristics were in agreement with the reported values for apoholarrhimine (212). An authentic sample, prepared following the Haworth's procedure⁴⁴ showed no depression in mixed melting point.



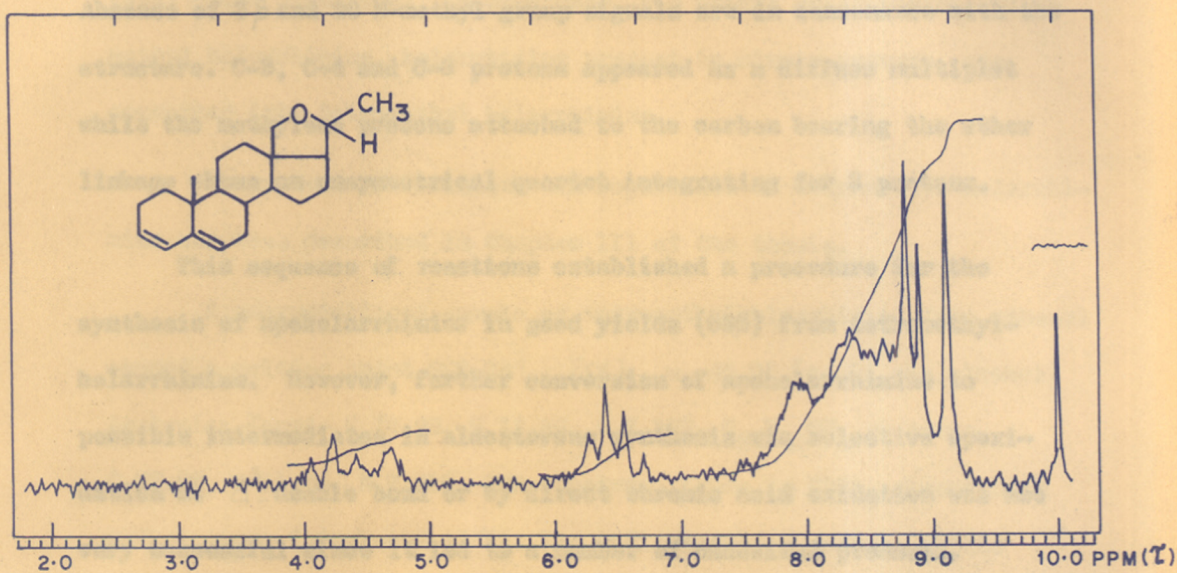


FIG.36

The PMR spectrum of apoholarrhimine (Fig.36) showed a three proton singlet for C-19 methyl group at 9.1τ . The secondary methyl proton at C-21 in ring E appeared as a doublet at 8.48τ , with $J=7$ cps. Absence of 3β and 20 N-methyl group signals are in consonance with the structure. C-3, C-4 and C-6 protons appeared as a diffuse multiplet while the methylene protons attached to the carbon bearing the ether linkage shows an unsymmetrical quartet integrating for 3 protons.

This sequence of reactions established a procedure for the synthesis of apoholarrhimine in good yields (65%) from tetramethylholarrhimine. However, further conversion of apoholarrhimine to possible intermediates in aldosterone synthesis via selective epoxidation at Δ^3 -double bond or by direct chromic acid oxidation was not very successful since it led to a number of undesired products.

EXPERIMENTAL

Tetramethylholarrhimine occurs in kurchi bark only in traces. However, it could be obtained in increased yields by methylating the total crude alkaloids. Holarrhimine, 3 N methyl holarrhimine, 20 N-methyl holarrhimine, holarrhidine present in minute amounts, are converted into tetramethyl holarrhimine.

The method of isolation and separation of tetramethyl holarrhimine has been described in Chapter III of the thesis.

A recrystallized sample of tetramethylholarrhimine from ethanol showed a melting point 226-30°. $[\alpha]_D = -45.5^\circ$ (c=0.01 abs.alcohol). Analysis: Found- C 76.92, H 11.03, N 7.18%. $C_{25}H_{44}ON_2$ requires C 77.02, H 11.34, N 7.21%. PMR spectrum, taken in $CDCl_3$ showed C-19 methyl group (singlet 8.97 τ). The C-21 secondary methyl protons (doublet at 8.95 τ , J= 7 cps). The 3 -N-methyl proton (a singlet at 7.70 τ); olefinic proton at C-6 (diffuse multiplet at 4.67 τ). Proton of the hydroxyl group resonates as at singlet at 6.47 τ . IR spectrum (Fig.5) showed a band at 3320 cm^{-1} (OH), shoulder at 2820 cm^{-1} (N-CH₃) and 1665 cm^{-1} (5:6 double bond).

Tetramethyl holarrhimine acetate

Tetramethylholarrhimine (250 mg) was treated with 3 ml pyridine and 3 ml acetic anhydride. After overnight reaction at room temperature the mixture was treated with benzene (25 ml) and partially evaporated under vacuum. Residue was then treated with excess of aqueous ammonia and the precipitate was taken in ether. The ether layer was washed free of alkali and evaporated to dryness. The solid compound was

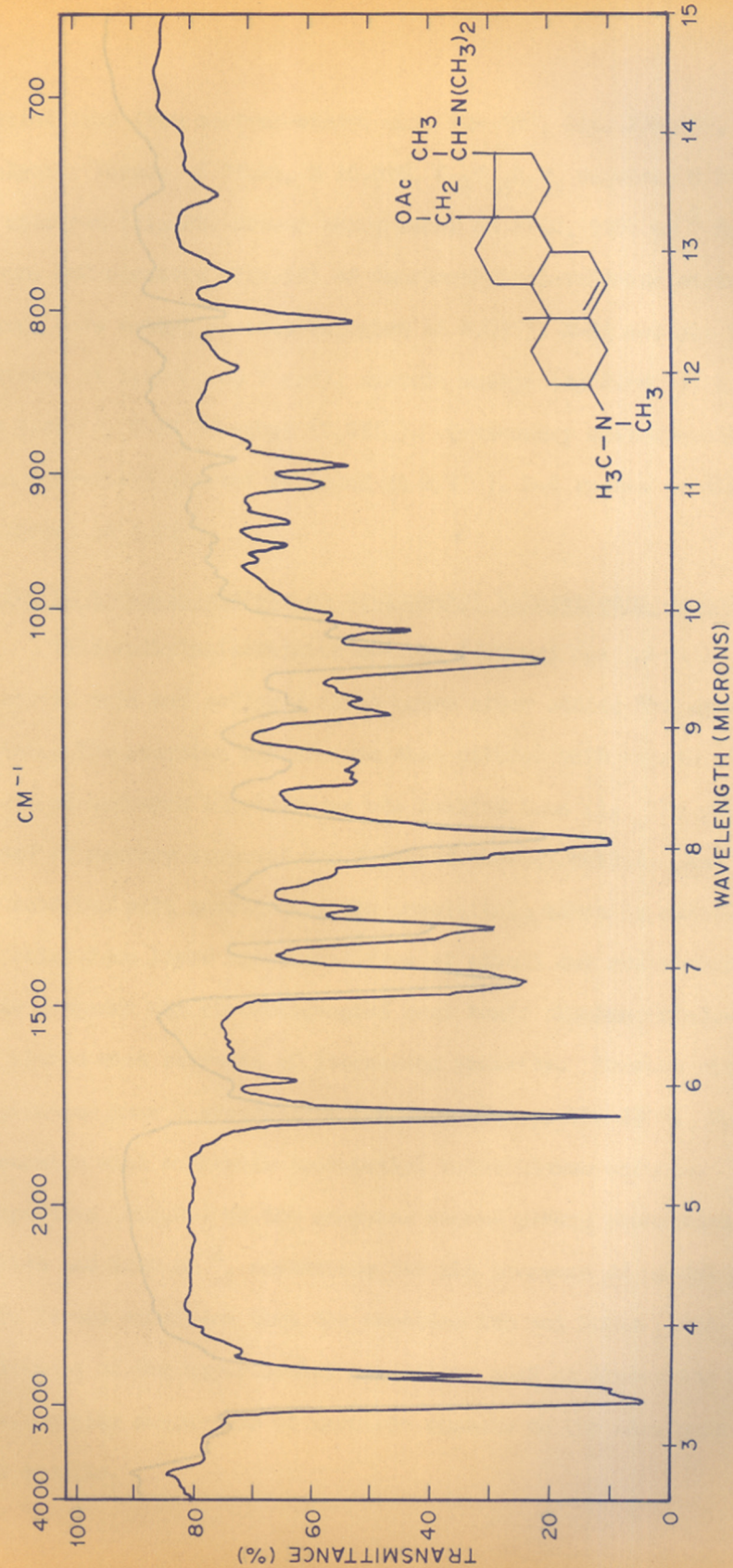


FIG. 28. IR SPECTRUM (NUJOL) OF ACETATE OF TETRAMETHYLHOLARRHIMINE.

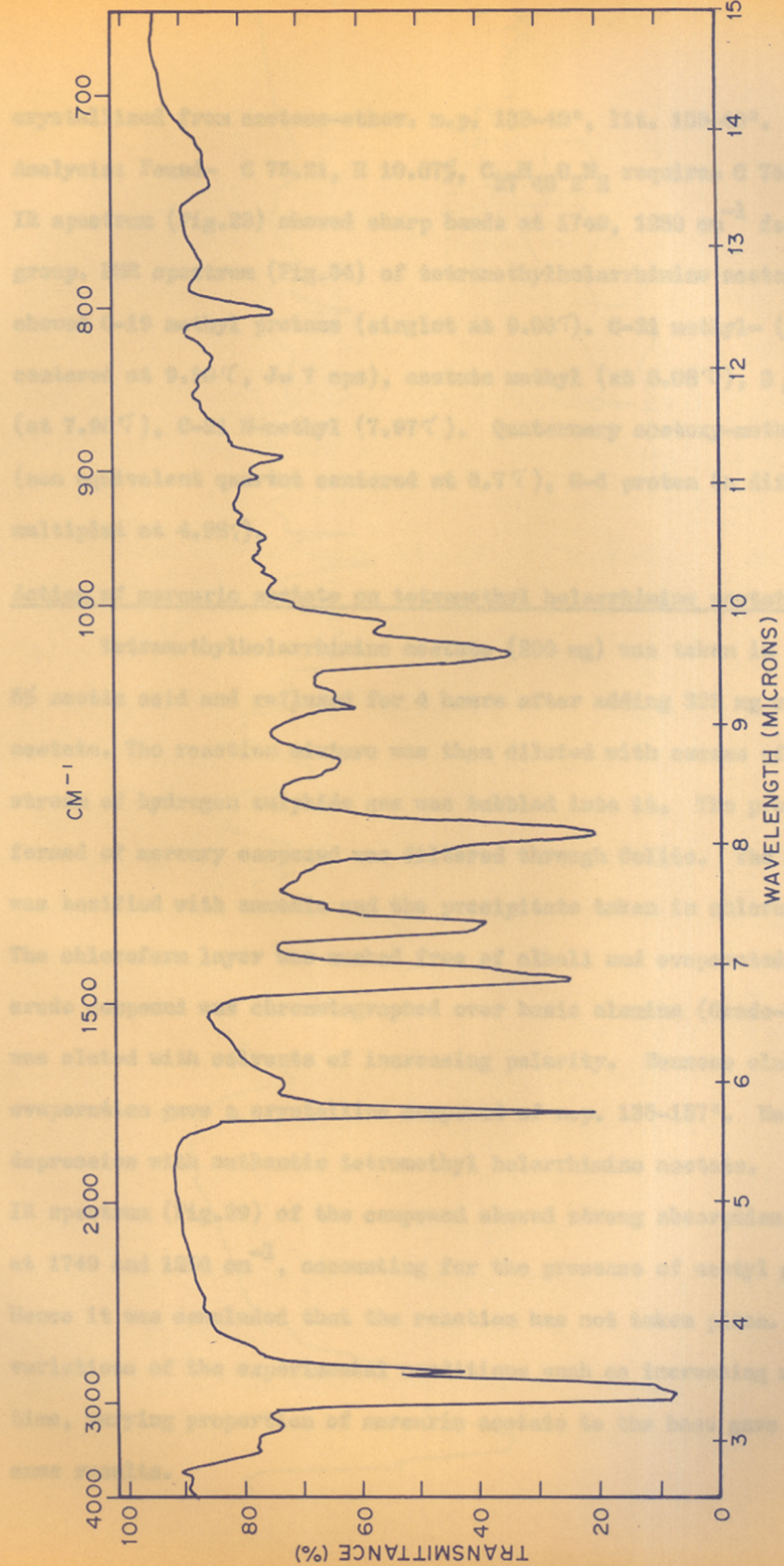


FIG. 29. IR SPECTRUM (NUJOL) OF REACTION PRODUCT OF
TETRAMETHYL HOLARRHINE AND MERCURIC ACETATE.

crystallized from acetone-ether. m.p. 138-40°, lit. 139-40°.

Analysis: Found- C 75.21, H 10.07%, $C_{27}H_{46}O_2N_2$ requires C 75.32, H 10.69%. IR spectrum (Fig.28) showed sharp bands at 1740, 1250 cm^{-1} for acetate group. PMR spectrum (Fig.34) of tetramethylholarrhimine acetate (CCl_4) showed C-19 methyl protons (singlet at 9.05 τ). C-21 methyl- (doublet centered at 9.10 τ , $J = 7$ cps), acetate methyl (at 8.08 τ), 3 β -N-methyl (at 7.90 τ), C-21 N-methyl (7.97 τ). Quaternary acetoxy-methyl $-C-CH_2-OAc$ (non equivalent quartet centered at 6.7 τ), C-6 proton (a diffuse multiplet at 4.98 τ).

Action of mercuric acetate on tetramethyl holarrhimine acetate

Tetramethylholarrhimine acetate (200 mg) was taken in 10 ml of 5% acetic acid and refluxed for 4 hours after adding 325 mg mercuric acetate. The reaction mixture was then diluted with excess of water, a stream of hydrogen sulphide gas was bubbled into it. The precipitate formed of mercury compound was filtered through Celite. The filtrate was basified with ammonia and the precipitate taken in chloroform. The chloroform layer was washed free of alkali and evaporated. The crude compound was chromatographed over basic alumina (Grade-II) and was eluted with solvents of increasing polarity. Benzene eluate on evaporation gave a crystalline compound of m.p. 135-137°. No depression with authentic tetramethyl holarrhimine acetate. The IR spectrum (Fig.29) of the compound showed strong absorption bands at 1740 and 1250 cm^{-1} , accounting for the presence of acetyl group. Hence it was concluded that the reaction has not taken place. Other variations of the experimental conditions such as increasing reaction time, varying proportion of mercuric acetate to the base gave the same results.

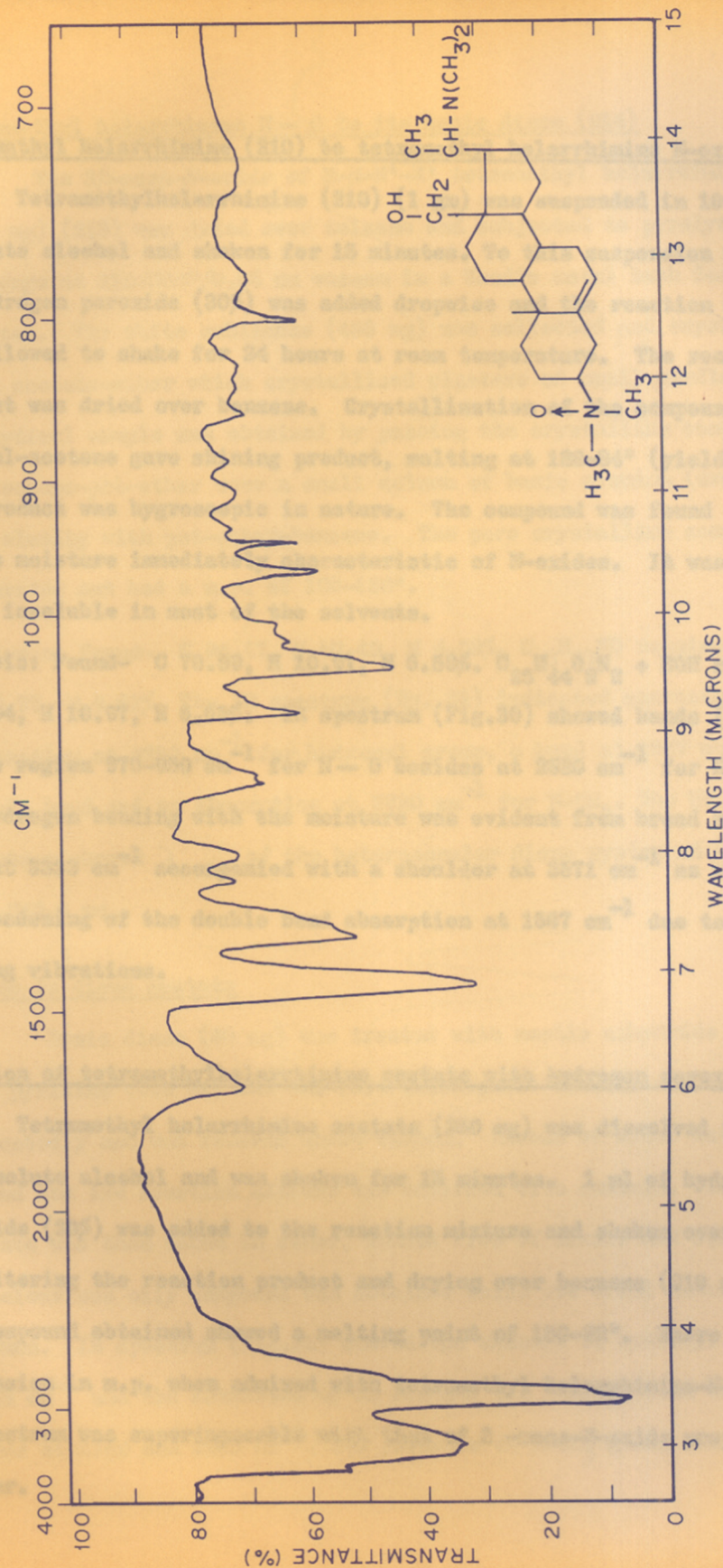


FIG. 30. IR SPECTRUM (NUJOL) OF 3 β -MONO-N-OXIDE OF TETRAMETHYLHOLARRHIMINE.

Tetramethyl holarrhimine (210) to tetramethyl holarrhimine N-oxide (213)

Tetramethylholarrhimine (210) (1 gm) was suspended in 10 ml of absolute alcohol and shaken for 15 minutes. To this suspension 0.66 ml of hydrogen peroxide (30%) was added dropwise and the reaction mixture was allowed to shake for 24 hours at room temperature. The reaction product was dried over benzene. Crystallization of the compound from alcohol-acetone gave shining product, melting at 182-84° (yield 0.95 gm). The product was hygroscopic in nature. The compound was found to absorb moisture immediately characteristic of N-oxides. It was practically insoluble in most of the solvents.

Analysis: Found- C 70.89, H 10.91, N 6.58%. $C_{25}H_{44}O_2N_2 + HOH$ requires C 71.04, H 10.97, N 6.63%. IR spectrum (Fig.30) showed bands (two) in the region $970-950\text{ cm}^{-1}$ for N—O besides at 2820 cm^{-1} for N—CH₃. The hydrogen bonding with the moisture was evident from broad hydroxyl band at 3333 cm^{-1} accompanied with a shoulder at 3571 cm^{-1} as well as by broadening of the double bond absorption at 1667 cm^{-1} due to HOH bending vibrations.

Reaction of tetramethylholarrhimine acetate with hydrogen peroxide

Tetramethyl holarrhimine acetate (250 mg) was dissolved in 5 ml of absolute alcohol and was shaken for 15 minutes. 1 ml of hydrogen peroxide (30%) was added to the reaction mixture and shaken overnight. On filtering the reaction product and drying over benzene (210 mg) the compound obtained showed a melting point of 180-82°. There was no depression in m.p. when admixed with tetramethyl holarrhimine-N-oxide. IR spectrum was superimposable with that of 3-mono-N-oxide prepared earlier.

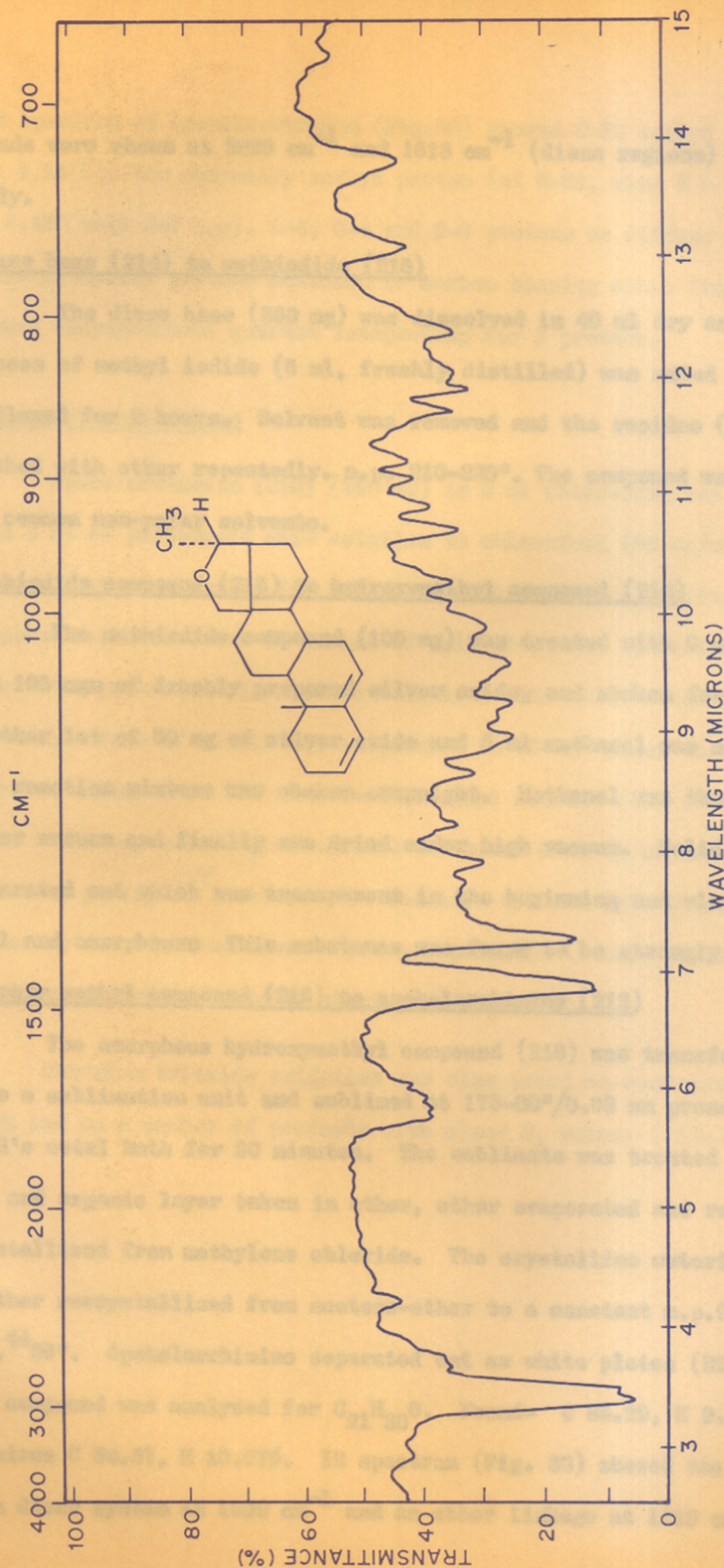
Tetramethyl holarrhinine N—O to its basic diene (214)

The 3 β -mono-N-oxide of N-N'-N' tetramethyl holarrhinine (500 mg) (213) was dried over toluene and subjected to pyrolytic cleavage at 210-220°/0.05 mm vacuum in a Wood's metal bath for 30 minutes. The white sublimate (456 mg) was collected and crystallized from acetone-ether which crystallized clusters of small needles. An analytical sample was obtained by passing the crystalline compound in benzene-pet-ether over a small column of basic alumina (Grade II) and eluting with pet-ether-benzene. The pure crystalline compound separated out had a m.p. of 126-128°.

Analysis: Found- C 80.17, H 10.49, N 4.58%, $C_{23}H_{37}NO$ requires C 80.44, H 10.77, N 4.82%. The IR spectrum (Fig. 31) indicated characteristic absorption at 3390 cm^{-1} for hydroxyl group, a band at 1667 cm^{-1} for double bond and an absorption at 2820 cm^{-1} for N-CH₃. The UV absorption spectrum showed λ_{max} of the heterocannular diene system at 233.5 m μ and 242.5 m μ .

Diene to diene acetate

Basic diene (50 mg) was treated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) and was left overnight. Benzene was then added repeatedly and was removed under vacuum. Excess of water was then added and the reaction mixture was basified with ammonia. The precipitate was then taken in ether, washed free of alkali and evaporated. A colourless oily compound was obtained which was dried under high vacuum. IR spectrum (Fig.32) showed the absence of hydroxyl band at 3330 cm^{-1} and the formation of an acetate was evident from absorption bands at 1740 and 1250 cm^{-1} . The presence of N-CH₃ group and 3,5 double



IR spectrum of apoholarrhimine (Fig. 36) showed C-H methyl protons. bonds were shown at 2820 cm^{-1} and 1613 cm^{-1} (diene regions) respectively. The secondary methyl protons (at C-11, vinyl 5 - doublet).

at $\delta 4.3$ (with $J=7\text{ cps}$). C-8, C-9 and C-10 protons at $\delta 4.5$ (multiplet).

Diene base (214) to methiodide (215)

The diene base (500 mg) was dissolved in 40 ml dry acetone and

excess of methyl iodide (5 ml, freshly distilled) was added and

refluxed for 2 hours. Solvent was removed and the residue (400 mg)

washed with ether repeatedly. m.p. $210-220^\circ$. The compound was insoluble

in common non-polar solvents.

The compound (215) (400 mg) in 1 ml chloroform was treated

Methiodide compound (215) to hydroxymethyl compound (216)

The methiodide compound (105 mg) was treated with 5 ml methanol

and 105 mg of freshly prepared silver oxide, and shaken for 6 hours.

Another lot of 50 mg of silver oxide and 5 ml methanol was added and

the reaction mixture was shaken overnight. Methanol was then removed

under vacuum and finally was dried under high vacuum. Solid material

separated out which was transparent in the beginning and slowly became

dull and amorphous. This substance was found to be strongly basic.

Hydroxy methyl compound (216) to apoholarrhimine (212)

The amorphous hydroxymethyl compound (216) was transferred

into a sublimation unit and sublimed at $175-80^\circ/0.02\text{ mm}$ pressure in

Wood's metal bath for 30 minutes. The sublimate was treated with 5%

HCl and organic layer taken in ether, ether evaporated and residue was

crystallized from methylene chloride. The crystalline material was

further recrystallized from acetone-ether to a constant m.p. $94-95^\circ$,

lit.⁴⁴ 96° . Apoholarrhimine separated out as white plates (85 mg).

The compound was analysed for $\text{C}_{21}\text{H}_{30}\text{O}$. Found- C 84.29, H 9.83%,

requires C 84.57, H 10.07%. IR spectrum (Fig. 33) showed the presence

of a diene system at 1639 cm^{-1} and an ether linkage at 1210 cm^{-1} .

PMR spectrum of apoholarrhimine (Fig.36) showed C-19 methyl protons (at 9.10 τ). The secondary methyl proton (at C-21, ring E - doublet at 8.48 τ with J=7 cps). C-3, C-4 and C-6 protons as diffuse multiplet while methylene protons attached to carbon bearing ether linkage showed unsymmetrical quartet integrating for 3 protons.

Attempted epoxidations

Apoholarrhimine (212) (100 mg) in 2 ml chloroform was treated with 2 ml of perbenzoic acid solution in chloroform (65 mg/ml) and left at room temperature for 4 days with intermediate shaking. The compound was then taken in excess of chloroform and treated three times with potassium bicarbonate solution. The chloroform layer was washed free of alkali and evaporated at 45-50° under vacuum. The reaction mixture, on TLC in pet-ether-ethyl acetate (5%) showed four spots with very close R_f values and the usual methods of separation failed to purify it further. The reaction did not take place on lessening the reaction time. Hydrogen peroxide (1 mol) was also tried but it failed to bring about any conversion.

Chromium trioxide oxidation was also tried on apoholarrhimine which led to a number of products with close R_f values (vide TLC).

SUMMARY

The discovery of the remarkable therapeutic effects of the Mineral corticoid hormone, aldosterone, has stimulated chemical research especially in the direction of its synthesis to such an extent that now there exist a number of methods of total as well as partial synthesis of this natural hormone and some of its closely related 18-oxygenated steroidal intermediates.

Chapter I

A brief review of literature describing the total as well as partial syntheses of aldosterone and other related 18-oxygenated steroids is the subject matter of this chapter.

Chapter II

This chapter describes the methods of preparation of the reagents and the Materials used in the course of this investigation.

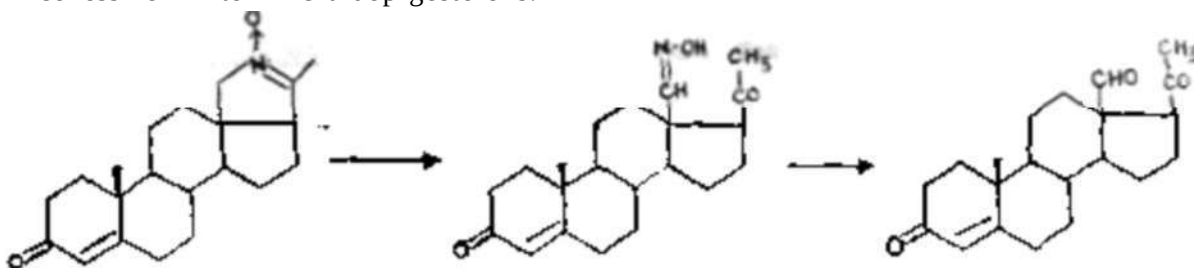
Chapter III

Isolation of alkaloids from the bark and seeds from Holarrhena antidysenterica was undertaken by Siddiqui and Pillay in 1932. Several different procedures have been reported since then. The method of isolation of the alkaloids in the present study is a modification of Cerny's procedure.

A systematic investigation of Holarrhena heartwood for its chemical constituents has also been carried out for the first time in this study. The basic fraction of the extract of the heartwood was found to contain two new alkaloids with strong absorption in the carbonyl region only in low yields besides conesine and other reported alkaloids. The neutral extract was found to contain (3-sitosterol and lupeol. However structural elucidation of these compounds could not be undertaken owing to paucity of the materials.

Chapter IV

Conessine has been converted into N-demethyl-conan-4-20(N) diene-3-one, a Schiff's base by a modification of the existing methods, in the present work. The Schiff's base was converted into its N-oxide, which was cleaved by sodium metaperiodate to give the corresponding oxime. The oxime on treatment with nitrous acid yielded 18-aldoprogesterone. This is a new approach of converting conessine into 18-aldoprogesterone.

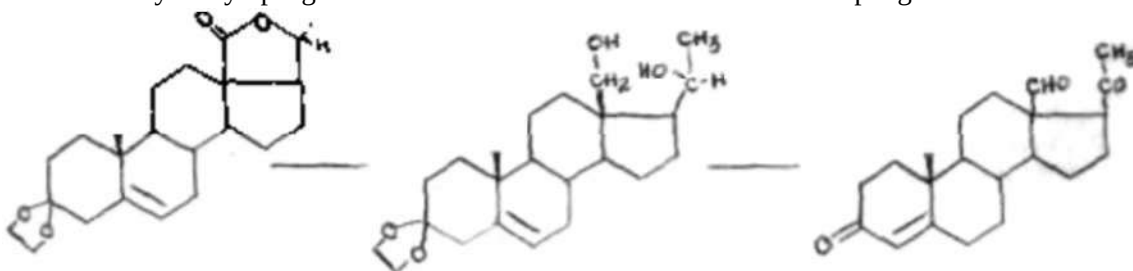


The generality of the cleavage reaction of the N-oxide with sodium metaperiodate was further extended to the N-oxide of 1-methyl-3:4-dihydro, 6:7-dimethoxy-isoequinoline. The latter, prepared from vanillin, was transformed to the corresponding oxime with sodium metaperiodate.

Chapter V

The 18-aldoprogesterone prepared by the above method was compared with the specimen obtained through an alternate route.

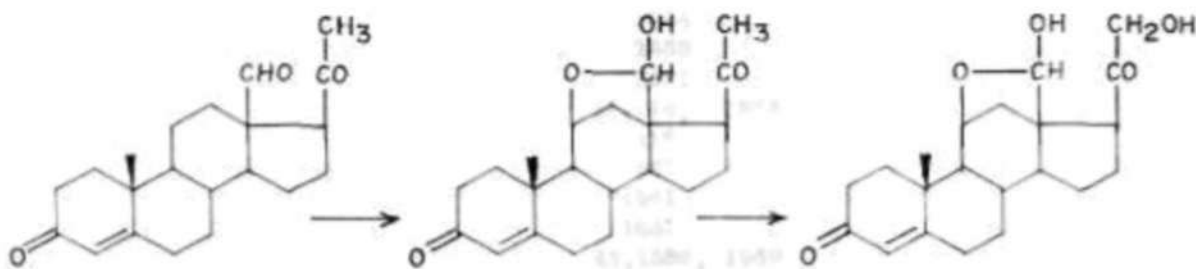
In the present study, the lactone, 3:3-ethylene dioxy-20 β -hydroxy-5-pregnen-18-oic acid was prepared by the known methods. The lactone was cleaved by lithium aluminium hydride to yield 3:3-ethylene dioxy-18:20-dihydroxy pregnene which was converted to 18-aldoprogesterone in excellent



yields (65% based on lactone) by Jones's reagent. This method is superior to an earlier one (30%, based on lactone) by Wettstein.

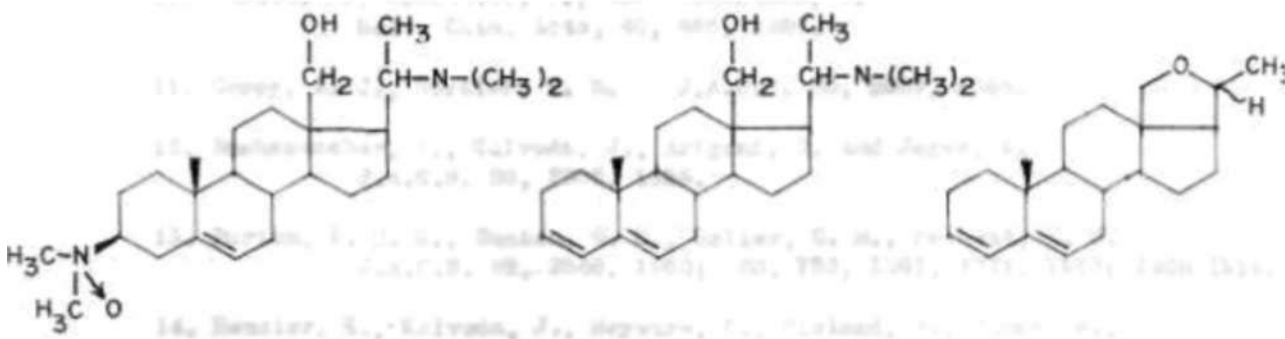
Chapter VI

The conversion of 18-aldoprogesterone obtained from conessine transformation into 21-desoxy-aldosterone by an *ii* P-hydroxylating fungus, Cunninghamella blakesleeana had been illustrated. Further transformation of this precursor into aldosterone had been brought about by Ophiobolus hexapotrichus, a C-21 hydroxylating fungus.



Chapter VII

Tetramethylholarrhimine was converted to its 3-mon-N-oxide which on Cope's elimination formed a diene base. The base on Hofmann degradation gave apoholarrhimine. This is a new and convenient route to apoholarrhimine, a promising intermediate in the synthesis of 18-oxygenated steroids.



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