TRANSFORMATIONS OF ALKALOIDS FROM HOLARRHENA ANTIDYSENTERICA

A Thesis

Submitted to

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for the Degree of

DOCTOR OF PHILOSOPHY

in Chemistry

by

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A_C_K_N_O_W_L_E_D_G_E_M_E_N_T

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December 1961.

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C O N T E N T S

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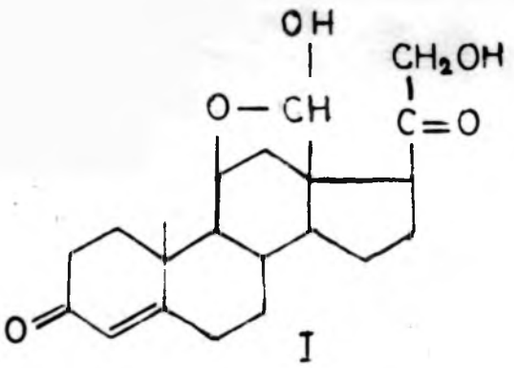
CHAPTER I

I_N_T_R_O_D_U_C_T_I_O_N

The interest in 18-substituted steroids dates back to the discovery of the mineralo-corticoid hormone aldosterone[1,2] (I, Plate I, page 2), and the elucidation of its remarkable therapeutic properties[3]. As a result of intensive research activity in this field for the past eight years several elegant methods of total synthesis of the natural hormone and some closely related 18-oxygenated analogues are known today[4-9]. It is, however, doubtful that any of these methods of total synthesis would be applicable to a large scale commercial preparation of the hormone. There are still too many steps involved including that of resolution of racemates.

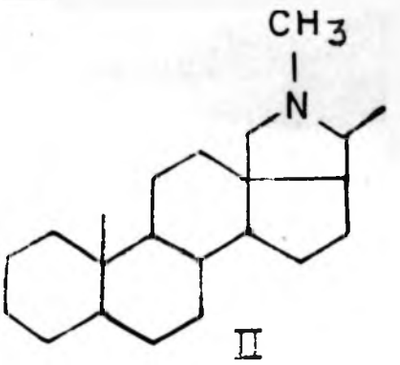
The search was, therefore, on for naturally occurring steroids which could serve as an inexpensive starting material for a partial synthesis. In this context the steroidal alkaloids from "kurchi" (Holarrhena), both the Indian and the African varieties, have attracted a considerable attention.

Very recently other approaches which appear to be commercially promising for the synthesis of aldosterone have been developed. Mention may be made in this connection of the elegant method of Barton and Beaton[10] which utilizes a photo-catalysed nitrosyl transfer reaction from position 11β to the angular methyl position 18 in corticosterone acetate and the intramolecular introduction of an oxygen function at position 18 from a 20-hydroxy group with the aid of lead tetraacetate by Heusler *et al*[11].



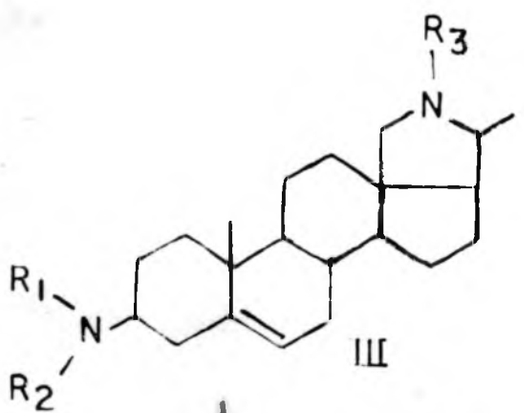
I

ALDOSTERONE



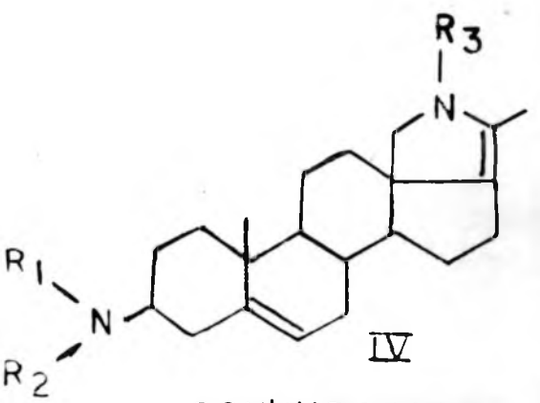
II

CONANINE



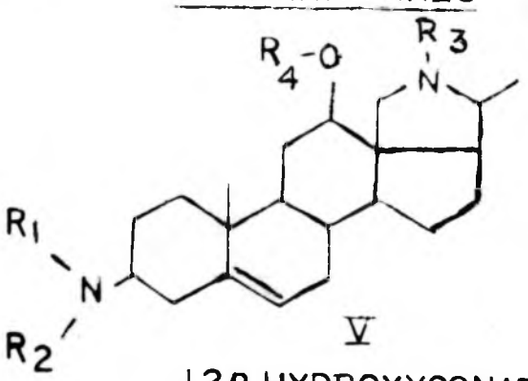
III

CONARRHIMINES



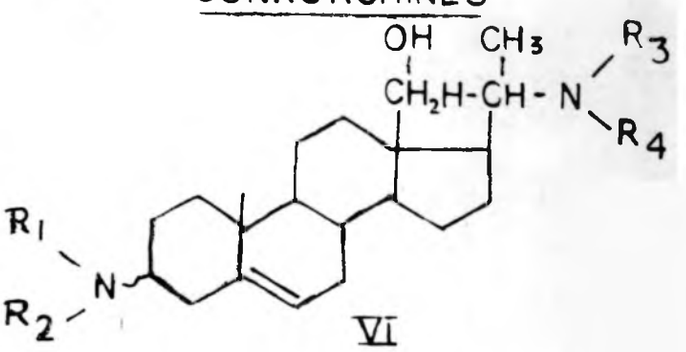
IV

CONKURCHINES



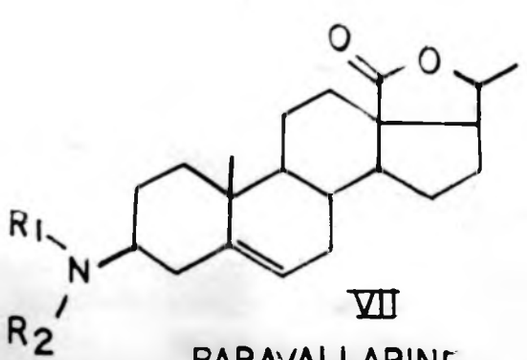
V

12β-HYDROXYCONARRHIMINES



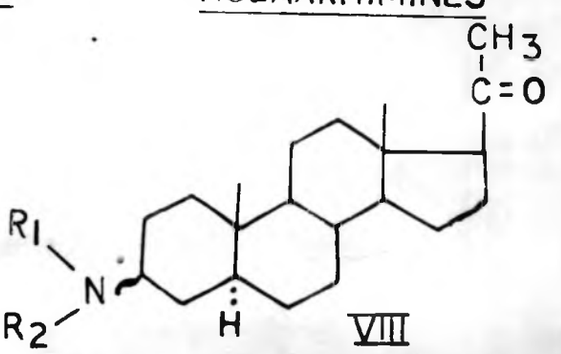
VI

HOLARRHIMINES



VII

PARAVALLARINE



VIII

3-AMINO-20-OXOPREGNANES

When this work was undertaken in October 1958, no partial synthesis of aldosterone was known - the intramolecular oxygen and nitrosyl transfer processes appearing in print at a much later date. Moreover, even if it is conceded that as starting material for aldosterone synthesis the kurchi alkaloids appear to be less attractive now than what they did in 1958, the number of steroids with different substitutions at positions 3, 18 and 20 obtainable from these alkaloids, by simple transformations would have justified the undertaking of the present investigation.

The Indian kurchi, Holarrhena antidysenterica, grows abundantly in India, especially in the state of Bombay, the Malabar region and the Himalayan belt. Extracts from kurchi have been used as a remedy for dysentery from time immemorial and the bark is commercially available ex Bombay at the rate of £1/- for 37 kg.

The Steroidal Alkaloids from the Holarrhena and the Apocyanaceae

Intensive work on the alkaloids of kurchi for more than one hundred years have resulted in the isolation of more than twenty-five alkaloids, having a steroidal basic skeleton. Some closely related alkaloids have been isolated in recent years from the family Apocyanaceae[12]. Structurally, the alkaloids belong to three broad classes viz. (A) the conanines (II, III, IV, V); (B) the 18-oxygenated 3-amino pregnanes

(VI, VII), and (C) the 3-amino-20-oxopregnanes (VIII). (Plate I, page 2).

(A) The conanine type of alkaloids constitute the major bases from kurchi. These can be further subdivided into three main groups differing only in some minor structural details.

(1) The connarrhimine type has the 3β-aminoconanine type of basic nucleus with a 5,6-double bond. The two nitrogens in the molecule can be unsubstituted as in conarrhimine (III; $R_1 = R_2 = R_3 = H$), monosubstituted as in conamine (III; $R_1 = R_2 = H$, $R_3 = CH_3$) and conimine (III; $R_1 = R_3 = H$, $R_2 = CH_3$) disubstituted as in conessimine (III; $R_3 = H$, $R_2 = R_1 = CH_3$) and isoconessimine (III; $R_1 = H$, $R_2 = R_3 = CH_3$) or fully substituted as in conessine (III; $R_1 = R_2 = R_3 = CH_3$). Six possible combinations arising out of methylation are possible. Of these, five are fully characterized. Conarrhimine has not been obtained in the pure form but is known through its N-nitroso-derivative[13].

(2) The conkurchines (IV) have all the basic structural features of the conarrhimines excepting for an extra double bond at position 17:20. Out of the six possible combinations on the basis of methyl substitutions on the two nitrogens, only three have been isolated so far, viz., conkurchine (IV; $R_1 = R_2 = R_3 = H$), conessidine (IV; $R_1 = CH_3$, $R_2 = R_3 = H$) and trimethyl conkurchine (IV; $R_1 = R_2 = R_3 = CH_3$). A fourth base of this

group, kurchamine, in which the 5:6 double bond is absent, has also been reported[14].

(3) Only three members[15] of the 12β-hydroxy conarrhimines have been isolated so far from the African variety of kurchi, viz., holarrhenine (V; $R_1 = R_2 = R_3 = CH_3$, $R_4 = H$), holafrine (V; $R_1 = R_2 = CH_3$, $R_3 = H$, $R_4 = (CH_3)_2 C = CHCH_2CO-$), and holarrhetine (V; $R_1 = R_2 = R_3 = CH_3$, $R_4 = (CH_3)_2C = CHCH_2CO-$). This group seems to be absent from the Indian kurchi.

(B) The 18-oxygenated 3-aminopregnanes[12] (VI and VII) can be subdivided into two groups. viz. (1) the holarrhimines (VI) and (2) paravallarines (VII).

(1) The holarrhimines are characterized by an 18--hydroxylated 3,20α-diamino-5-pregnene structure[14]. Out of the nine combinations possible by methyl substitution of 3β,20α-aminonitrogens only four have been isolated from kurchi. These are holarrhimine (VI; $R_1 = R_2 = R_3 = R_4 = H$), 3N-methylholarrhimine (VI; $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$), 20-methylholarrhimine (VI; $R_1 = R_2 = R_4 = H$, $R_3 = CH_3$), and tetramethylholarrhimine (VI; $R_1 = R_2 = R_3 = R_4 = CH_3$). A base of the 3α-series, holarrhidine[16] (VI (3α); $R_1 = R_2 = R_3 = R_4 = H$), has also been isolated from the Indian kurchi.

(2) The Apocyanaceae alkaloids, paravallarines[12] (VII), have a 3β-amino 18,20-dioxygenated pregnane as the basic skeleton where the 18 and 20 positions are connected by a

lactone bridge. Two members of this class are known, paravallarine (VII; $R_1 = H$, $R_2 = CH_3$) and methylparavallarine (VII; $R_1 = R_2 = CH_3$).

(C) The 3-amino-20-oxo-pregnanes lack any substitution at position 18. Several members of this class are known[12] of which three, viz. holaphylline (VIII, $^5(3\beta)$; $R_1 = CH_3$, $R_2 = H$), holaphyllamine (VIII, $^5(3\beta)$; $R_1 = R_2 = H$), and holamine (VIII, Δ^4 or $\Delta^5(3\alpha)$; $R_1 = R_2 = H$) have been isolated from the African variety of kurchi. Several other bases have been obtained from the family Apocyanaceae[12].

From a cursory examination of the structures (Plate I) holarrhimine appears to be one of the most attractive starting material for the syntheses of 18-oxygenated steroids. The conanines which occur more abundantly in kurchi suffer from the disadvantage of having a nitrogen bridge which sometimes offers difficulties in the elimination of nitrogen[17].

The present studies are, therefore, restricted to holarrhimine and 20-methyl holarrhimine only.

The Chemistry of Holarrhimine

Holarrhimine, $C_{21}H_{36}ON_2$, was first isolated by Siddiqui and Pillay[18] in 1932 from the bark of Indian kurchi. Preliminary studies indicated that holarrhimine has two primary amino groups, a hydroxyl group and a double bond. From a comparison of elemental analyses of conarrhimine type of

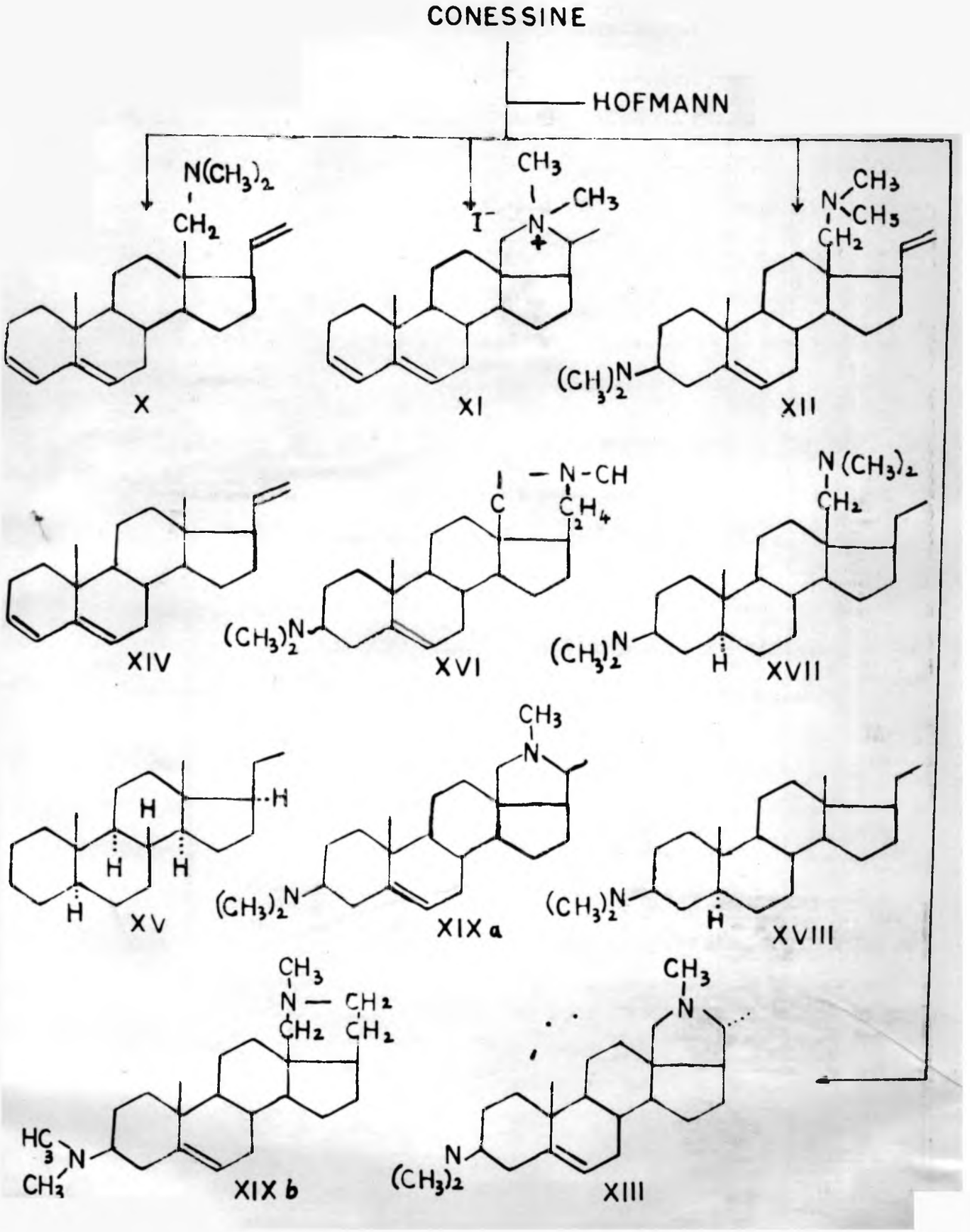
alkaloids with that of holarrhimine it was obvious that the imino bridge nitrogen in the conarrhimines is missing from holarrhimine. A peculiar interrelationship between connarrhimine and holarrhimine was reported by Siddiqui[19]. It was found that the crude conarrhimine fraction on refluxing with moist ethyl acetate was transformed into holarrhimine. Now that the chemistry of the kurchi alkaloids has been well established, it is difficult to envisage a mechanism for such a transformation.

The most significant contributions towards the elucidation of structure of holarrhimine came from the work of Haworth and coworkers, and Sorm and coworkers.

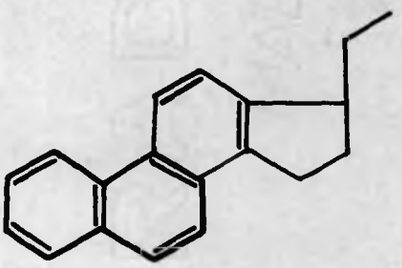
At the initial phase as the work on the determination of structure of holarrhimine was closely linked up with that of conessine, it will be necessary to present a brief summary of the chemistry of conessine.

On dry distillation of conessine dihydroiodide, Siddiqui and Sharma[20] obtained a hydrocarbon conessene, $C_{21}H_{30}$. Haworth and coworkers[21] demonstrated that conessene on selenium dehydrogenation yielded 3'-ethyl-1,2-cyclopentanophenanthrene (IX

PLATE II


CONESSINE
HOFMANN
N(CH3)2
CH2
X
CH3
I-
N
CH3
CH3
XI
CH3
N
CH3
CH2
(CH3)2N
XII
XIV
C
N-CH
CH2
CH2
(CH3)2N
XVI
N(CH3)2
CH2
(CH3)2N
H
XVII
H
H
H
H
XV
CH3
N
(CH3)2N
XIX a
(CH3)2N
H
XVIII
CH3
N
CH2
CH2
CH2
HC3
N
CH3
XIX b
CH3
N
N
(CH3)2
XIII

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



IX

From earlier work[19] it was known that conessine had a dimethylamino group and a methylimino group. On this basis the structure was developed to $C_{21}H_{31}N^{a}Me_2 - N^{b}Me$ (N^{a} and N^{b} representing the dimethylamino nitrogen and the methylimino nitrogen respectively).

After exhaustive methylation and Hofmann degradation, conessine yielded four products[22] (Plate II, page 9) :-

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

(b) a methiodide, $C_{21}H_{30}N^{b}Me_2I$ (XI), with two double bonds;

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

(d) heteroconessine, $C_{21}H_{30}N^{a}Me_2\text{-}N^{b}Me_2$ (XIII), with one double bond, isomeric with conessine.

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

(a) Apoconessine (X) was converted by Emde degradation to 3, 5, 20 (21) pregnatriene (XIV) which was hydrogenated to the known 5α-pregnane (XV).

Since apoconessine (X) and the methiodide (XI) retained only the N^{b} nitrogen and the elimination of the N^{a} nitrogen left a double bond in conjugation with the existing double bond, a partial structure for conessine could be formulated as XVI.

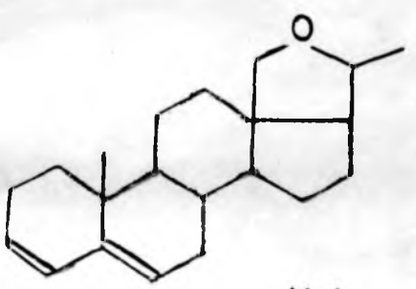
(c) Conessimethine (XII) was hydrogenated to the tetrahydroderivative (XVII) which on Emde degradation yielded the known 3β,N-dimethyl-5α-pregnane (XVIII). The assignment of 3β-configuration for N^{a} was thus justified.

(d) Heteroconessine, (XIII) on Kuhn-Roth degradation gave the same number of C-methyls as conessine. It was, therefore, possible to select structure (XIXa) for conessine out of two possibilities, (XIXa) and (XIXb).

The only uncertainty about the structure (XIXa) was the orientation of the methyl group at C_{20}. This problem was later unequivocally resolved by Sorm and coworkers[23] from

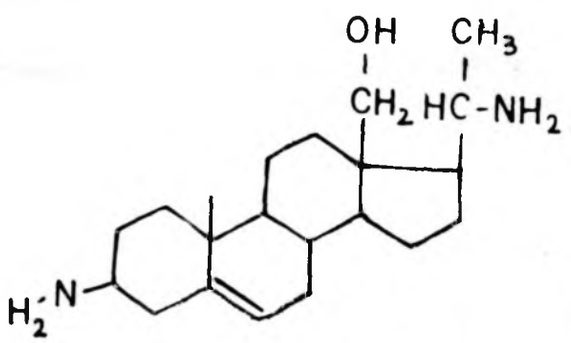
the data on the structure of holarrhimine.

On Hofmann degradation, tetramethyl holarrhimine yielded a nitrogen-free ether assigned the structure (XX) which gave the characteristic absorption of a 3,5-diene system[24].

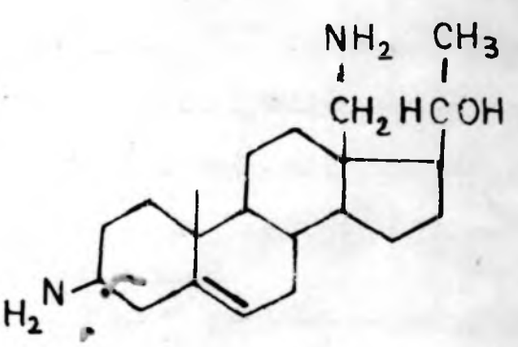


XX

On this basis two possible structures could be assigned to holarrhimine, (XXIa) and (XXIb).



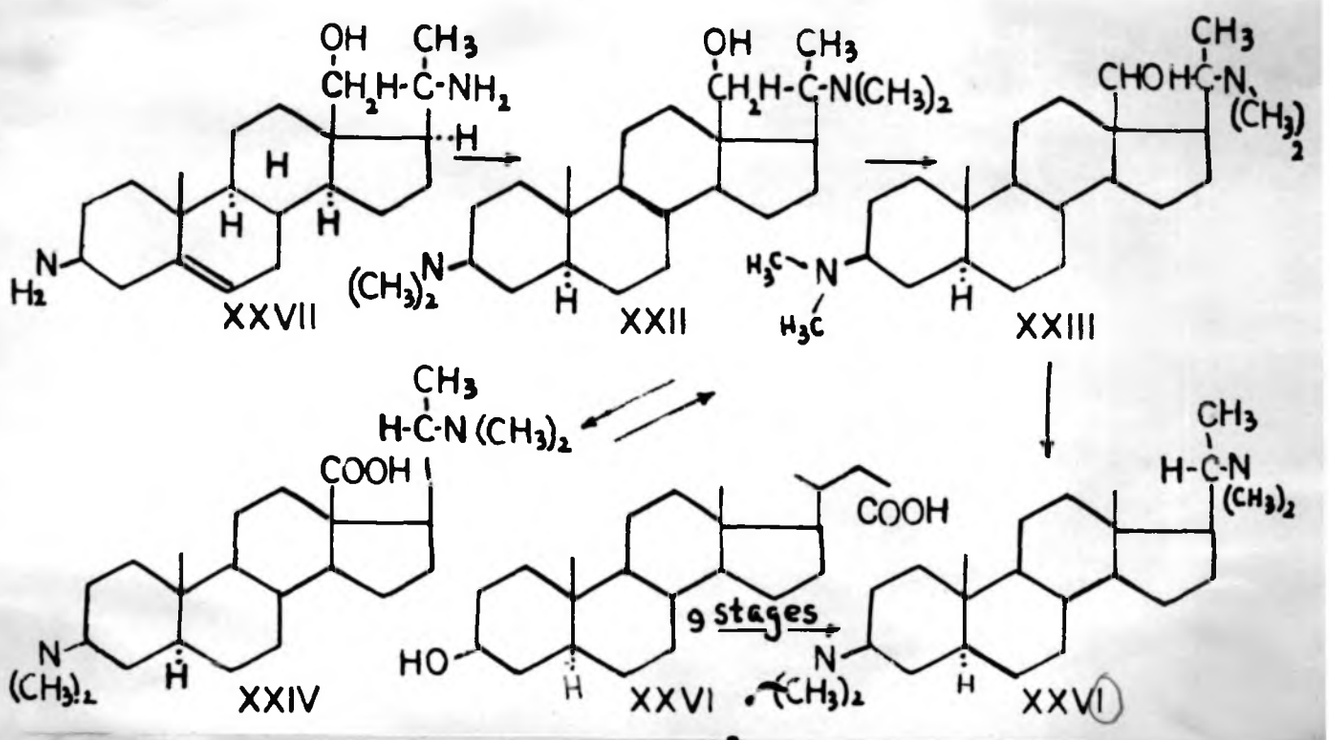
XXI a



XXI b

The next development in the chemistry of holarrhimine came from the work of Sorm and coworkers.

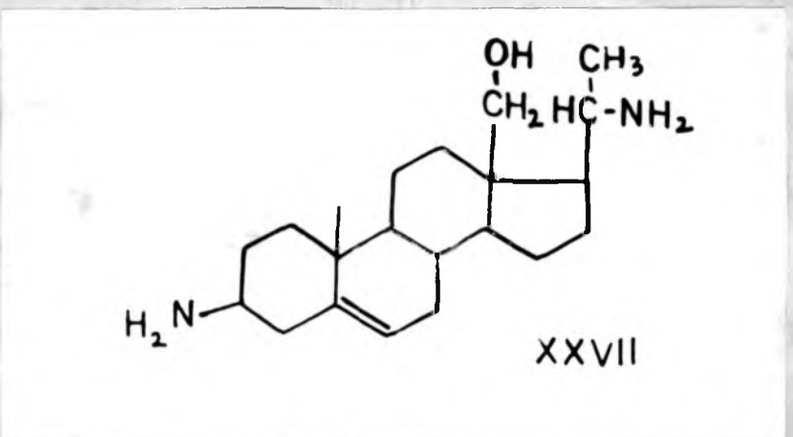
Cerny et al.[25] were able to establish that the hydroxyl group in holarrhimine was primary by oxidation of dihydrotetramethylholarrhimine (XXII) to an aldehyde (XXIII) and an acid (XXIV). The aldehyde (XXIII) was characterized by its oxime and the acid by its methyl ester. On lithium aluminium hydride reduction the ester gave back dihydrotetramethylholarrhimine. The data supported structure(XXIa) of Haworth.



On Wolff-Kishner reduction the aldehyde (XXIII) yielded 3β,20α-bis-dimethylamino-5α-pregnane (XXV). The

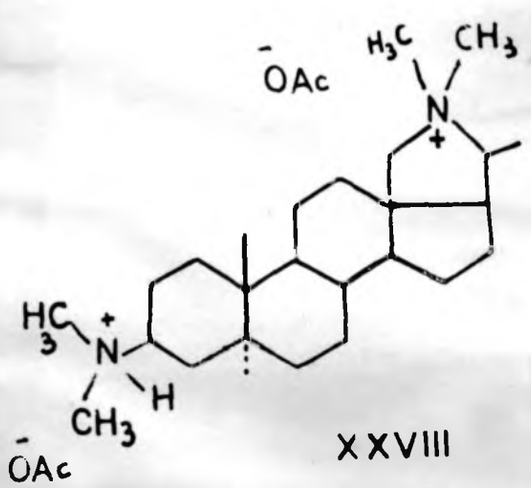
structure and stereochemistry of compound (XXV) was established by its unequivocal synthesis in 9 stages from 3β-hydroxyallocholanic acid (XXVI).

These studies completely established the structure and stereochemistry of holarrhimine as 18-hydroxy-3β,20α-diamino-5-pregnene (XXVII).

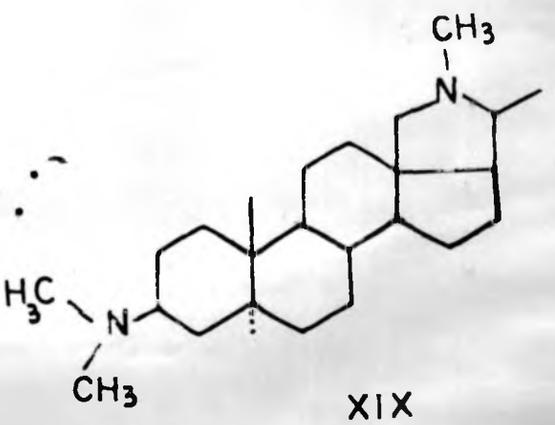


XXVII

Refluxing of dihydrotetramethylholarrhimine with thionyl chloride followed by a thermal degradation of the diacetate salt of the tertiary-quaternary base (XXVIII) resulted in the formation of dihydroconesine[23] (XIX).



XXVIII



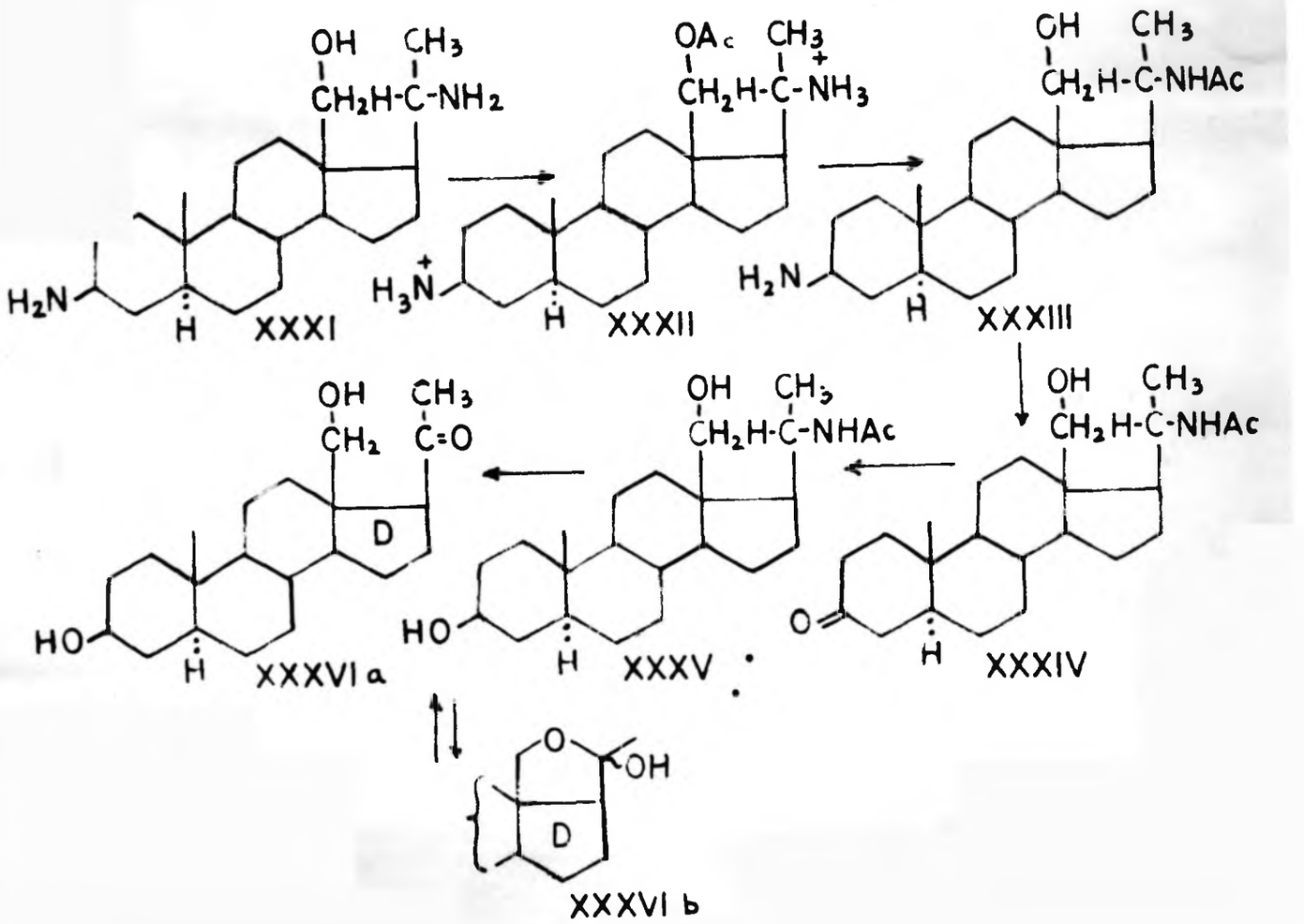
XIX

This established that the orientation of the methyl group in position 20 is 20β.

The structure and stereochemistry of conessine have been confirmed by the recent syntheses of dihydroconessine[27,28] and conessine[29,30].

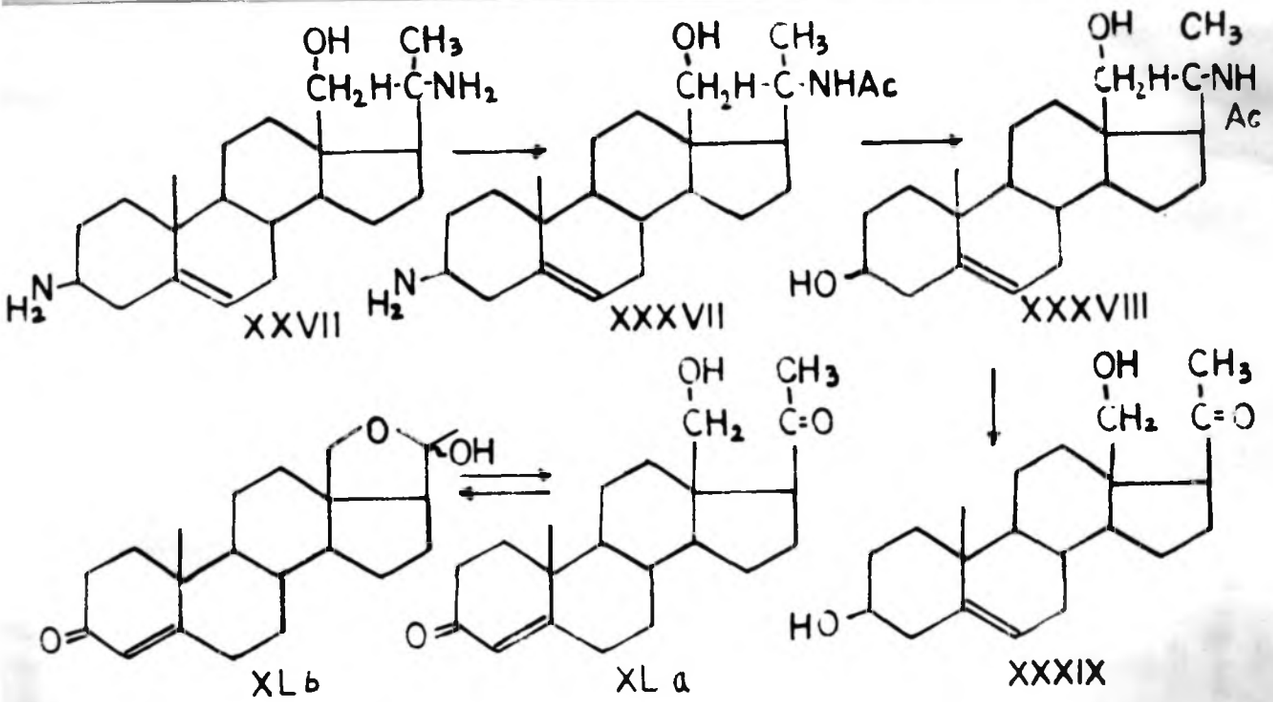
Transformations of Holarrhimine

The conversions of holarrhimine to 18-oxygenated neutral steroids have been reported exclusively by Sorm and coworkers.



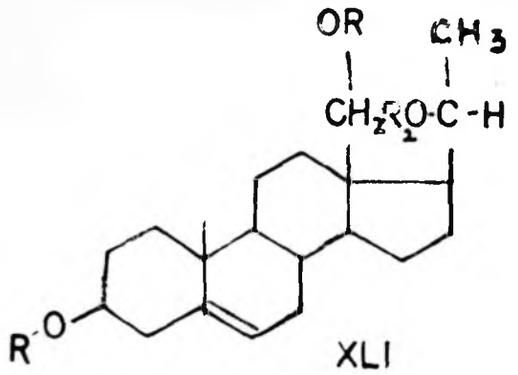
Dihydroholarrhimine (XXXI) was converted to the O-acetyl dihydrobromide (XXXII) by acetylation with acetic acid-hydrobromic acid[31]. The acetyl derivative (XXXII) on basification with ammonia underwent an O → N acetyl migration to yield 20-acetyl dihydroholarrhimine (XXXIII). N-Chlorination, dehydrochlorination and hydrolysis by the Ruschig procedure converted the amino group at position 3β to an oxo group (XXXIV). The 3-oxo compound (XXXIV) was reduced by tri-t-butoxyaluminohydride to the corresponding 3β-hydroxy compound (XXXV). Hydrolysis of the 20-acetamido group in compound (XXXV) followed by another Ruschig deamination on the resulting 20-amine, yielded 3β,18-dihydroxy-20-oxo-5α-pregnane (XXXVIa) which existed in the tautomeric hemiketal form (XXXVIb).

In a parallel series of degradations holarrhimine was converted into 18-hydroxyprogesterone[32].

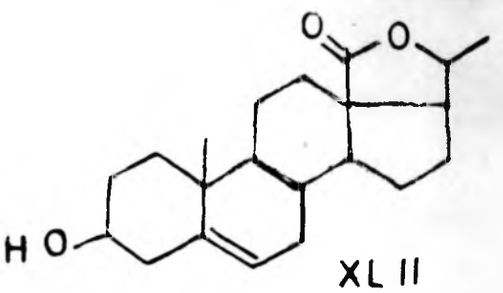


The O-acetyl derivative was prepared from holarrhimine by treatment with acetic acid-perchloric acid, isolated as the diperchlorate salt and converted to the 20-monoacetyl holarrhimine (XXXVII) by treatment with ammonia. Nitrous acid treatment converted compound (XXXVII) to the 3β-hydroxy compound (XXXVIII). The 20-acetamido group was then hydrolysed and the free base deaminated by the Ruschig procedure to yield 3β,18-dihydroxy-20-oxo-5-pregnene (XXXIX). On Oppenauer oxidation compound (XXXIX) yielded 18-hydroxyprogesterone (XL).

PLATE III



XLI



XLII

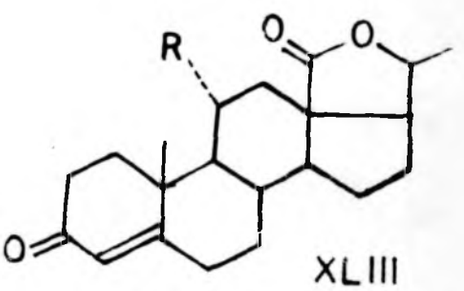
a : R_1 = R_2 = H

b : R_1 = R_2 = Ac

c : R_1 = H ; R_2 = Ac

d : R_1 =  ; R_2 = Ac

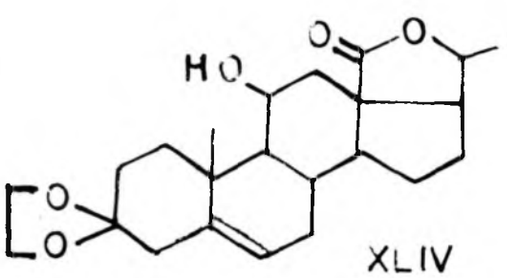
e : R_1 =  ; R_2 = H



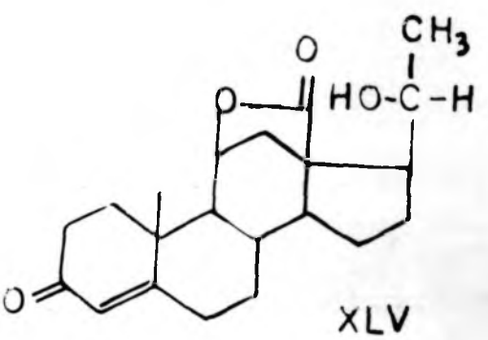
XLIII

a : R = H

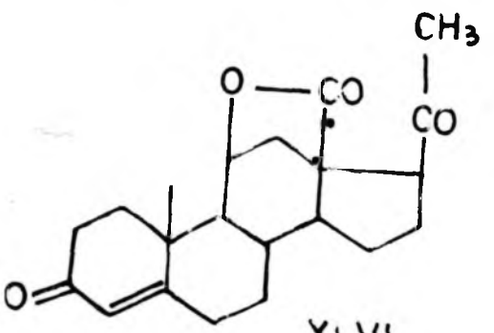
b : R = OH



XLIV



XLV



XLVI

Reduction of 3β,18-dihydroxy-20-oxo-5-pregnene with lithium aluminium hydride yielded 3β,18,20β-trihydroxy--5-pregnane[33] (XLIa; $R_1 = R_2 = H$, Plate III, page 18). The triacetoxy derivative (XLIb; $R_1 = R_2 = Ac$) was partially hydrolysed at the 3β-position to give the diacetoxy derivative (XLIc; $R_1 = H$, $R_2 = Ac$). The 3β-hydroxy group was protected by a pyranoxy residue and compound (XLId; $R_1 =$  , $R_2 = Ac$) was hydrogenolysed with lithium aluminium hydride to the corresponding diol (XLIe; $R_1 =$  , $R_2 = H$). Chromic acid oxidation of this diol gave the 18 → 20 lactone (XLII) which was converted by Oppenauer oxidation to 20β-hydroxy-3-oxo--18-carboxy-4-pregnene (18 → 20 lactone) (XLIII).

After the present investigations were completed in August 1961 a route to aldosterone from compound (XLIIIa) was established by Sorm and coworkers[34]. They were able to hydroxylate compound (XLIIIa; R=H) to (XLIIIb; R=OH) with the help of Rhizopus nigricans. The 11α-hydroxy group was oxidized to the corresponding 11-ketone, the 3-keto group was protected by dioxolane formation and the 11-keto function was reduced to 11β-hydroxyl function (XLIV) with sodium borohydride. The 18 → 20 lactone was opened by base and the 11 ⟶ 18 lactone (XLV) formed on acidification.

The 20β-hydroxyl group was finally oxidized by chromic acid to yield the 20-oxo-compound (XLVI) a key intermediate in the synthesis of aldosterone[35].

Present Investigation

The present work comprises two main parts.

(A) Development of a suitable extraction procedure for holarrhimine and methylholarrhimine.

(B) Conversion of holarrhimine into 18-substituted steroids.

The chemical studies on holarrhimine were channelised in three different directions.

(1) Holarrhimine was deaminated with nitrous acid and six different crystalline compounds were isolated. Two of them were identified as 3β-hydroxy,18-20β-oxido-5-pregnene and 3β,18-dihydroxy-20α-amino-5-pregnene. Tentative structures were assigned to three other compounds. The majority of these products yielded 3β,18,20α-triacetoxy-5-pregnene on treatment with boron trifluoride and acetic acid.

(2) A single step conversion of holarrhimine to 18-hydroxyprogesterone and its microbiological transformation to 11α,18-dihydroxy-3-oxo-4-pregnene.

(3) Synthesis of several 18-oxygenated and 18,20-dioxygenated steroids through a stepwise hydrolysis of triacetyl holarrhimine to the di- and 3-monoacetyl holarrhimine and studies on the proximity relation of the hydroxyl group at position 18 and the amino group at position 20.

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CHAPTER II

METHODS AND MATERIALS

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All melting points are uncorrected. The rotations unless otherwise stated, were taken in chloroform and the concentrations are expressed in g./100 ml of the solution.

For chromatography, acid washed neutral alumina (100 to 200 mesh) was used. This was prepared from a commercial grade of alumina by washing with nitric acid and then with distilled water until neutrality and igniting at 400 to 420° for 6 hours. The subsequent desired gradations were accomplished by mixing with the requisite quantity of water and homogenising over a roller-mixer for 8 hours. The standardization of the various grades were carried out according to the procedures described in the literature. All solvents used in the experimental were purified according to the conventional methods.

The ultraviolet spectra were measured in ethanol solution on a Beckmann Ratio-recording spectrophotometer Model DK_2. The infrared spectra were recorded in nujol suspension on a Grubb-Parson double beam spectrophotometer with sodium chloride optics.

Infrared spectra of compounds and were taken in nujol mull on a Perkin-Elmer Infracord with sodium chloride prism.

N-chlorosuccinimide was prepared according to the method of Tscherniac[2] and standardised iodometrically.

Cyanogen bromide was prepared according to the method described in Organic Synthesis.[3]

Buffer solutions :

The buffer solutions used in the extraction of holarrhimine were prepared according to McIlvain.[4]

Van Slyke amino nitrogen

The van Slyke amino nitrogen determinations were carried out according to the method described by Van Slyke.[5]

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CHAPTER III

ISOLATION OF HOLARRHIMINE AND 20N-METHYLHOLARRHIMINE

Various procedures have been described in literature regarding the extraction and isolation of kurchi alkaloids. A brief comparative survey of only four of these relatively simple procedures is presented here.

The first procedure is the one used by Siddiqui and Pillay[1] who isolated pure holarrhimine for the first time. The dried bark is ground, soaked for a week with a mixture of ether, ethanol, and ammonia, and percolated. The second extract is drawn three days after soaking with a fresh solvent mixture. Hydrochloric acid gas is bubbled through the extracts which precipitate the alkaloidal hydrochlorides together with ammonium chloride. The supernatant solvent mixture is basified with ammonia and used for further extractions. Eight such percolations are carried out. The total hydrochlorides are dissolved in water and treated with a solution of sodium sulphate which precipitates the insoluble alkaloidal sulphates. These insoluble sulphates contain holarrhimine as the major alkaloid. Purification of holarrhimine is achieved by reprecipitating the sulphates and by fractional crystallization of the free bases obtained from the sulphates.

Haworth's group[2] use Soxhlet extraction for obtaining the total alkaloids. The bark is mixed with calcium oxide and extracted with ethanol on a Soxhlet extraction unit. The yields of total alkaloids are not satisfactory and the yield of holarrhimine is not reported.

The third procedure is due to Tschesche and Peterson[3] who use chloroform in addition to the solvent mixture used by Siddiqui and Pillay. A single percolation after soaking the bark for a period of eight days appears to be enough to procure the total alkaloids in somewhat satisfactory yields. The concentrated extract is dissolved in chloroform and the alkaloids are extracted in hydrochloric acid solution. The hydrochlorides are basified and re-extracted in chloroform. The chloroform solution is concentrated and chromatographed over alumina. The chromatographic fractions contain overlapping mixtures of alkaloids which are further separated by aqueous extraction of the alkaloids from their hydrochlorides at their pK values. The pH is adjusted by the addition of dilute ammonia solution. They have also suggested further purification of the bases through their perchlorate salts. Holarrhimine is then purified from extracts of pH 7.2.

An economic method for extraction is due to Labler and Cerny[4], who extracted the bark with ammoniacal ethanol. The total time required is two weeks for two such extractions. The extract is acidified with sulphuric acid. Bulk of the ammonia is precipitated as ammonium sulphate. The extract is concentrated, treated with 20 per cent sodium hydroxide till alkalinity and the bases are extracted in ether. After concentrating the ether solution, the bases are separated into petroleum ether-soluble and petroleum ether-insoluble alkaloids. The petroleum ether-insoluble alkaloids are treated

with an alcoholic solution of p-nitrobenzaldehyde when the dibenzylidine Schiff's base of holarrhimine is precipitated out, while other bases remain in solution. The insoluble Schiff's base is treated with sulphuric acid to convert it to the water-insoluble holarrhimine sulphate which is freed from the liberated p-nitrobenzaldehyde by washing with alcohol. The sulphate is basified and the base extracted in organic solvents and purified through the perchloric acid procedure described above. This method gives very pure holarrhimine.

A choice was to be made among these procedures for the extraction of alkaloids. The procedure used by Siddiqui and Pillay was found to be eminently suitable in getting the total alkaloids in best yields. A modification of the method was, however, necessary to obtain holarrhimine in good yields in a short time. The objective was to develop a procedure for the isolation of holarrhimine which avoids the necessity of time consuming fractional crystallization methods or chromatography. In this context the procedure of Labler and Cerny, suggested itself for the recovery of holarrhimine in the later stages. Also the extraction of the alkaloids at their pK values, using buffer solutions of different pH values, was found to be convenient.

A procedure for the extraction of total alkaloids and isolation of holarrhimine was finally developed, which achieved the objective of cutting of number of steps and time.

In a preliminary experiment on five kg. of bark four extractions were found to be enough to extract almost all alkaloids from the bark. Instead of soaking the bark for a number of days, it was stirred in the solvent mixtures for four hours only and the extract was taken off.

The alkaloids were precipitated as hydrochlorides by passing dry hydrochloric acid gas through the extract till the supernatant was acidic. The insoluble sulphates were precipitated according to Siddiqui and Pillay. These sulphates were worked up by (a) following the procedure of Cerny and Labler or (b) extraction with phosphate-citrate buffer of pH 7.

The results are compared in Table No.1.

Table No.1

Procedure by	Solvents used	Time required	Total alkaloids %	Holarrhimine %
1. Siddiqui & Pillay	Ether:ethanol:liquor ammonia 8:1:1	4 weeks	2.1	0.12 fresh bark 0.03 old bark
2. Haworth et al.	Alcohol (+ Calcium oxide)	2 days	1.3	-
3. Tschesche & Peterson	Ether:Ethanol: Chloroform Liquor ammonia 15:15:10:4	8 days	1.1	0.05
4. Cerny & Labler	Ethanol:liquor ammonia 10:1	14 days	1.39	0.028
5: Present work	Same as 1	20 hours	1.96	0.11 fresh bark 0.035 old bark

20*N*-Monomethylholarrhimine accompanied holarrhimine in the insoluble sulphates and the buffer extracts in method (b). When purified it gave a water-soluble sulphate and an alcohol-soluble p-nitrobenzylidine Schiff's base.

The salient features of the present method can be summarized as follows :

(1) Four extractions of the bark require only twenty hours and the recovery of the total bases is satisfactory.

(2) The bases could be obtained as solid hydrochlorides by passing hydrochloric acid gas through the extracts. In this way concentration of the extracts and further fractionation to obtain the bases are avoided.

(3) Precipitation as sulphates avoids the time-consuming method of chromatography. After basification recovery of the alkaloids in organic phase presents no undue difficulty.

(4) Holarrhimine precipitates out as insoluble phosphate from the buffer extracts, almost quantitatively along with some impurities.

(5) The procedure involving precipitation as *p*-nitrobenzylidine Schiff's base on the other hand is a little more elaborate compared to the buffer extraction method. However, it yields practically pure holarrhimine in one step.

Out of the mother liquor from the insoluble phosphate

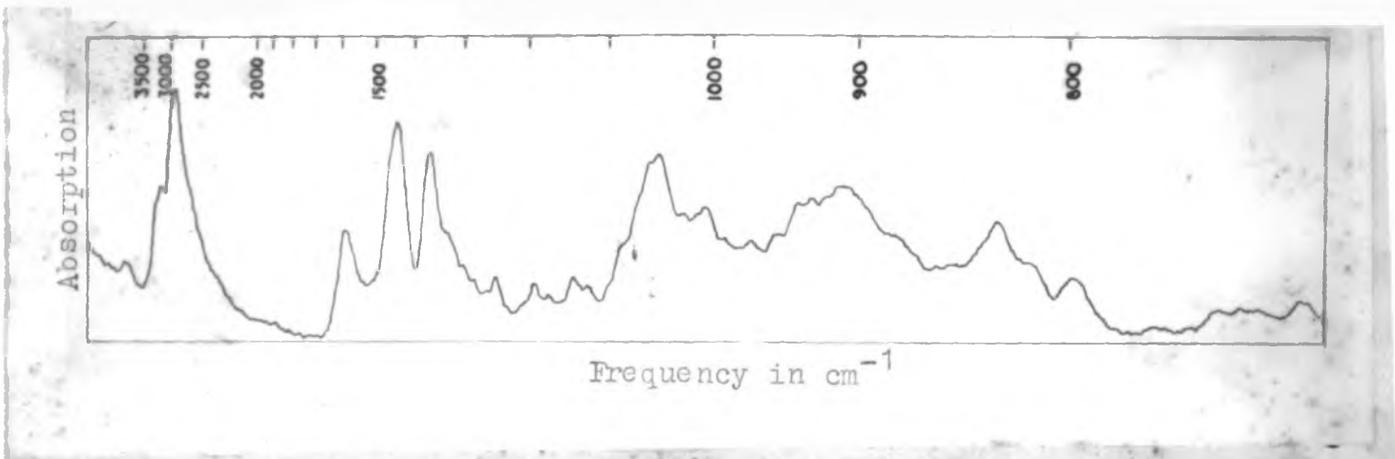

Fig. 1. Infrared spectrum of holarrhimine.

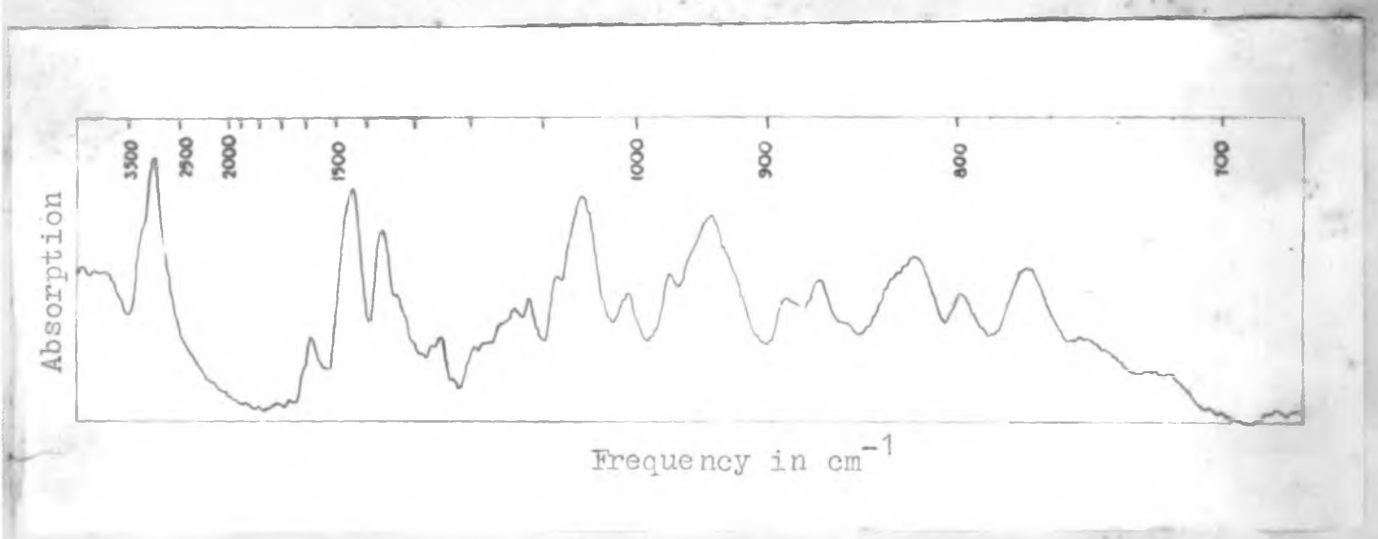

Fig. 2. Infrared spectrum of 20N-monomethylholarrhimine.

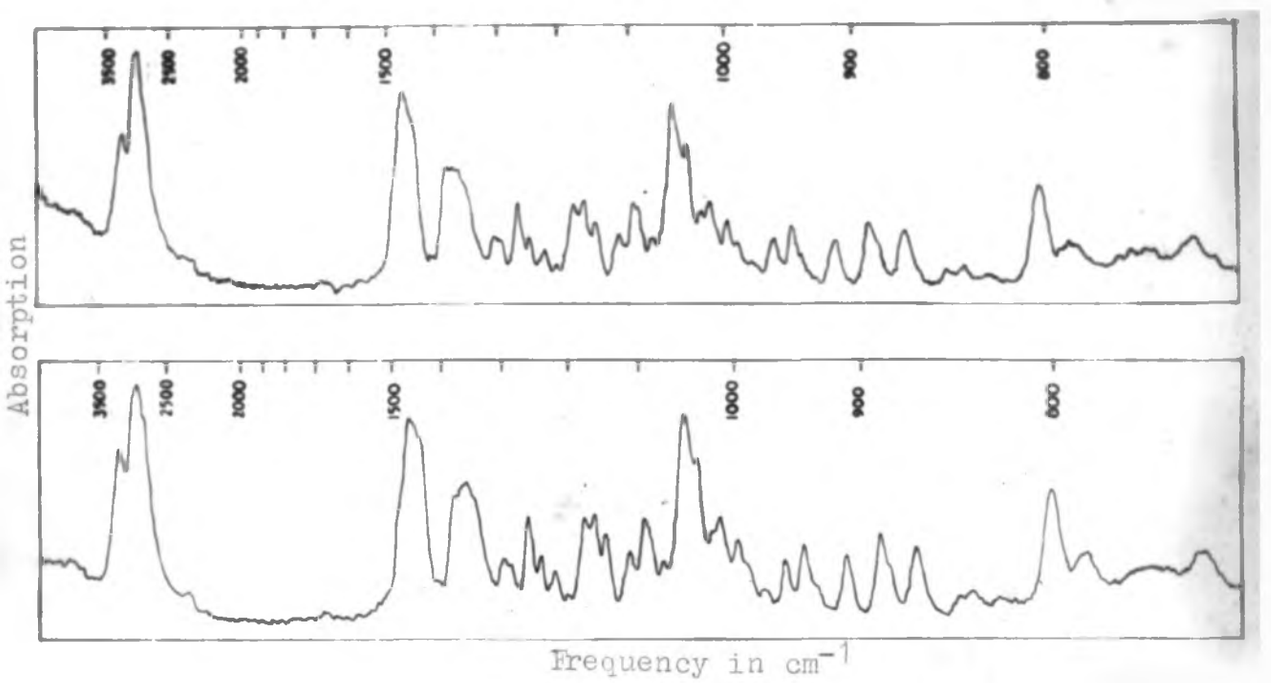

Fig. 3. Infrared spectra of

(1) Tetramethylholarrhimine from holarrhimine.

(2) Tetramethylholarrhimine from 20N-methyl holarrhimine.

of holarrhimine, 20*N*-methylholarrhimine was isolated. At first it was presumed to be impure holarrhimine, but it was later found to differ from holarrhimine in the following properties :-

(1) The sulphate of this base is soluble in water but insoluble in alcohol. Holarrhimine sulphate is insoluble in both.

(2) Its *p*-nitrobenzylidine Schiff's base is soluble in alcohol; that of holarrhimine is insoluble.

(3) Its optical rotation is different from that of holarrhimine.

(4) The salicylidine and the picrate derivatives have melting points different from those of holarrhimine.

(5) Its infrared spectrum shows a distinct difference in the finger-print region. (Fig.1 and 2, page 29).

The melting points of the base and its derivatives suggested that it was 20*N*-methylholarrhimine which was first isolated by Tschesche and Wienz[5].

N-Methyl estimation showed that it contains one methyl group. The base on methylation gave tetramethyl--holarrhimine which was identical with that obtained from holarrhimine. (Fig.3 , page 30).

E_X_P_E_R_I_M_E_N_T_A_L

Fresh kurchi bark was procured from M/s Jadavji Lallubhai and Company, Bombay. It was dried in an oven at 50° for 16 hr and ground in a Wiley mill with a sieve of mesh size - 20.

(1) Preliminary experiment on a 5 kg lot

The powdered bark (5 kg) was vigorously stirred for 4 hr in a 6 gallon-stainless steel drum provided with a perforated false bottom and a stopcock at the bottom with a precooled mixture (20 l.) of ether, alcohol and ammonia in proportions of 8:1:1. After settling for half an hr the first percolate was drawn (10 l.). Five successive similar extractions were carried out, using fresh solvent mixture (10 l.) each time. Each of these six extracts was treated separately with a stream of dry hydrochloric acid gas till the supernatant was acidic to litmus. At this stage the green colour of the extract changed to red-brown. The supernatants were decanted off from the precipitated alkaloidal hydrochlorides and ammonium chloride. The precipitates were washed with ether, and each lot was separately dissolved in water (200 ml each), heated on a steam-bath, treated with a hot saturated solution of sodium sulphate (50 ml each) and kept at 0° overnight. The precipitated insoluble sulphates were filtered and washed with water. The residue and filtrate from each lot were separately basified and extracted in chloroform. The chloroform layers were washed with water, dried over sodium sulphate and evaporated to dryness. Each fraction was weighed.

Table 2 shows the amount of alkaloids in the soluble and insoluble sulphate fraction.

Table 2. 5 kg. bark extracted

Extraction No.	Alkaloids in the soluble sulphates (g)	Alkaloids in the insoluble sulphates (g)
1	33.3	5.980
2	28.5	3.540
3	12.6	1.402
4	6.0	0.074
5	3.0	0.000
6	2.5	0.000
Total :	85.3	10.998

Total alkaloids extracted - 96.3 g; yield - 1.93%.

The Table shows that four extractions are enough to exhaust 94.5% total alkaloids and all the bases that give insoluble sulphates.

(2) Large scale extraction on a 35 kg lot

(A) Extraction of total alkaloids

First extraction : Dry powdered kurchi bark (35 kg) was charged in a stainless steel drum (capacity 45 gal) provided with a false botton covered with muslin and a stopcock.

It was vigorously stirred with a solvent mixture containing ether (24 gal), ethanol (3 gal) and liquor ammonia (sp.gr.0.88, 3 gal).

After stirring for 4 hr the first percolate was tapped off (20 gal) and was treated with a stream of hydrochloric acid gas in the cold until the extract became acidic to litmus. The supernatant was decanted off from the precipitated hydrochlorides and ammonium chloride, and left aside for the third extraction.

Second extraction : A fresh solvent mixture consisting of ether (16 gal), ethanol (2 gal) and liquor ammonia (2 gal) was added to the bark. The second extraction was carried out in the same manner as described above. After precipitation of the hydrochlorides from the extract (19 gal), the supernatant was set aside for the fourth extraction.

Third and fourth extractions : The bark residue was extracted for a third and fourth time using alkaloid-free supernatants from the first and second extractions respectively, after the addition of liquor ammonia (2 gals) to each lot.

After the fourth extraction the bark was pressed to get some more extract (1.5 gal) and then discarded.

(B) Fractionation of the alkaloids

The precipitated hydrochlorides along with ammonium chloride were pooled together and washed with ether to remove

the green colouring pigments and some neutral material. The precipitate was divided into 4 lots. Each lot was dissolved in water (5 l.) heated to 80° to remove ether and treated with hot saturated solution of sodium sulphate (1.5 l. per lot). After heating the mixture for another hr on a steam-bath it was left at 15° overnight. The insoluble sulphates from four lots pooled were filtered, washed with a little water, ethanol and then ether and finally dried at the water pump. (108 g).

The insoluble sulphates were slurried with sodium hydroxide (3 l., 1N) and extracted in chloroform (5 x 1 l.). Any emulsion formation during separation was destroyed either by addition of a little methanol or by filtration with Hyflo Super-Cel. The chloroform layer was washed with water, dried with sodium sulphate and concentrated to dryness (66 g).

Similarly the soluble sulphates were treated with sodium hydroxide (20 l., 2N) and extracted with chloroform (4 x 3 l.). The chloroform layer was washed with water, dried over sodium sulphate and concentrated to dryness (residue - 620 g).

The recovery of the total bases was thus 686 g (1.96%).

(C) Isolation of holarrhimine

(i) Buffer extraction method : The bases (30 g) from insoluble sulphates were dissolved in chloroform (1.2 l.).

This was shaken with McIlvain's phosphate-citrate buffer solution (3 x 250 ml) of pH 8.0. It was subsequently extracted four times with buffer solution (500 ml each time) of pH 7.0. From the aqueous layer an insoluble phosphate separated out. This was filtered and dried (32 g).

The aqueous phase from the buffer extract of pH 8.0 was added to chloroform (200 ml). The pH of the solution was lowered to 7.0 under rapid mechanical agitation by addition of citric acid solution (1 Molar). The phosphates from the aqueous layer were filtered and dried (weight - 3 g).

The filtrates from the above operations after separation from precipitated phosphates were kept aside for the extraction of methylholarrhimine.

The total insoluble phosphates (35 g) were suspended in water (250 ml) and the bases were liberated by stirring with dil sodium hydroxide (150 ml, 1N). The bases were extracted with chloroform (3 x 200 ml). The chloroform layer was washed with water, dried over sodium sulphate and evaporated to dryness. The pale yellow residue (21 g) was crystallized from ethyl acetate. Two crops of practically pure holarrhimine were obtained. The first crop (11.8 g) melted at 179-181° and the second crop (5.9 g) melted at 176-178°.

The yield corresponds to 39 g of free base from the total amount of insoluble sulphates or 0.11% calculated on total amount of bark.

(ii) p-Nitrobenzylidine Schiff's base precipitation method : The bases (30 g) from the insoluble sulphates were dissolved in ethanol (300 ml, 95%) and refluxed for 30 min with a solution of p-nitrobenzaldehyde (25 g) in ethanol (200 ml). A crystalline precipitate appeared and the mixture was kept at room temp overnight and filtered by suction. The residue was slurried and warmed with ethanol (100 ml). After cooling, it was filtered and washed with the solvent. The crude Schiff's base (41 g) melted at 260°.

The Schiff's base was suspended in sulphuric acid (800 ml, 2N) and kept on a steam-bath for 45 min with stirring. The reaction mixture was then allowed to stand overnight. The precipitate containing insoluble sulphate of holarrhimine and p-nitrobenzaldehyde was filtered and washed with water. It was suspended in ethanol (300 ml, 95%) and refluxed to dissolve p-nitrobenzaldehyde. After cooling, the undissolved holarrhimine sulphate was separated by filtration, washed with ethanol and finally with ether (32 g).

The dry sulphate was suspended in ammonium hydroxide solution (500 ml, 10N) and vigorously stirred with chloroform (200 ml). The chloroform layer was separated and the aqueous phase was extracted twice more with chloroform (100 ml each). The combined chloroform extracts were washed with water. From the chloroform solution the alkaloid was extracted with perchloric acid solution (1%) in three lots (600, 400 and 200 ml). A little methanol was added to remove emulsions at this stage.

The combined perchlorate extract was washed with chloroform (3 x 150 ml) and filtered. The colourless filtrate was then basified with sodium hydroxide (150 ml, 1N) and the base was extracted in chloroform (3 x 200 ml). The colourless chloroform extract was washed with water, dried over sodium sulphate and concentrated to dryness, when a colourless crystalline mass of holarrhimine appeared on the surface of the flask. It was crystallized from ethyl acetate. The first crop (14.2 g) melted at 183-184° and the second crop (2.2 g) melted at 180-181°. The yield by this procedure amounts to 0.103%.

Physical constants - m.p.183-184°(lit.[4] 185°), $[\alpha]_D$ = -14.0 (C, 1.2), m.p, of p-nitrobenzylidine Schiff's base 263°; lit.[4] - 264°,m.p. of tetramethylholarrhimine 226°; lit.[4] 230-235°. $[\alpha]_D$-32.0°; lit.[4] -35.0°. [Found: C, 75.4; H, 11.0; N, 8.9. $C_{21}H_{36}ON_2$ requires : C, 75.9; H, 10.8; N, 8.4%].

(D) Isolation of 20N-methyl holarrhimine

The filtrates from the insoluble phosphates were strongly basified with 10N sodium hydroxide and extracted with chloroform (3 x 200 ml). The chloroform extract was washed with water and the solvent evaporated. The residue was refluxed with p-nitrobenzaldehyde (10 g) in ethanol (200 ml) for half an hr. On cooling a small amount of insoluble p-nitrobenzylidine derivative was obtained. It was removed by filtration. The filtrate was refluxed with dil sulphuric

acid (20 ml, 2N) for 15 min. On cooling, a colourless precipitate was obtained. This was filtered, washed with ethanol until free from acid and dried (4.3 g).

The residue was stirred with sodium hydroxide (25 ml, 1N) and the base was extracted with chloroform. The chloroform layer was extracted thrice with dil hydrochloric acid (3 x 15 ml, 0.5N). The combined acid layer was washed with a small amount of chloroform and then decolourized with activated charcoal (2 g). The colourless hydrochloride solution obtained by filtration was basified and the base extracted with chloroform (3 x 25 ml). The chloroform extract was washed with water, dried over sodium sulphate and concentrated to dryness. The residue on crystallization from benzene-ethyl acetate gave two crops of 20N-methylholarrhimine. The first crop (1.1 g) melted at 163-164° and the second crop (0.6 g) at 160-162°. Yield 0.0049%.

Physical constants - m.p.163-164°; lit.[3] 164°, $[\alpha]_D$ - 17.0°(C, 1.5); lit. - 17.0°, m.p. of picrate 90-95°; lit. 98-99°, m.p. of salicylate 254°; lit. 264°, m.p. of tetramethylholarrhimine 226°; lit. 230-235°. It gave no depression in the mixed m.p. with tetramethylholarrhimine obtained from holarrhimine and both showed identical infrared spectra. [Found : C, 75.7; H, 10.7; N, 8.12; N-Me, 4.1. $C_{22}H_{38}ON_2$ requires : C, 76.25; H, 11.05; N, 8.08; N-Me, 4.52%].

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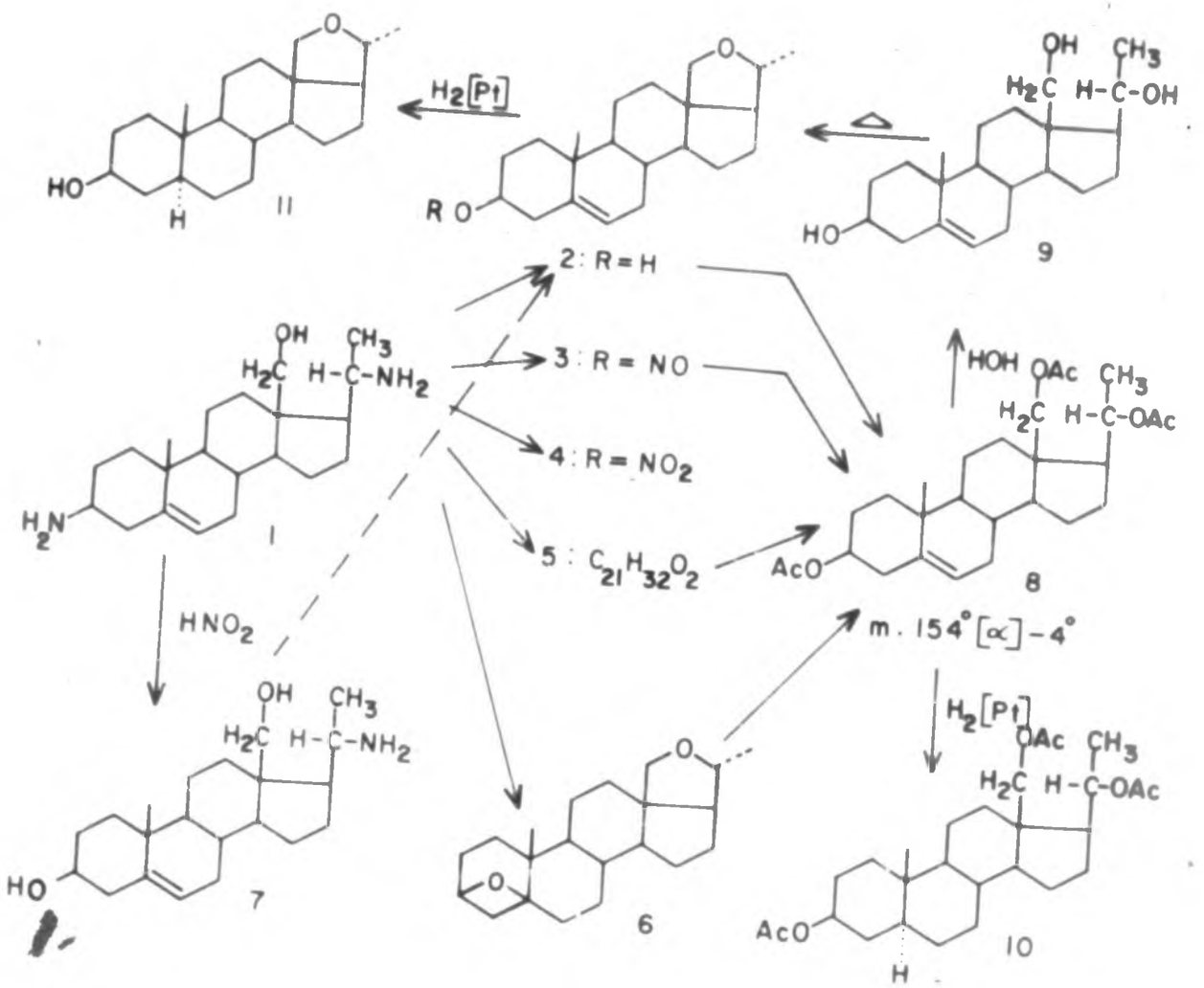
CHAPTER IV

REACTION OF HOLARRHIMINE WITH NITROUS ACID

The initial problem of the conversion of holarrhimine to some 18-substituted neutral steroids is the removal of nitrogen atoms at the 3β-and 20α-positions and their substitution by useful oxygen functions. One of the earliest approaches in this direction dates back to Siddiqui and Siddiqui[1] who obtained a nitrogen-free oily product by treatment of holarrhimine with nitrous acid. The material did not give correct analysis for the expected trihydroxy compound and was obviously a mixture.

A reinvestigation of the problem seemed desirable on the basis of certain assumptions. It is known that a 3β-equatorial primary amino-group leads exclusively to the 3β-equatorial hydroxy compound in the case of cholesterylamine[2]. It was expected that in the deamination of the 20α-amino group an anchimeric assistance from the neighbouring 18-hydroxyl group would result in a 18 → 20 oxide bridge formation. A resultant of these two processes would lead to the formation of [20β] 3β-hydroxy-18 → 20 oxido-5-pregnene (2, Chart I, page 44) as one of the major products. The nitrous acid reaction was, therefore, run under different conditions of acidity, but although the expected compound (2) was detected and isolated under each condition, the yield hardly exceeded 10 per cent. Furthermore, the crude reaction products contained varying amounts of nitrogen reaching upto 2 per cent in some cases. This could not be removed by a further nitrous acid

CHART I


H2[Pt]
2: R=H
3: R = NO
4: R = NO2
5 : C21H32O2
HNO2
m. 154° [α] -4°
H2[Pt]
HO
RO
AcO
HOH OAc CH3
H2C H-C-OAc
OH CH3
H2C H-C-NH2
H2N
11
9
1
8
7
6
10

treatment. It is worth recording that under the conditions of Van Slyke primary amino group determination on the other hand, a nearly quantitative yield of nitrogen was obtained from holarrhimine.

A yellowish semi-solid mass was obtained as a product when holarrhimine was subjected to nitrous acid reaction in acetic acid. On a 100-transfer Craig distribution in a solvent system consisting of hexane, ethyl acetate, ethanol and water (2:1:2:1) the product was separated into polar and non-polar components. Compound (2) was directly crystallized from the pooled contents of tube No. 60-69. Column chromatography of fractions 91-96 on alumina yielded a nitrogen containing crystalline material (4).

When mineral acids were used in place of acetic acid in the nitrous acid reaction, the products were somewhat different. Besides compound (2) four other crystalline compounds (3), (5), (6) and (7) were isolated from the reaction mixture by solvent extraction and partition chromatography over magnesium oxide. The basic compound (7) was isolated only when the nitrous acid reaction was run under very mild conditions of acidity and temperature. Compound (6) was not detected among the products from acetic acid-nitrous acid mixture. It appeared in appreciable amounts only with mineral acids. Some physical properties of these compounds are listed in Table 1.

Table No.1

Compound No.	Name assigned	m.p.	$[\alpha]_D$
2	3β-hydroxy-18 – 20β--oxido-5-pregnene	165-166°	- 44°
3	3β-O-nitroso-18 – 20β-oxido-5-pregnene	149-150°	- 44°
4	3β-O-nitro-18 – 20β--oxido-5-pregnene	133°	- 49°
5	--	107-108°	- 47°
6	3β – 5β,18 – 20β--dioxido-5-pregnene	87°	+ 14°
7	3β-,18-dihydroxy-20α--amino-5-pregnene	225°	

Compound (2), $C_{21}H_{32}O_2$, contained one atom of active hydrogen and was precipitable with digitonin. The infrared spectrum (fig. 4, page 47) indicated besides the bands due to hydroxyl group (3320 cm^{-1}) and a trisubstituted double bond (840 cm^{-1}), a strong ether band (at 1062 cm^{-1}) and complete absence of any carbonyl function. On Oppenauer oxidation, compound (2) was converted to a steroid containing a Δ^4 3-keto chromophore (fig.5, page 47) (λ_{max} 241 mμ , ϵ = 17,070, IR bands at 1670, 1615 cm^{-1}). The monoacetate of compound (2) was obtained by acetylation with pyridine and acetic anhydride. On treatment with boron trifluoride and acetic anhydride,

Fig. 4. Infrared spectrum of 3β-hydroxy-18 — 20- -oxido-5-pregnene.

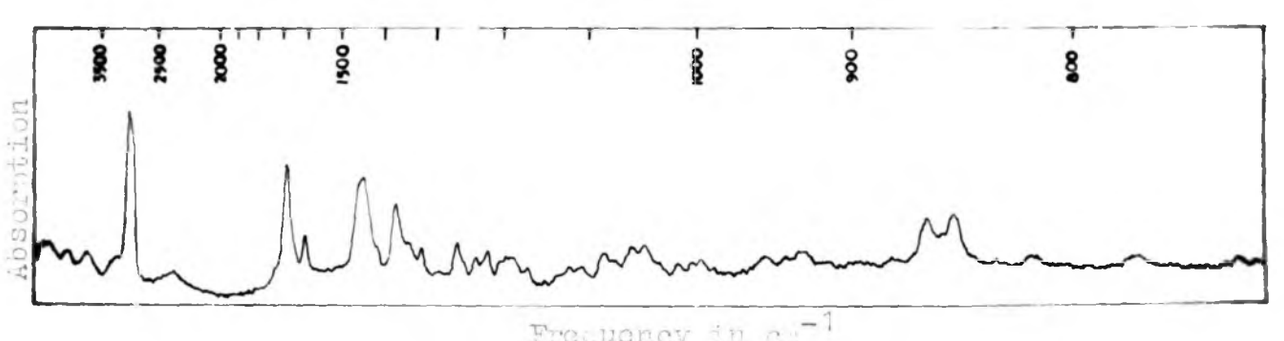


Fig. 5. Infrared spectrum of 3-keto-18 — 20- -oxido-4-pregnene.

compound (2) or its monoacetate was converted to a triacetoxy compound (8). (fig. 6, page 49). On hydrogenation over platinum in glacial acetic acid compound (2) yielded the known [20β] 3β-hydroxy-18 → 20-oxido-5α-pregnane (11) first obtained by Cainelli et al.[3] by lead tetra-acetate oxidation of 3β-acetoxy-20β-hydroxy-5α-pregnane followed by hydrolysis.

On the basis of these properties compound (2) was identified as [20β] 3β-hydroxy-18 → 20-oxido-5-pregnene (structure 2). The assignment of the 20β-configuration of the carbon oxygen linkage is consistent with the expected inversion at C-20 in the intramolecular S_N 2'displacement of the 20α-diazonium intermediate from the rear by the C-18 oxygen.

Both the triacetate (8) and the corresponding triol (9) obtained by its saponification had different physical constants from those reported by Labler et al.[4] for the 3β-, 18-,20β-triacetoxy-5-pregnene and the corresponding triol. The molecular rotation difference (M_D 8 - M_D 9) was positive in sign in contrast with that of the corresponding two 20-epimers of Labler et al.[4] and is consistent with the 20α configuration for compounds (8) and (9). The stereochemistry at C-20 of compound (8) is also in accord with the mechanism of ring opening assisted by a S_N2 displacement by acetate anion resulting in another inversion.

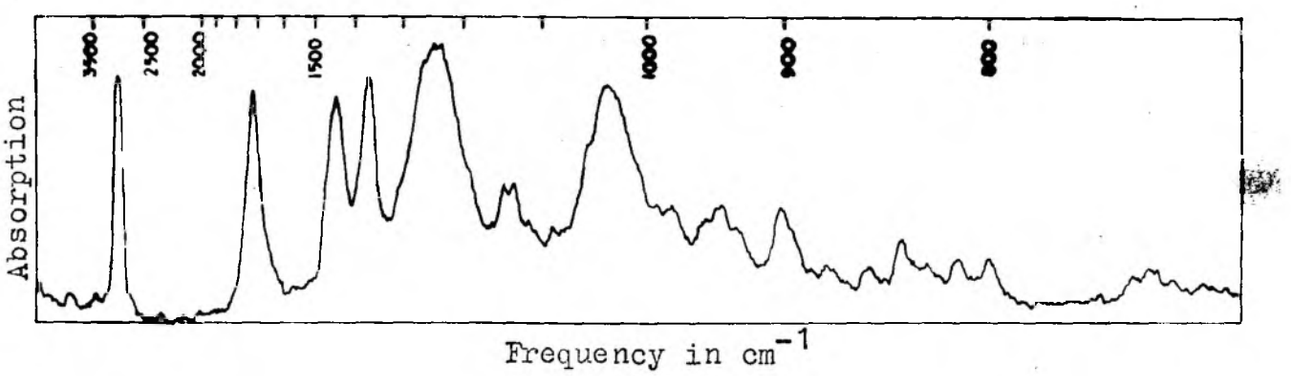


Fig. 6. Infrared spectrum of 3β-, 18, 20α-triacetoxy--5-pregnene.

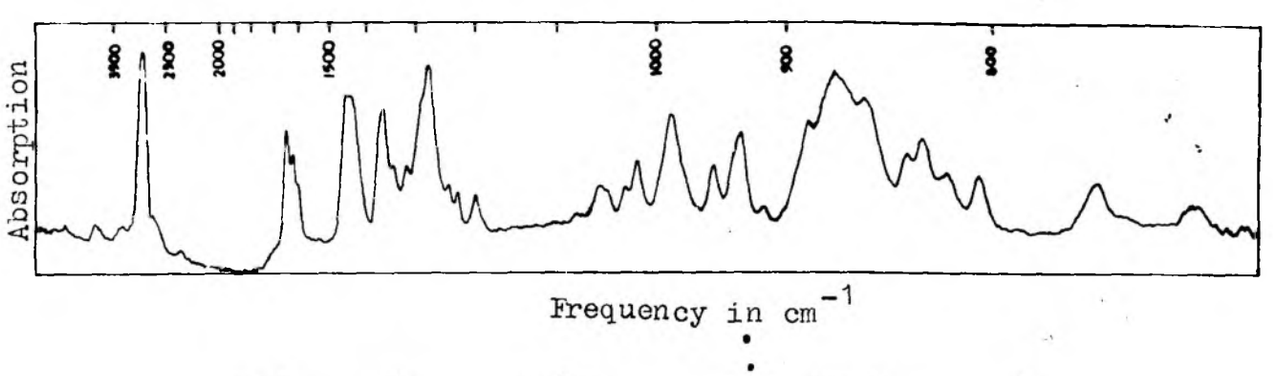


Fig. 7. Infrared spectrum of 3β-O-nitro-18 — 20--oxido-5-pregnene.

Furthermore, on hydrogenation of compound (8) over platinum in glacial acetic acid the known 3β-, 18-, 20α-triacetoxy-5α-pregnane[5] (10) was obtained.

Vacuum sublimation of the triol (9) afforded back compound (2) by elimination of a water molecule. Such an unusual transformation in a thermal reaction is probably due to the proximity effect in the 1,4-diol system.

The nitrogen containing compound (4), $C_{21}H_{31}O_4N$, was obtained when the reaction was run in acetic acid. The same compound was obtained among the nitrous acid reaction products of 18 O-acetylholarrhimine perchlorate.[4] The compound did not show any peak corresponding to a hydroxyl or carbonyl group in the infrared spectrum but showed the presence of bands at 1654, 870 and 684 cm^{-1} (fig. 7, page 49) indicative of a nitrate ester grouping. One of the products of alkaline hydrolysis of compound (4) exhibited identical mobility with compound (2) on a paper chromatogram. These data support the structure (4) for compound (4).

Compound (3), $C_{21}H_{31}NO_3$, had an oxygen atom less than that in compound (4) but behaved in the same manner towards boron trifluoride acetic anhydride reagent and alkaline hydrolysis as compound (4) yielding the same triacetoxy compound (8) and compound (2) respectively. In the infrared spectrum there was no evidence of a hydroxyl or a carbonyl group. A distinctive infrared band at 1527 cm^{-1}

(fig. 9 , page 52) was assigned to the nitrite ester formation.

Another interesting compound (6), isomeric with (2) was isolated when the reaction was run with mineral acids particularly in presence of sulphuric acid. Unlike compound (2), compound (6) did not give any active hydrogen and was the least polar product from the nitrous acid reaction. The infrared spectrum indicated absence of any hydroxyl or carbonyl functions, but the presence of strong ether bands at 1096, 1062 cm^{-1}. (fig. 9 , page 52).

On treatment with borontrifluoride-acetic anhydride the triacetoxy compound (8) was obtained. One of the logical structures that fits in with the data would be (6). The mechanism of its formation and conversion to the triacetoxy compound (8) may be formulated as in (Scheme 1, page 53).

It may be noted that the stereochemistry at position 20 of the triacetate, (8), is the same as that in holarrhimine in all these transformations because of two successive inversions in steps 4 and 10.

The positive rotation of compound (6) is consistent with the 3β-, 5β-oxido structure. It may be mentioned in this connection that a 3α-, 5α-cholestane oxide prepared by Henbest[6] also had a strong positive rotation. The M_D value difference between compounds (6) and (2) was however different from that between 3α-, 5α-oxido cholestane and cholesterol.

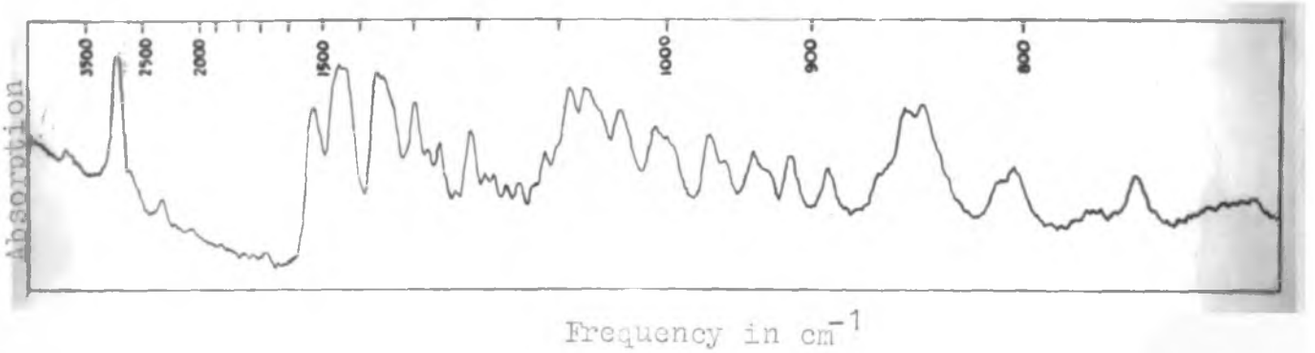


Fig. 8. Infrared spectrum of 3β-O-nitroso-18 — 20--oxido-5-pregnene.

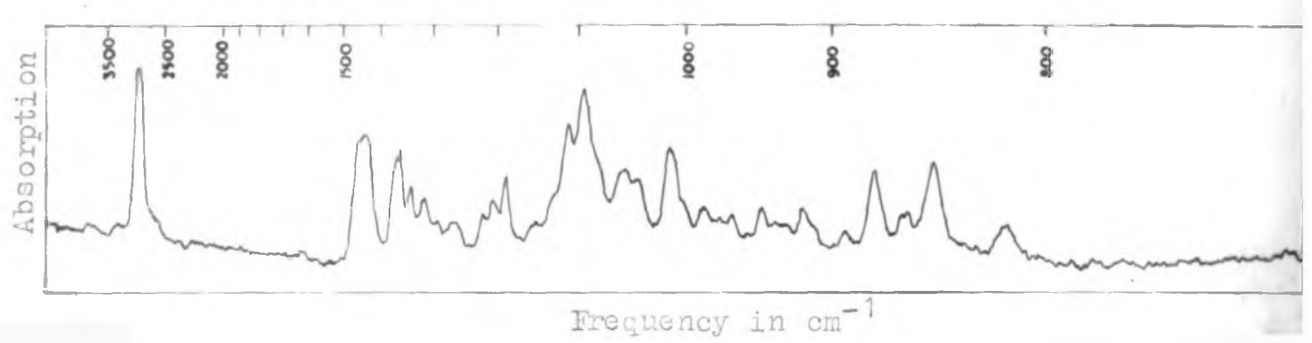
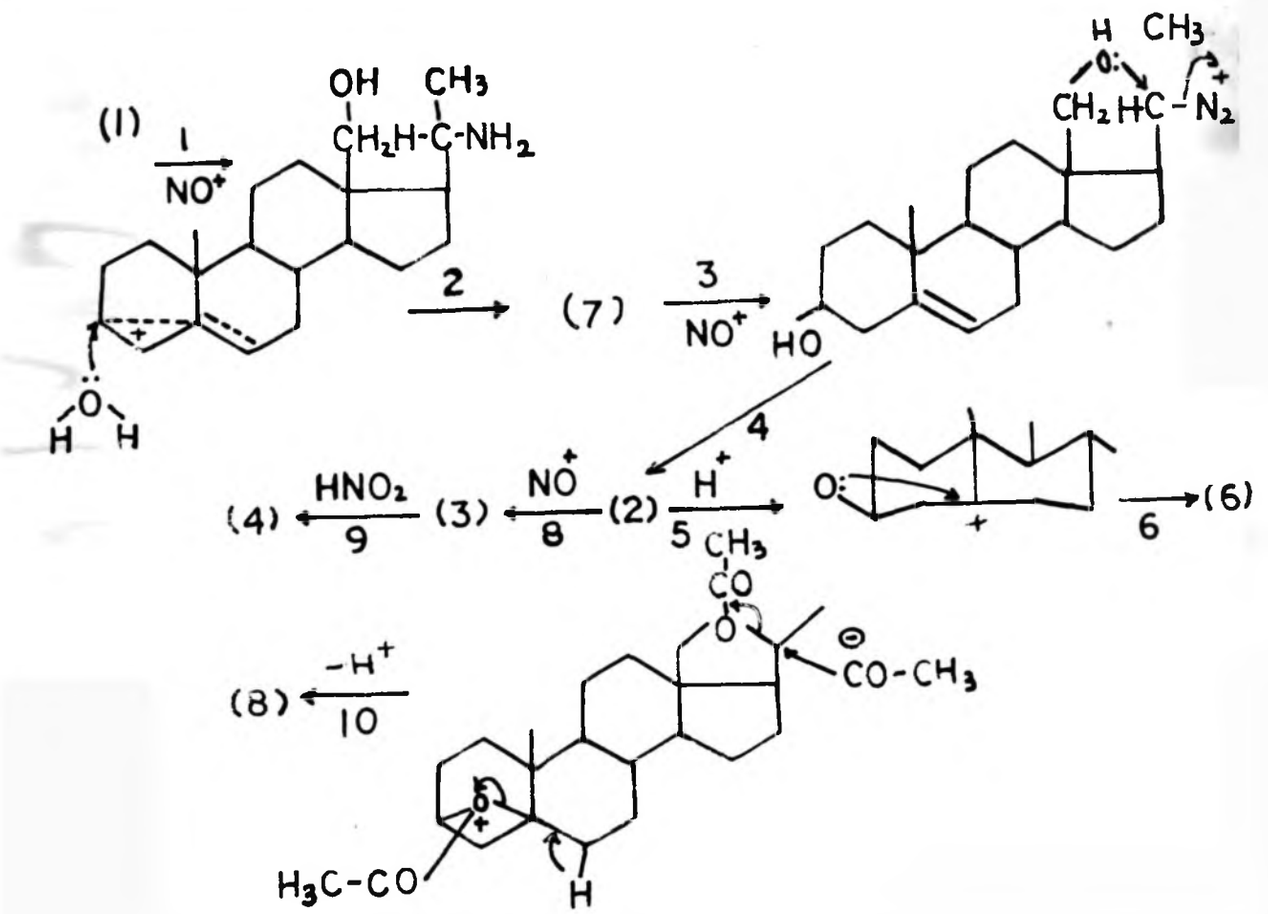


Fig. 9. Infrared spectrum of 3β-,5β-, 18-20β--dioxido pregnane.



There is, however, no known analogy of the steps 2 and 3 in 3β-hydroxy-Δ^5-steroids. No 3β-, 5β-oxido coprostane could be detected in the product from a model reaction run with cholesterol and nitrous acid in aqueous dioxane system.

The basic compound (7) was isolated in 5-10 per cent yield when the nitrous acid reaction was run at a lower temperature and under mild acid conditions.

It gave a precipitate with digitonin and was converted to a Δ^4, 3-keto steroid (λ_{max} 241 mμ) on Oppenauer oxidation. A treatment of compound (7) with nitrous acid afforded compound (2) in very good yields. The structure (7) could be assigned to compound (7) on the basis of these properties. Compound (7) showed similar physical data with 3β,18-dihydroxy-20α--amino-5-pregnene prepared by Labler et al.[7] (Fig. 9 , page 52).

No logical structure could be assigned to compound (5).

There is a difference in the relative reactivities of the two amino groups at 3β- and 20α-amino groups in holarrhimine. This is obvious by the isolation of the 20α-monoamino compound (7) as the intermediate from the nitrous acid reaction mixture. Even after the 3β-amino group has completely reacted with nitrous acid, the steroid remains in solution in the acidic medium until the 20α-amino group is deaminated finally resulting in an insoluble neutral product. The intermediate such as compound (7) is subject to further action by NO^+ cations resulting in the formation of a nitrite ester and a nitrate ester. The formation of a nitrite ester[8] in quite substantial yields has been observed in the action of nitrous acid on a water soluble amine as methylamine. Nitrate ester[9]

formation has also been observed in the case of diamination of n-butylamine with nitrous acid. In the case of cholesteryl amine, however, the replacement of the 3β-amino group by a hydroxyl group precipitates the resulting cholesterol out of the acidic solution and hence further attack on the hydroxyl group is prevented.

The course of nitrous acid reaction on holarrhimine may be depicted in the overall scheme presented in Scheme I. (page 53).

E_X_P_E_R_I_M_E_N_T_A_L

(A) Nitrous Acid Reaction in Acetic acid

Holarrhimine (2g) in aqueous acetic acid (50 ml, 50%) was treated with sodium nitrite (3g) in water (10 ml) with stirring. The reaction mixture was warmed on a water--bath for 5 min cooled and extracted with chloroform (3 x 30 ml). The combined chloroform layer was thoroughly washed with water, dried over anhydrous sodium sulphate and evaporated under vacuum. The dried neutral gummy product (1.9 g) still contained ca. 2% nitrogen which could not be removed by a further nitrous acid treatment.

(1) Isolation of compound (2)

The product was subjected to a 100-transfer Craig distribution in a solvent system consisting of hexane, ethyl acetate, ethanol and water (2:1:2:1).

The tubes 60-69 yielded a crystalline fraction (0.125 g) which on crystallization from dilute acetone gave the pure product, precipitable from an alcoholic solution as a digitonide. The same substance was also obtained when the nitrous acid reaction was run in mineral acids.

It gave the following physical constants : m.p. 165-166°; $[\alpha]_D$ - 44 (C, 2.02%). [Found : C, 80.21; H, 10.07; 10.19; Active hydrogen 0.36. $C_{21}H_{32}O_2$ requires : C, 79.90; H, 10.19; Active hydrogen 0.32%]. Infrared

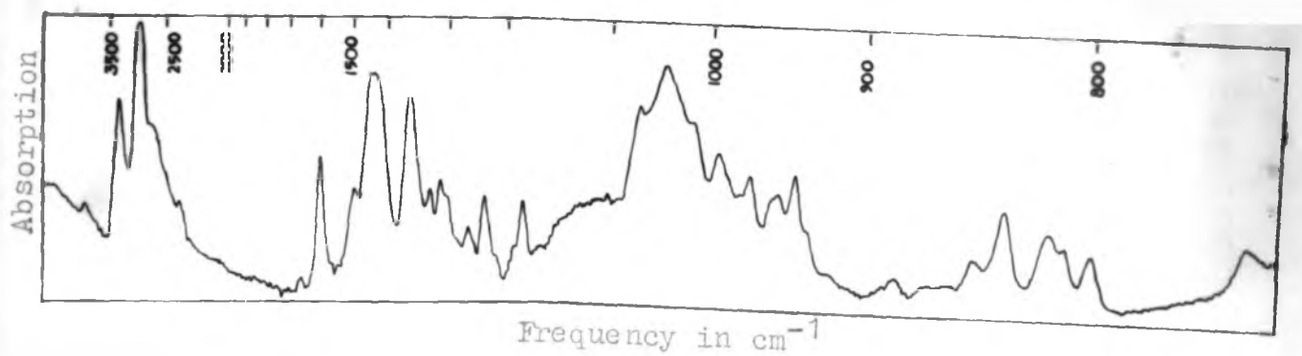


Fig.10. Infrared spectrum of 3β-,18-dihydroxy--20α-amino-5-pregnene.

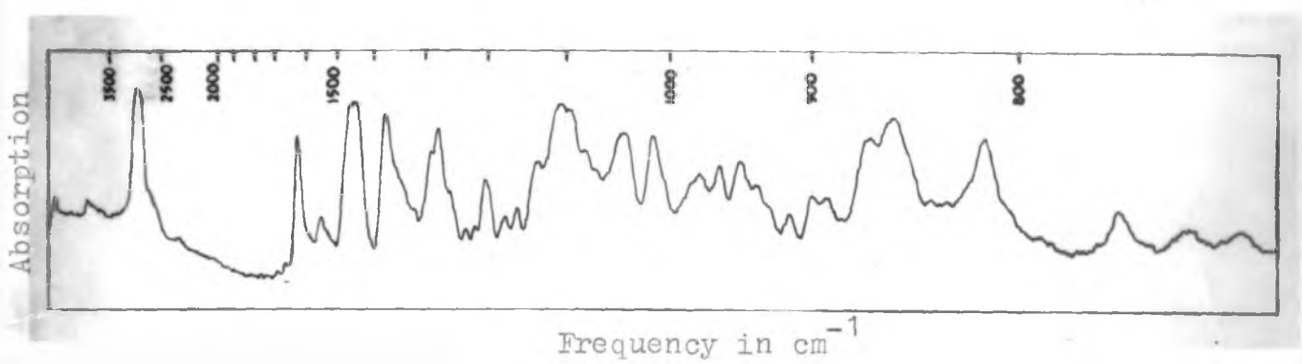


Fig.11. Infrared spectrum of compound (5) $C_{21}H_{32}O_3$

bands at 3390, 1062, 840 (cm^{-1}). (Fig. 4 , page 47).

(2) Isolation of Compound (4)

The non-polar fractions (92-100) from the Craig distribution were combined. The solvent was evaporated off, the residue (0.940 g) was dissolved in a small quantity of benzene and loaded on to an activated alumina (Grade II, 30 g) column. The substance (0.35 g) which was eluted with benzene was crystallized from methanol. It had the following constants : m.p. 133°; $[\alpha]_D$ - 49 (C, 1.4%). [Found : C, 69.1; H, 8.8; N, 3.2; Active hydrogen, nil. $C_{21}H_{31}O_4N$ requires : C, 69.8 ; H, 8.6 ; N, 3.85%]. Infrared bands at 1654, 1628, 856 (cm^{-1}). (Fig. 7 , page 49).

The substance gave a positive Liebermann and antimony trichloride reactions. Hydrolysis of compound (4) with alcoholic potassium hydroxide gave three compounds, one of which had the same mobility on a papergram as that of compound(2).

Compound (4) was also obtained in slightly lower yields when 18 O-acetyl holarrhimine was treated with nitrous acid in a similar way.

Other products of the nitrous acid reaction on holarrhimine in acetic acid were obtained as gums and could not be resolved further into crystalline components.

(B) Action of nitrous acid on holarrhimine in dil hydrochloric acid

Holarrhimine (1.4 g) was dissolved in dil hydrochloric acid (50 ml, 0.2N) and cooled to 0-5° and treated with sodium nitrite (1.5 g) in water (4 ml) with stirring. The reaction was allowed to proceed at 5° for 10 min and then at 10-15° for another 10 min. The neutral product was extracted with chloroform (3 x 25 ml) - Extract A.

The aqueous acidic layer was made alkaline with sodium hydroxide (6 ml, 2N) and re-extracted with chloroform (3 x 25 ml) - Extract B.

(1) Isolation of compound (7)

The extract B was washed with water (3 x 10 ml), dried over sodium sulphate and evaporated to dryness. The residue was taken up in alcohol (15 ml) and carefully neutralized with dil sulphuric acid when unreacted holarrhimine separated out as the insoluble sulphate. It was filtered and washed with little alcohol.

The mother-liquor was diluted with water (50 ml), made alkaline with sodium hydroxide (0.5N) and extracted with chloroform. The chloroform extract yielded a gummy product which was crystallized from ethanol to yield the monobasic compound (7) (50 mg). It had the following properties : m.p. 225°; lit.[7] 232-233°. (Found : C, 75.4; H, 10.6; N, 4.0.

$C_{21}H_{35}NO_2$ requires : C, 75.63; H, 10.58; N, 4.2%].

The compound gave up its entire content of nitrogen in Van Slyke primary amino group determination. It was digitonin precipitable. On Oppenauer oxidation it gave a gummy substance which showed a band in ultraviolet spectrum at 241 mμ characteristic of a Δ^4, 3-keto steroid. On further treatment with nitrous acid it gave compound (2) in 80% yield.

(C) Action of nitrous acid in hydrochloric acid on holarrhimine sulphate

Holarrhimine (5 g) in ethanol (50 ml) was converted into its insoluble sulphate by neutralization with sulphuric acid (4N). The insoluble sulphate was filtered and suspended in water (250 ml) and brought into solution by addition of dil hydrochloric acid (220 ml, 1N). The solution was treated at 20° with a cold solution of sodium nitrite (30 g) in water (100 ml) with stirring during a period of 5 min and allowed to stand at room temp for 2 hr. The clear mother-liquor was decanted off from the semi-solid precipitate, allowed to deposit overnight in the refrigerator some more insoluble material and decanted off. The combined residue was washed with water and extracted in chloroform (50 ml). The chloroform extract was washed with dil hydrochloric acid, then thoroughly with water, dried over sodium sulphate and concentrated in vacuo. The gummy product (4.8 g) was separated into polar and non-polar

components by distributing between hexane and 80% aq. ethanol. The hexane layer yielded the non-polar fraction (3.45 g) and the alcohol layer the polar fraction (1.25 g).

(1) Isolation of Compounds (3) and (5)

The non-polar fraction (3.45 g) dissolved in methanol (15 ml) was subjected to partition chromatography over magnesium oxide (85 g) which was equilibrated with hexane phase from a mixed solvent system consisting of hexane, methanol and water (5:4:1). The column was eluted successively with (a) hexane (200 ml) from the mixed solvent system as described above, (b) hexane (100 ml) containing 20% ethanol, and (c) hexane (100 ml) containing 50% ethanol. The eluate was collected in 5 ml portion at the rate of 5 ml in 3 min. From the 60th fraction the alcoholic phase started separating out.

The first three fractions crystallized from ethanol gave the crystalline product, (5) (156 mg) m.p. 95-105°. On repeated recrystallizations the pure compound was obtained. It showed the following properties : m.p. 107-108°, $[\alpha]_D$ - 47°, [Found : C, 75.16; H, 9.54; Active hydrogen, nil. $C_{21}H_{32}O_3$ requires : C, 75.86; H, 9.7%).

The infrared curve did not show any band due to hydroxyl or carbonyl absorption but showed bands at 1618, 1271, 1104, 1039, 1013, 854, 813 cm^{-1} (Fig.11 , page 57).

The mother-liquor from the Compound (5) contained compound (6) as an impurity.

Crystallization of eluate fractions 4-8 (700 mg) from acetone yielded small quantities of a substance (22 mg, m.p. 165-175°). The mother-liquor (0.677) was rechromatographed on magnesium oxide (20 g) in a similar way as described above. The major component from the eluate fraction was recrystallized from methanol to yield compound (3). It showed the following properties : m.p. 149-150°, $[\alpha]_D$ - 44°. [Found : C, 73.31; H, 10.07; N, 3.5. $C_{21}H_{31}O_3N$ requires : C, 73.00; H, 9.05; N, 4.05%]. Infrared bands at 1527, 1296, 850 cm^{-1}. On hydrolysis with alcoholic potassium hydroxide it gave a mixture of three products, one of which had identical mobility on a papergram with compound (2).

(2) Isolation of compound (2)

Following fraction 10 a substance was obtained spread over 40 eluate fractions. On crystallization from acetone these fractions yielded compound (2) (0.3 g).

(C) Characterization of compound (2)

Acetylation : Compound (2) (50 mg) in pyridine (1.5 ml) was treated with acetic anhydride (0.25 ml). After standing overnight acetic anhydride and pyridine were evaporate off under vacuum and the product was recrystallized from methanol. It had the following properties : m.p. 134°,

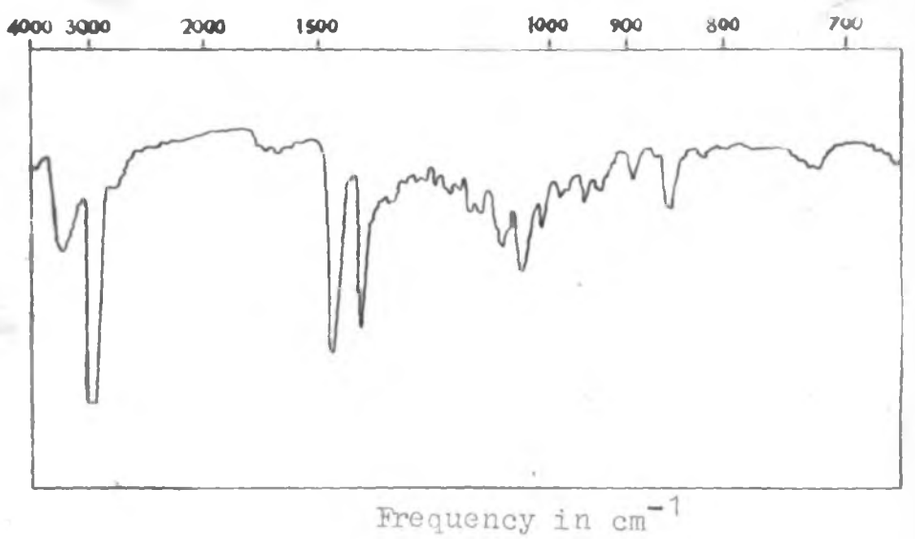


Fig.12. Infrared spectrum of 3β-hydroxy-18 — 20--oxido-5α-pregnane.

$[\alpha]_D$ - 59°. [Found : C, 77.11; H, 8.97; Acetyl, 11.3, $C_{23}H_{34}O_3$ requires : C, 77.05; H, 9.56; Acetyl, 12.0%). Infrared bands at 1735 and 1246 cm^{-1}. (Fig. , page).

Oppenauer oxidation

Compound (2) (30 mg) was taken in acetone (0.22 ml) and benzene (0.3 ml) and refluxed with 25 mg aluminium tertiary butoxide under anhydrous conditions for 9 hr. The reaction mixture was cooled, diluted with water (2 ml), shaken with dilute sulphuric acid (5 ml, 4N) and extracted with benzene. The benzene extract was washed with water, dehydrated over sodium sulphate and the solvent evaporated off to give a gummy product. On chromatography over neutral activated alumina (Gr. II, 1 g) the crude product yielded a crystalline fraction (6 mg). Its ultraviolet spectrum showed a band at 241 mμ with log ϵ_{max} = 4.21 and the infrared spectrum showed bands at 1678 and 1608 characteristic of a Δ^4, 3-oxo-steroid. (Fig. 5 , page 47).

Hydrogenation

Compound (2) (30 mg) was hydrogenated in glacial acetic acid (5 ml) with platinum oxide catalyst. It absorbed one mole of hydrogen in about 2 hr. The mixture was centrifuged to remove the catalyst and the supernatant was decanted, diluted with water and extracted with ether. The ether extract after washing with sodium bicarbonate solution and

water and drying over sodium sulphate yielded a crystalline solid. It was recrystallized from acetone (6 mg). m.p. 143°, lit.[3] 137-138°, $[\alpha]_D$ - 4°, lit.[3] + 3°.

3β,18,20α-Triacetoxy-5-pregnene (8)

Monoacetate of compound (2) (50 mg) was treated with acetic anhydride (1.5 ml) and boron trifluoride ether complex (freshly distilled, 12 drops) at room temp. After 2 hr the mixture was poured into water. The reaction product was extracted with ether and the ether extract after washing with aqueous sodium bicarbonate and water, was dried over sodium sulphate. The residue (60 mg) obtained after evaporation of the solvent, was chromatographed on alumina (neutral, grade II, 500 mg). The fraction which was eluted with pet. ether benzene 1:1, crystallized out (35 mg). It was recrystallized from methanol. m.p. 154°, $[\alpha]_D$ - 4°. [Found : C, 70.2; H, 8.0; $COCH_3$, 29.77. $C_{27}H_{40}O_6$ requires : C, 70.4; H, 8.75; $COCH_3$, 28.1%]. Compound (2) also was converted directly to the triacetate without preliminary acetylation at position 3 using the above procedure, but the yields were somewhat poorer.

Similarly compounds (3), (5), (6) on treatment with boron trifluoride-acetic anhydride according to the above procedure gave the same 3β,18,20α-triacetoxy compound (8) in 50-60% yields. The identity was established by infrared spectra, rotation and mixed m.p. determination.

3β, 18, 20α-Trihydroxy-5-pregnene (9)

The triacetoxy derivative (8.40 mg) was saponified with alcoholic potassium hydroxide solution (1 ml, 1N) at room temp for overnight. The resulting substance which separated out on addition of water, was filtered, and recrystallized from a mixture of ethanol and acetone (25 mg). m.p. 215-225°, $[\alpha]_D$ - 14° (ethanol). [Found : C, 76.81; H, 10.09. $C_{21}H_{34}O_3$ requires : C, 75.4; H, 10.25%]. It gave positive tetranitromethane and digitonin precipitation tests. On Oppenauer oxidation it gave a gummy compound which showed an ultraviolet band at 241 mμ (ϵ, 10, 130).

It was observed that in the drying procedure for the preparation of an analytical sample of the trihydroxy compound (9), there was a loss in weight and depression in melting point. This behaviour may account for a higher carbon value in the above analysis.

Compound (2) from 3β, 18, 20α-trihydroxy-5-pregnene (9)

Sublimation of the triol, (9) (10 mg), under vacuum resulted in the formation of compound (2). The sublimed compound was found identical with compound (2) by paper chromatography and a comparative infrared spectra.

Chromic acid oxidation of triol (9)

The triol (9) (25 mg) was oxidized with chromium trioxide (20 mg) in acetic acid (5.0 ml) according to the

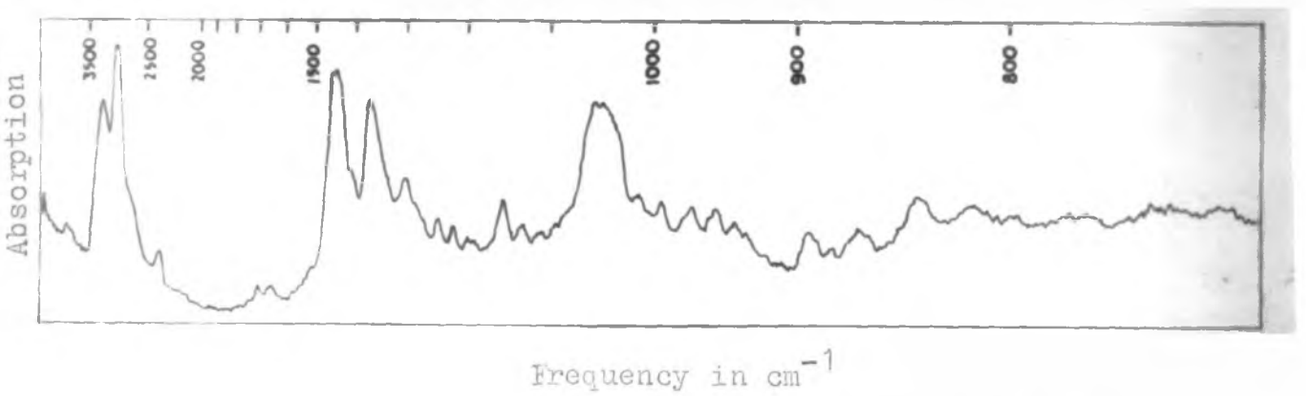


Fig.13. Infrared spectrum of 3β-,18-,20α-trihydroxy--5-pregnene..

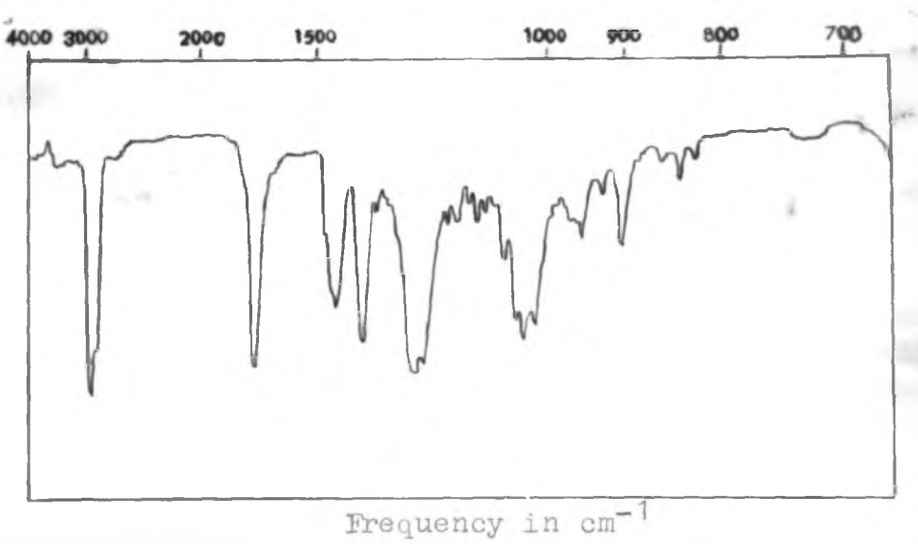


Fig.14. Infrared spectrum of 3β-,18-,20α-triacetoxy -5α-pregnane.

procedure of Kambler et al.[5] After an overnight reaction the excess chromic acid was decomposed by addition of methanol (2 drops) and the mixture was poured into water (25 ml) and extracted with ether (3 x 15 ml). The ether extract was washed with water, dried over sodium sulphate and evaporated to dryness. The gummy residue (19.5 mg) was chromatographed on activated alumina (neutral, grade II, 0.5 g). A crystalline compound (2 mg) was obtained with chloroform. It gave infra-red bands at 1754 (Lactone) 1671, 1604 (α,β-unsaturated ketone) similar to those of 20-hydroxy-3-oxo-4-pregnene-18-oic acid, (18 → 20 lactone).

3β, 18, 20α-Triacetoxy-5α-pregnane (10)

The triacetoxy compound (8) (70 mg) was hydrogenated in acetic acid (5 ml) with platinum oxide catalyst (10 mg). One mole of hydrogen was absorbed in two hr. The catalyst was removed by centrifugation and the supernatant diluted with water (50 ml), and extracted with chloroform (3 x 20 ml). The chloroform extract was washed with sodium bicarbonate and then with water. After drying with sodium sulphate it was evaporated to dryness when the reaction product (65 mg) was obtained. It was repeatedly crystallized from methanol to yield the pure saturated product (26 mg). m.p. 111° ; lit.[5] 105-106°; $[\alpha]_D$ + 24; lit. + 23°.

Isolation of compound (6)

The nitrous acid reaction in mineral acid on

holarrhimine sulphate, was repeated with 10 g quantity of holarrhimine. The product extracted by the usual procedure was separated by a four transfer distribution in 95% methanol and hexane (60 ml each phase each transfer). The non-polar fractions (5.76 g) were chromatographed on a partition column of magnesium oxide (130 g) in the manner described already. The first three fractions (10 ml each) gave a new compound (0.780 g) which on recrystallization with methanol gave compound (6) which was isomeric with compound (2). m.p. 87°; $[\alpha]_D$ + 14°. [Found : C, 79.95; H, 10.1; Active hydrogen, nil. $C_{21}H_{32}O_2$ requires : C, 79.70; H, 10.19%]. Tetranitromethane test:- negative.

It was found to be resistant to mineral acids and lithium aluminium hydride. Chromic acid oxidation yielded a mixture of several products which could not be separated.

On treatment with boron trifluoride and acetic acid in the manner described earlier a yield of 50% of 3β, 18-, 20α--triacetoxy-5-pregnene (8) was obtained and the compound was found identical with that obtained from compounds (2), (3) or (5).

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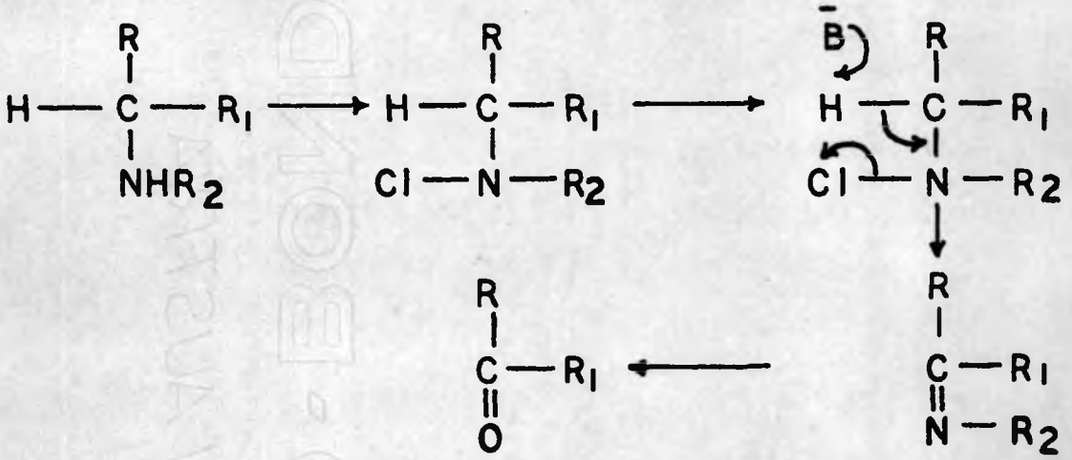
CHAPTER V

SYNTHESIS OF 18-HYDROXYPROGESTERONE AND 11α-, 18-DIHYDROXYPROGESTERONE

One of the more convenient methods of oxidative deamination of a primary or a secondary amino group to a carbonyl compound comprises of replacing one of the hydrogen atom on the amino nitrogen with a halogen. On treatment with a base, dehydrohalogenation occurs involving the halogen atom and a hydrogen atom on the carbon atom α-to the amino group resulting in the formation of an aldimine or ketimine. On subsequent hydrolysis the corresponding oxo compound is obtained. The yields by this method are generally excellent.



The first step, N-chlorination can be accomplished by various reagents such as hypochlorous acid, chloramine T, t-butyl hypochlorite, N-chlorosuccinimide etc.

Hypochlorous acid was successfully used with a steroid in the N-chlorination of cholesterylamine in a German patent in 1937[1]. It was later shown that this method was also effective in the oxidative removal of an amino group

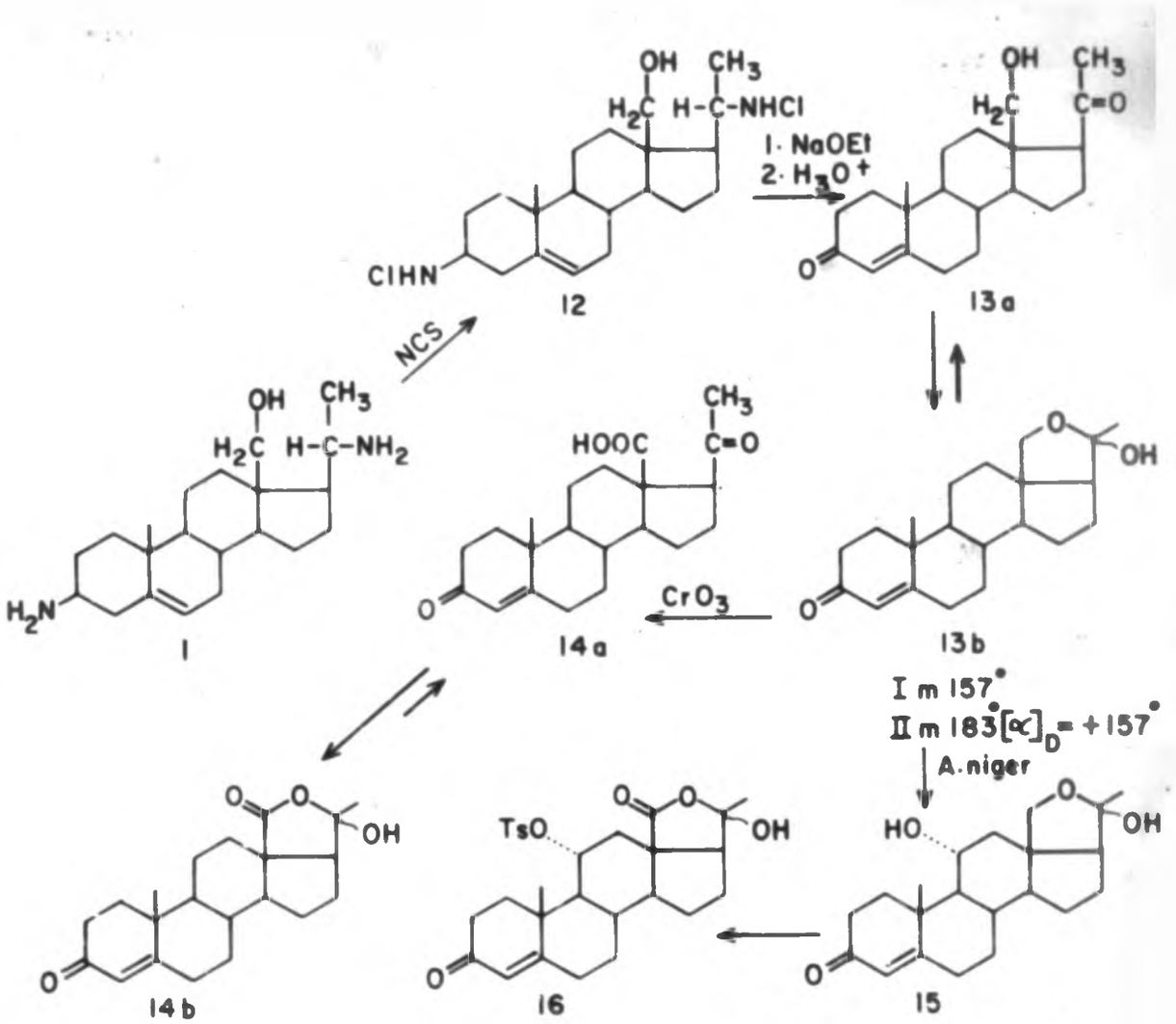
in the side chain position 20 to yield 20-oxo steroids[2].

Bachmann et al.[3] modified the method and used tert. butyl ester of hypochlorous acid to minimize the side reactions. This reagent had the advantage of being stable for several days. From the stand point of stability and convenience the best reagent in this class is N-chloro-succinimide[4], which is a crystalline solid stable for a few months at room temperature.

Simultaneous deamination of both the amino groups of holarrhimine presented some difficulty at the beginning, the reactivities of both the amino groups being not of the same order. A local excess of N-chlorosuccinimide resulted in the formation a trichloro derivative along with the expected N-N' dichloroderivative. This introduced compli-cations in the subsequent dehydrochlorination reaction. After a few trial and error runs the best condition for obtaining high yields of N-N' dichloroderivative was worked out. It is important to maintain a high rate of agitation while adding N-chlorosuccinimide in methylene chloride solution at a uniform rate.

The di-N-chloro derivative (12) 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

CHART I


OH CH3
H2C H-C-NHCl
ClHN
12
1. NaOEt
2. H3O+
OH CH3
H2C C=O
O
13a
NCS
OH CH3
H2C H-C-NH2
H2N
1
CH3
HOOC C=O
O
14a
CrO3
O
OH
13b
I m 157°
II m 183° [α]D = +157°
A. niger
O O
OH
O
14b
TsO
O O
OH
O
16
HO
O O
OH
O
15

methoxide and ethoxide on the other hand gave satisfactory results, provided the alcohols employed 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.

Hydrolysis with dilute sulphuric acid was accomplished at water bath temperature in two hours. The basic ketimine went rapidly in solution and the neutral product, 18-hydroxyprogesterone (13a or 13b) precipitated out as a brownish yellow gum.

In early experiments the crystalline 18-hydroxy progesterone (13) was isolated after chromatography over alumina in two forms - one melting at 157° (Form I) and other at 182° (Form II). Both forms had identical optical rotation and superimposable infrared spectra which did not indicate the presence of a free carbonyl group at position 20. Moreover, attempts at acetylation or tosylation of the 18 hydroxyl group did not succeed. It was presumed, therefore, that Form I and Form II were 20 epimeric 18 ⟶ 20 hemiketals as their chromatographic behaviour indicated a difference in their polarities. Furthermore, on crystallization from aqueous acetone the higher melting form, II, gave the lower melting form. Analogous hemiketal formation has been observed in most of the 18-hydroxy-20-keto compounds known so far and such behaviour was first reported for 17α,18-dihydroxy-3,11,20--triketo-4-pregnene by Wieland *et al*.[5]

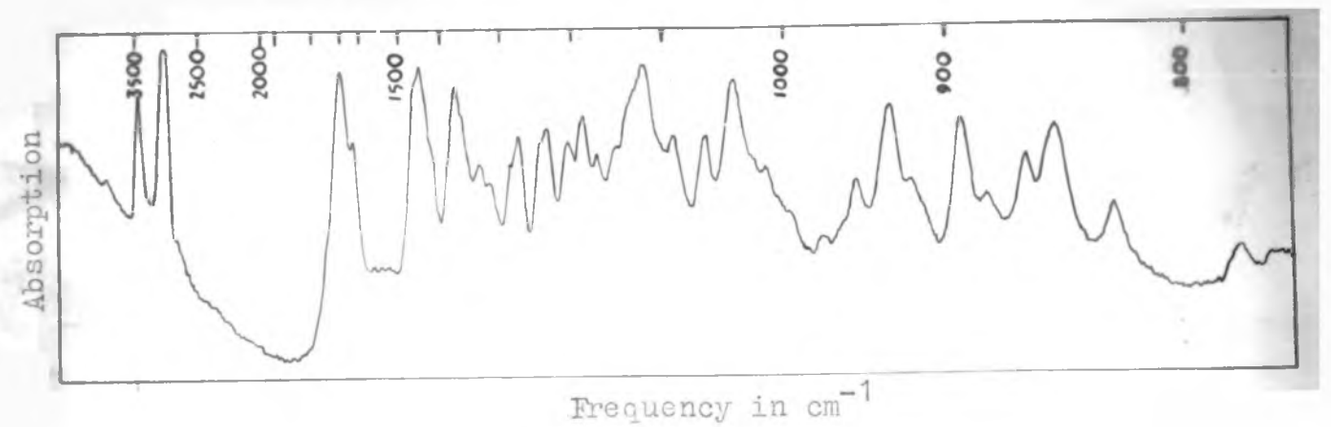


Fig.15. Infrared spectrum of 18-hydroxy-progesterone.

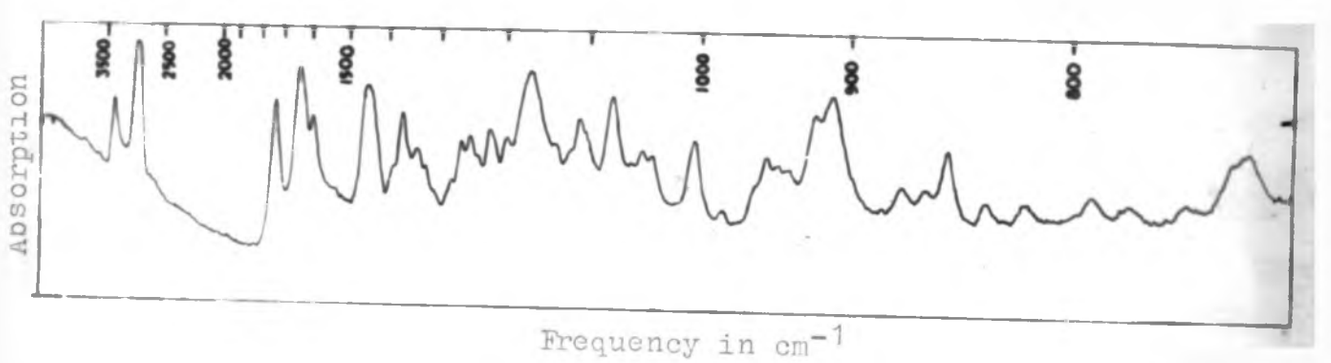


Fig.16. Infrared spectrum of 3-,20-diketo-18-carboxy--4-pregnene (18 — 20 lactol).

The identity of compound (13) was established by a comparison of its physico-chemical behaviour, mixed melting point and comparative infrared spectra with 18-hydroxy--progesterone synthesized from conessine according to the procedure of Jeger and coworkers[6].

In later experiments compound (13) could be obtained in a single stage reaction from holarrhimine in 45-50% yield by direct crystallization of the acid hydrolysis product from 2-butanone. It has been mentioned earlier that Labler and Sorm[7] synthesized 18-hydroxy progesterone from holarrhimine in five stages. It has also been obtained by Pappo[8] from conessine in 10 stages and by the homolytic degradation of cortexone acetate by Jeger and coworkers[9].

Progesterone 18-carboxylic acid (14a or b)

On oxidation with chromium trioxide in acetic acid at room temperature 18-hydroxyprogesterone (13) was converted to progesterone-18-oic acid (14)in 30% yield and probably to the neutral 18-aldehyde. Attempted purification of the aldehyde through alumina column converted it to a product m.p. 204°. Characterization of this product was discontinued, since at this time it was reported by Pappo[10] that the product was an internal condensation product of the aldehyde.

The crystalline acidic product (14) was found to be insoluble in aqueous bicarbonate. It did not exhibit

any infrared bands characteristic of a free carboxylic acid (14a)(absence of bands at 2700 and 1700 cm^{-1}) but of a 5 membered lactol. (14a) (1744, 3340 cm^{-1}). This compound was also prepared by Pappo[8] by chromic acid oxidation of 18-hydroxy progesterone.

Attempted microbiological transformations of progesterone 18-carboxylic acid :

Progesterone 18-carboxylic acid (14) was fermented with strains of Cunninghamella blackesleeanas and A.niger known to introduce 11β-or 11α-groups in progesterone respectively. C.blackesleeanas was used with a view to getting the 11→18 lactone (16a) directly. Compound (14) was recovered virtually unchanged from acidic components of the fermentation mixture in both these fermentations. Very little neutral compounds were detected in these experiments. Further attempts, were therefore, abandoned.

Hydroxylation of 18-hydroxy progesterone :

18-hydroxyprogesterone (13) on the other hand was converted in 25% yield to a dihydroxy compound (15a, R = H) which could be separated from the unreacted starting material by repeated column chromatography over alumina. The assignment of 11α-18-dihydroxy progesterone structure to compound (15a) was made on the following grounds :

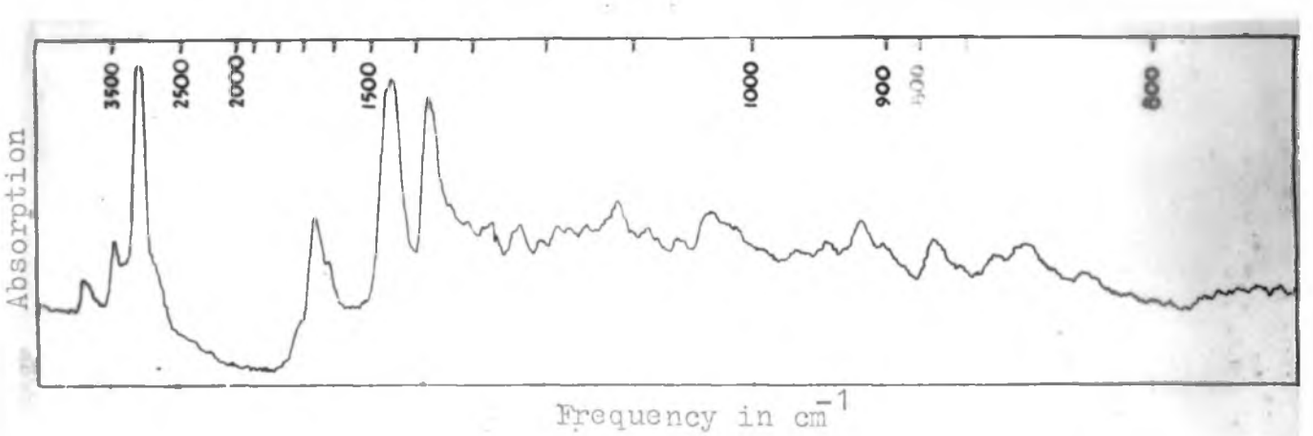


Fig.17. Infrared spectrum of 11α,18-dihydroxyprogesterone.

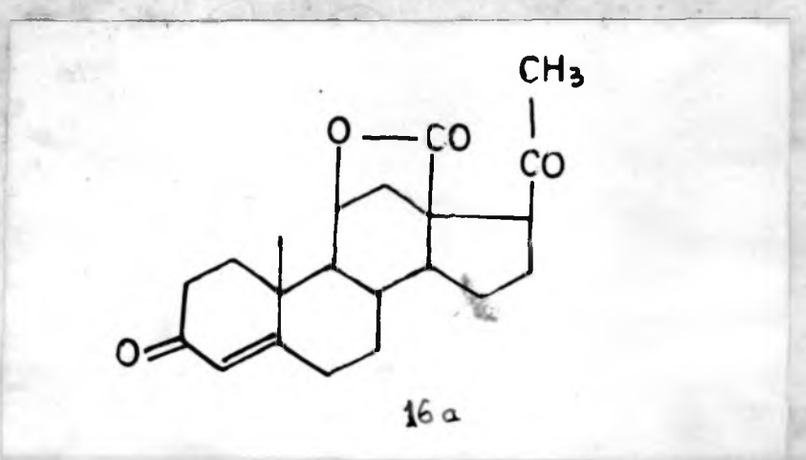
(1) The strain of A.niger used is known to hydroxylate Δ^4 3-keto-pregnenes at positions 11α or 6β. The ultraviolet spectrum of the compound (15a) did not exhibit any hypsochromic shift characteristic of a 6β-hydroxy, Δ^4 3-keto steroid.

(2) The molecular rotation difference between 18--hydroxyprogesterone and compound (15a) was of the same order as that between progesterone and 11α-hydroxyprogesterone. From the molecular rotation data[11] both 7α-and 7β-hydroxy structures could also be excluded.

(3) The difference in mobility between compound (14) and 18-hydroxy progesterone on a paper chromatogram was of the same order expected between a steroid and its 11α-hydroxy derivative.

Attempted synthesis of aldosterone :

Compound (15a) gave a tosylate (14b, R = Ts) when treated with p-toluene sulfonyl chloride in pyridine. It was anticipated, that oxidation of the 18-hydroxy group in the tosylate (15b) to a carboxyl group by chromic acid oxidation followed by the treatment of the 11α-tosyloxy--progesterone 18-carboxylic acid (16) by mild base would lead to the formation of lactone (16a) by an intramolecular S_N2' displacement of the tosylate by the carboxylate.



16a

The tosylate was oxidized by chromic acid and the acidic fraction without purification was refluxed with (a) methanolic potassium bicarbonate and (b) methanolic potassium carbonate. Neither of these procedures yielded any significant amount of neutral lactone.

Further work in this direction had to be discontinued due to lack of material.

It may be mentioned here that after this work was completed an intramolecular tosylate displacement of the type mentioned above has been demonstrated by Wettstein and coworkers[12].

E_X_P_E_R_I_M_E_N_T_A_L

N-N'-dichloroholarrhimine (12)

Holarrhimine (1.1 g) was dissolved in 16 ml methylene chloride. A solution (8%) of N-chlorosuccinimide in methylene chloride was added dropwise avoiding local excess to the above solution with vigorous stirring. The end point was followed with a bromo-thymol-blue indicator paper which indicated neutralization of the basic functions. The total amount of the solution required was 10.4 ml.

The reaction mixture was washed four times with water (10 ml each). The aqueous washings were tested with starch iodide indicator paper to ensure removal of the water soluble N-chlorosuccinimide and of course, succinimide. The solution was dried with sodium sulphate and concentrated to dryness under vacuum keeping the bath temp below 40°. The white crystalline product (1.15 g or 84%) decomposed at 130°. [Found : Cl, 17.16; required for $C_{21}H_{34}ON_2Cl_2$: Cl, 17.7].

18-Hydroxy-3,20-dioxo-4-pregnene (18 — 20 hemiketal) (18 hydroxy progesterone) (13)

N,N'-dichloroholarrhimine (1.09) was dissolved in absolute methanol (10 ml) and refluxed for one hour with a solution of sodium methoxide (prepared by dissolving 1.1 g sodium in 40 ml absolute methanol). The completion of the reaction was indicated by a negative test with acidic starch iodide indicator paper.

The solution was concentrated under vacuum and the steroid was extracted with chloroform (100 ml) and washed with water. The extract was dried over sodium sulphate and concentrated to dryness. Attempts to crystallize the pale yellow residue (0.89) did not succeed.

The residue was dissolved in ethanol (80 ml) and refluxed with dil sulphuric acid (2N, 80 ml), for 1 hr. Alcohol was evaporated off and the steroid was extracted in chloroform (3 x 40 ml). The chloroform layer was washed with water, dried over sodium sulphate and evaporated to dryness when a yellowish brown gum (0.75 g) was obtained. The crude compound (13) showed a band in ultraviolet at 240 mμ , ϵ_{max} = 11,000.

Purification of 18-hydroxy progesterone by chromatography

The gum (0.75 g) was dissolved in benzene (5 ml) and loaded on to 22 g of activated neutral alumina (Gr.II) and developed with the sequence of solvents indicated in Table I. The column was run as follows :

Table I

Fr. No.	Eluent	Wt. of the fraction (mg)	Remark
1	Benzene	0.0	
2	"	11.1	Crystalline
3	"	13.0	"
4	"	13.5	"
5	"	55.0	"
6	Benzene : CH_2Cl_2 (9:1)	32.0	"
7	"	25.0	Gummy
8	"	18.0	"
9	CH_2Cl_2	10.5	"
10	"	9.2	"
11	"	04.7	"
12	CH_2Cl_2: Methanol (99:1)	60.0	"
13	"	78.0	Semicrystalline
14	"	105.0	"
15	"	65.8	"
16	"	42.1	"
17	CH_2Cl_2: Methanol (19:1)	48.0	Gummy
18	" (1:1)	98.0	"
19	" (1:1)		"
	Total :	688.9	

Fractions 2 to 6 (124.6 mg) were pooled together and recrystallized twice from aqueous acetone to yield 90 mg of material (m.p.159-160°). Fractions 12 to 16 (342.9 mg) were pooled

and crystallized thrice from aqueous acetone. The first crystallization took about one week and yielded a product with m.p.182° (Form II). It showed no depression in the mixed melting point with the non-polar (Form I). The infrared spectra, analyses and rotations of both the forms were identical. Subsequent crystallizations of this product removed the yellowish tinge in the crystals and reduced the melting point to 162° (Form I, yield 200 mg). The total yield of pure 18-hydroxy-progesterone was thus 290 mg (38%).

In a subsequent run the residue was not chromatographed but dissolved in butanone and kept for 10 days when crystals were formed. Recrystallization of this material gave 18-hydroxy progesterone in 45% yield based on holarrhimine.

It had the following constants. m.p.162°; lit. 159[6], 172-182[8]; $[\alpha]_D$ + 159° (C, 2.0); λ_{max} 241, log ϵ_{max} 4.21 . [Found : C, 76.45; H, 9.08. $C_{21}H_{30}O_3$ requires : C, 76.3; H, 9.09].

Attempts to prepare acetyl or p-toluene sulfonyl derivatives did not succeed.

Chromic acid oxidation of 18-hydroxy progesterone :

To a solution of 18-hydroxy progesterone (530 mg) in glacial acetic acid a solution of chromic acid (22 ml, 1%

in 98 acetic acid) was added dropwise, during a period of 30 minutes. It was kept overnight at room temperature. Methanol (2 ml) was added to destroy the excess chromic acid. The mixture was then poured into cold water (100 ml) and the steroid extracted in chloroform (3 x 30 ml). The chloroform solution was washed with water (2 x 50 ml). The water washings were extracted with a little chloroform (10 ml) and the solvent extract was added to the main extract. The combined chloroform layer was extracted with sodium carbonate (5%) washed with water, dried over sodium sulphate and evaporated <u>in vacuo</u> to yield a gummy product (290 mg), the infrared spectrum of which indicated that it is a mixture of unreacted compound (13) and an aldehyde. (ν_{max} at 3300, 2670 cm^{-1} and 1720 cm^{-1}) Chromatography of the product yielded a crystalline solid m.p. 204-206°, lit.[10] 225-229°.

The sodium carbonate extract was acidified with sulphuric acid (2N) and extracted with chloroform (3 x 20 ml). The chloroform layer was washed with water (3 x 25 ml), dried over sodium sulphate and concentrated to dryness (228 mg). The residue was crystallized from aqueous-acetone (150 mg). It had the following constants : m.p.226-228°; lit.[8]; $[\alpha]_D$ + 115°(C, 0.75); λ_{max} 241, $\log\epsilon_{max}$ 4.21. [Found : C, 73.36; H, 8.45. $C_{21}H_{28}O_4$ required : C, 73.2; H, 8.14].

<u>Fermentation of 18-hydroxy-progesterone</u>

A growth medium (4 l.) of Czapek-Dox (consisting of glucose - 40 g; potassium chloride 0.50 g; magnesium sulphate (anhydrous) 0.25 g; potassium dihydrogen phosphate 1.0 g;

sodium nitrate 2.0 g; ferrous sulphate 0.01 g; yeast extract 0.50 g; corn-steep liquor 5.0 g per l. (distilled water) pH 4.8) in a 8.1. Chain fermentor (previously sterilized at 20 lbs for 1 hour) equipped with a paddle and baffles for stirring, was sterilized at 15 lbs for 45 minutes, and cooled to room temperature. A.niger (612 N.C.I.M. of this laboratory) spores from a week old slant culture on potato dextrose agar were suspended in sterile water and inoculated through an inoculating porthole under aseptic conditions. The incubation temperature was 28° and sterile air was introduced through a tube extending below the surface at a moderate rate (0.3 vol/vol./min) while stirring the medium at 220 r.p.m.

After 24 hr growth period the substrate, 18-hydroxy progesterone, (1.1 g) in acetone (50 ml) was added to the culture. The agitation was continued for a further period of 24 hr.

After this period the fermentation was stopped and the mycelium was separated from the broth by filtering through a muslin cloth. The mycelium was acetonized and the acetone extract was added to the broth. The mycelium and the broth were extracted separately with methylene chloride (3 x 100 ml and 3 x 300 ml) respectively. Both the extracts were pooled together, washed with sodium carbonate solution (3 x 100 ml, 5%), distilled water, (3 x 100 ml) dried over sodium sulphate and concentrated to dryness. The residue (0.984 g) was dissolved in benzene (8 ml) and loaded onto an

activated alumina (Grade III, 30 g) column. The fractions (each fraction 40 ml) were collected as follows :

Fr.No.	Eluent	wt. of residue (mg)
1	Benzene only	0.0
2	"	102.0
3	"	28.2
4	"	25.4
5	"	10.3
6	Benzene : Methylene Chloride (9:1)	19.1
7	"	20.9
8	Benzene : Methylene Chloride (17:3)	15.0
9	"	13.1
10	" (1:1)	28.0
11	"	32.5
12	"	25.0
13	"	24.5
14	"	25.0
15	Methylene chloride only	80.8
16	"	88.3
17	"	52.4
18	Methylene chloride : Methanol (99:1)	25.3
19	"	44.0
20	"	50.1
21	"	22.0
22	"	5.8
23	Methanol only	4.1
24	"	97.0
25	"	41.3

Fraction No.15 to 22 (368.7 mg) were pooled together. This contained two substances having different mobilities on a papergram. It was dissolved in benzene--methylene chloride (1:1) and loaded onto another column activated alumina (Grade IV, 11 g). The fractions were collected as follows :

Fr.No.	Eluent (20 ml each)	Wt. of residue (mg)
1	Benzene : Methylene chloride (1:1)	09.8
2	"	18.2
3	Methylene chloride only	25.4
4	"	40.8
5	"	50.7
6	"	31.2
7	"	12.8
8	Methylene chloride : Methanol (9:1)	28.3
9	"	35.2
10	"	28.0
11	"	24.7
12	"	8.5
13	Methanol only	20.0

Fractions 8 to 12 (124.7 mg) were pooled together and crystallized through aqueous acetone.

The substance (15a, R = H) (98 mg) after recrystallization showed the following physical properties :

m.p. 192-193°; $[\alpha]_D$ + 152.4° (C, 0.840); λ_{max} 241 m $\log \epsilon_{max}$ 4.21. [Found : C, 72.1; H, 9.20. $C_{21}H_{30}O_4$ requires : C, 72.8; H, 8.67].

Tosylation of 11α-, 18-dihydroxyprogesterone (15a)

To compound 5 (70 mg) in pyridine (2 ml) p-toluene sulphonyl chloride (50 mg) in pyridine (1 ml) was added and the mixture kept at room temperature overnight. Water (25 ml) was added to the mixture and the steroid was extracted in chloroform (3 x 10 ml). The chloroform layer was washed with water, dilute hydrochloric acid (3 x 5 ml, 2N), dilute sodium bicarbonate and finally with water. It was dried on sodium sulphate and concentrated to dryness. The residue (68 mg) was dissolved in methylene chloride (5 ml) and petroleum ether was added drop-by-drop till turbidity. The solution was then warmed and kept in cold. A semi-crystalline tosylate separated out which was filtered and dried (50 mg). The tosylate 6 showed the following properties. m.p. 163-167°. [U.V. bands at 241 mμ. (ε, 12,000) and 2.68 (ε, 2,000)].

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CHAPTER VI

SYNTHESIS OF DIACETYL HOLARRHIMINE AND ITS FURTHER CONVERSIONS

The presence of three reactive functional groups in holarrhimine often present difficulties in many reactions. The amino groups differ in their reactivities to most reagents and the presence of the hydroxyl group vicinal to the 20α amino group leads to complications, especially with reagents such as nitrous acid. It was therefore considered desirable to study the reactions of the polar functional groups of holarrhimine individually after a selective protection of either the 3β-or the 20α-amino groups.

Fortunately, the proximity of the 20 amino group and the 18-hydroxyl also presents an advantage in this respect. Sorm and coworkers[1] were able to utilise this proximity relationship to protect the amino group at position 20 selectively by a base catalyzed acyl-migration from the oxygen at position 18. Since the selective transformations at position 3 were studied in detail by these workers, it was decided to restrict the present investigations to studying the transformations at positions 18 and 20 by protecting the 3β-amino group.

3-20 Diacetyl Holarrhimine and the reactions of the 18-hydroxyl

As the preliminary step a selective hydrolysis of the O-acetyl group in triacetyl holarrhimine (17) was accomplished under mild alkaline conditions to obtain the neutral 3, 20 diacetylholarrhimine (18a). In this compound the

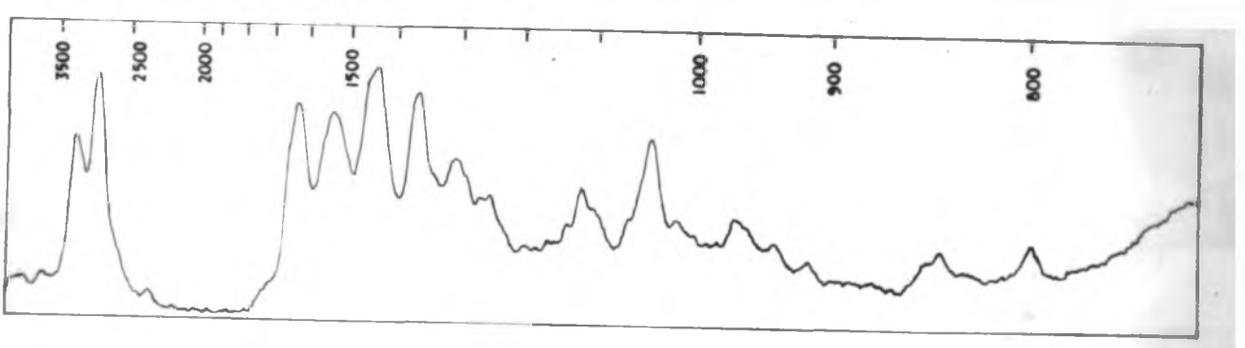

Fig.18. Infrared spectrum of 3β-, 20α-diacetamido--18-hydroxy-5-pregnene.

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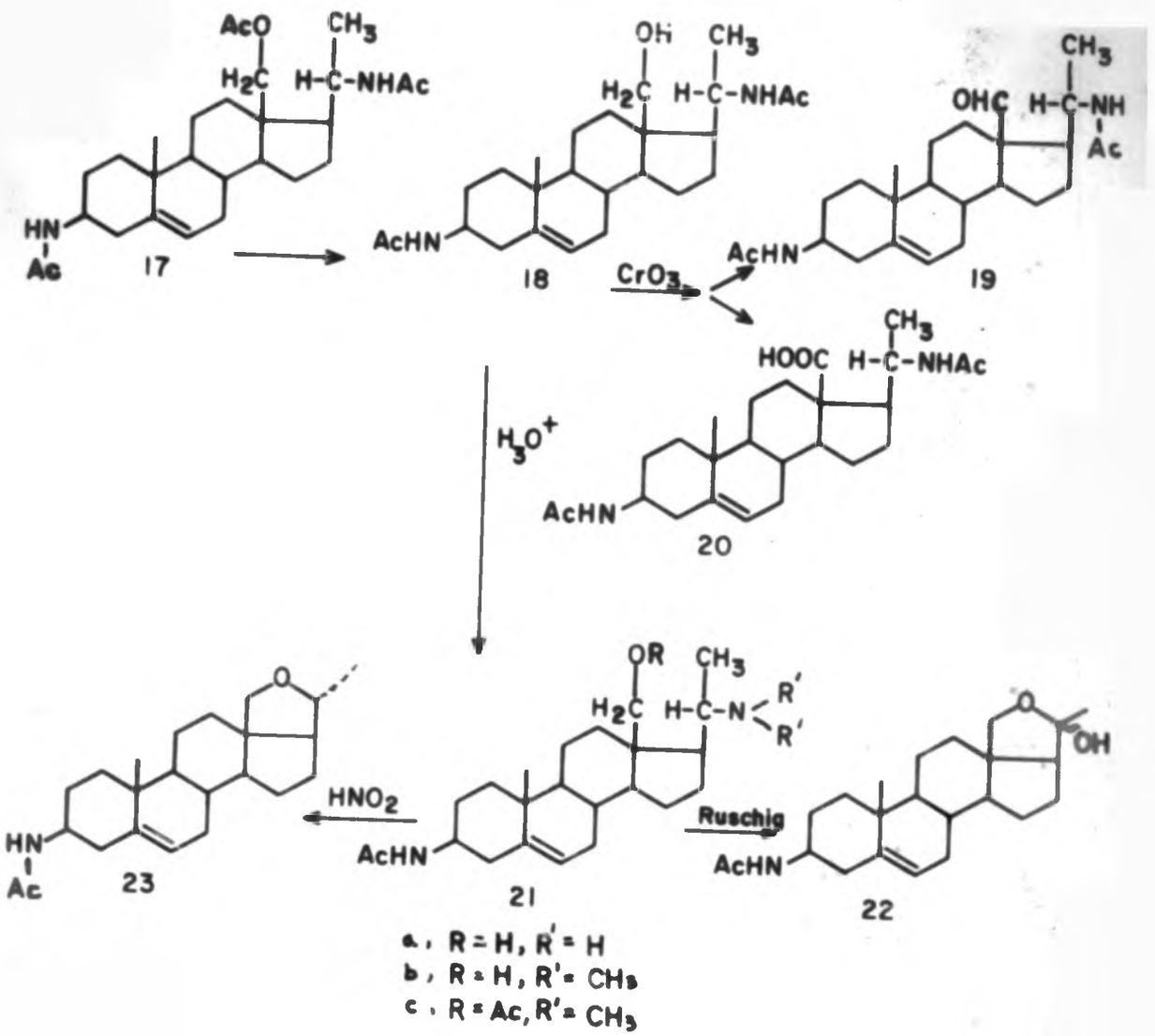
AcO
CH3
H2C
H-C-NHAc
HN
Ac
17
OH
AcHN
18
CrO3
OHC
19
HOOC
20
H3O+
OR
R'
HNO2
Ruschig
OH
23
21
22
a, R = H, R' = H
b, R = H, R' = CH3
c, R = Ac, R' = CH3

18-hydroxy group was free and could be easily oxidised to the 18-aldehyde (19) and the 18-carboxylic acid (20) by chromium trioxide. In contrast with progesterone 18-carboxylic acid (14b) and its 11α-tosyloxy derivative (16) both of which behave like lactols and are insoluble in bicarbonate, the acidic compound (20) was a true carboxylic acid. It showed a carbonyl band at 1710 cm^{-1} and bonded hydroxyl absorption at 2680 cm^{-1} characteristic of free carboxylic acids and was soluble in aqueous bicarbonate.

The neutral diacetyl holarrhimine was considered to be a potential intermediate in the synthesis of both progesterone and aldesterone. Attempted tosylation of the free 18-hydroxyl group did not succeed. An 18-O-mesyl (18b) derivative was, obtained by treatment with methane sulphonyl chloride in pyridine. The yields of this derivative were, however, not encouraging and it was not possible to study its conversion to progesterone.

Lead tetraacetate oxidation of diacetyl holarrhimine

The action of lead tetraacetate on diacetyl holarrhimine yielded some interesting results. The objective of this reaction was to introduce an 11β-oxygen function with the intramolecular assistance of the hydroxyl function at position 18.

Such an intramolecular oxygenation has been recently achieved in the case of 3α-acetoxy-11β-hydroxy-20-oxo-5β-pregnane

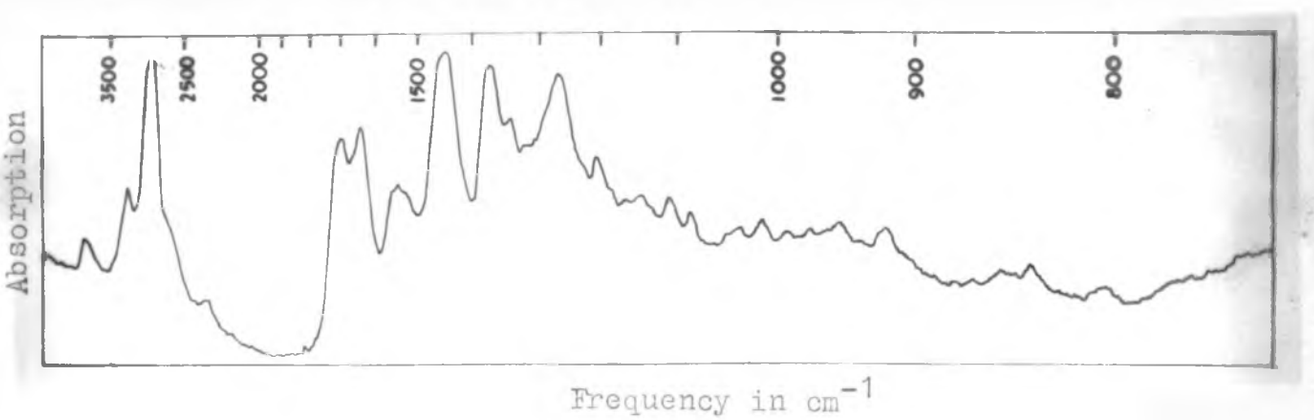

Fig.19. 3β-,20α-diacetamido-18-aldo-5-pregnene.

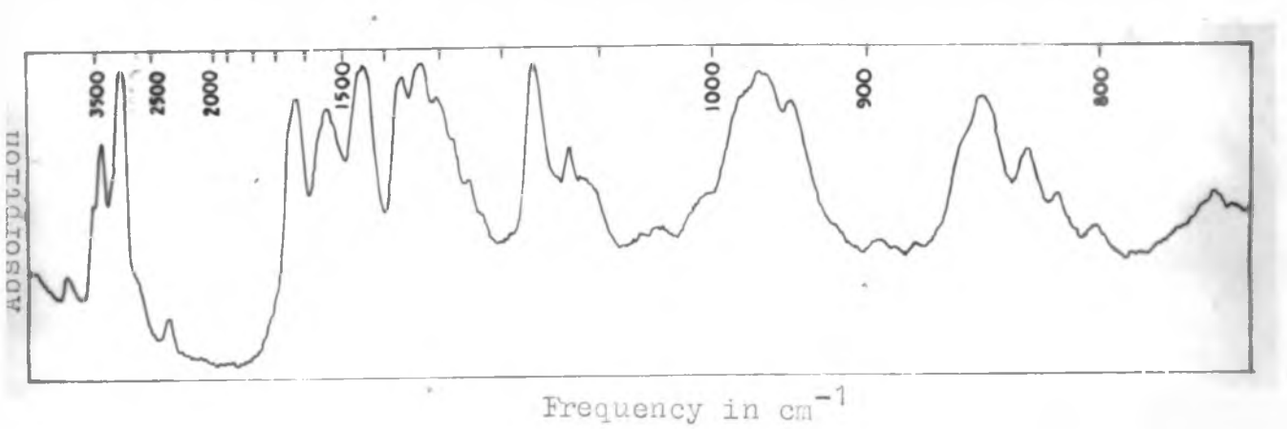

Fig.20. 3β-,20α-,diacetamido-18-mesyloxy-5-pregnene.

where intramolecular oxygenation of the angular methyl position 18 from the 11β-hydroxy position through the action of lead tetraacetate and iodine[2].

From a conformational stand point a cationic oxygen can extract a hydride from 3 different positions, viz., (a) 11β, (b) 8β and (c) 20β.

However, the extraction of 8β hydrogen appears to be unlikely from Bredt's Rule and the removal of the 20β tertiary hydrogen would be rendered difficult due to the electron defficiency on the 20α-amide nitrogen[3]. It was therefore speculated that at least theoretically there was a chance of intramolecular oxygenation at 11β position with the formation of 11β→18 ether mediated by lead tetraacetate.

The major product from the lead tetraacetate oxidation of holarrhimine was, however holarrhimine triacetate. Acetylation by lead tetraacetate is not unknown, that of cholesterol being one of the common examples[4].

Another product associated with the triacetate was isolated in extremely variable yields from the reaction mixture. It analysed for $C_{25}H_{39}O_4N_2$ and showed strong hydroxyl bands at indicating that (a) either the hydroxyl function at position 18 is unchanged or (b) if the hydroxyl group at position 18 has been involved in ether formation another hydroxyl group has been introduced somewhere in the molecule.

On mild acid hydrolysis the product gave rise to a basic gummy product indicating that the hydroxyl group is located in the vicinity of the 20α-amide nitrogen and possibly on position 18.

On chromic acid oxidation the oxidation product yielded a neutral compound with an IR band at 1745 cm^{-1} which may have been due to a five membered ketone or a lactone. The product was recovered unchanged after refluxing for one hour with aqueous alkali indicating that it may be a five membered ketone. It is not unlikely that lead tetraacetate could extract a hydride from the 15 or the 16 position of Ring D, from the tetrahedral orthoester type intermediate. Lack of material did not permit further studies on the characterisation of the second product from lead tetraacetate oxidation.

3-mono-acetylholarrhimine and reaction of the 20 amino groups

On mild acid hydrolysis diacetyl holarrhimine was converted to the 3 monoacetyl holarrhimine (21a, $R_1 = R_2 = H$). In this reaction anchimeric assistance was presumably obtained from the 18-hydroxyl function resulting in a N → O acyl migration followed by the acid hydrolysis of the ester. The 18-hydroxy-20-amino system was thus available for further studies, the 3β-acetamido group being resistant to the action of mild acid or alkali.

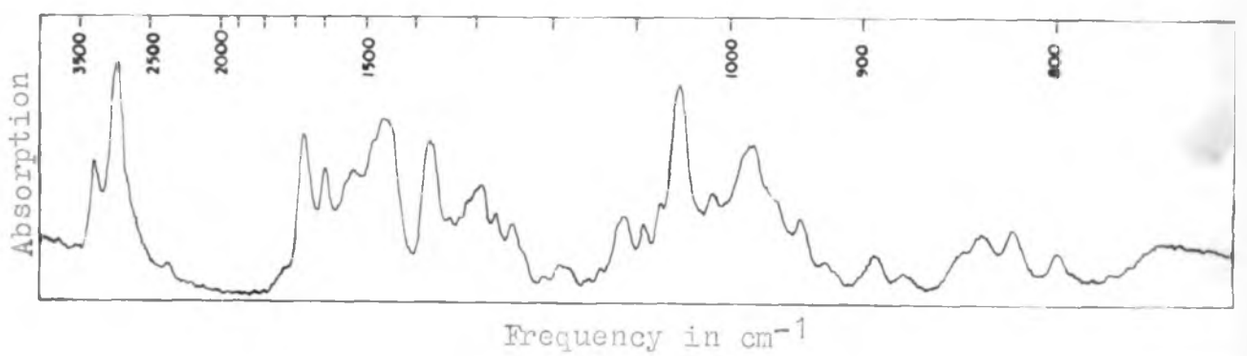

Fig.21. 3β-acetamido-18-hydroxy-20α-amino-5-pregnene.

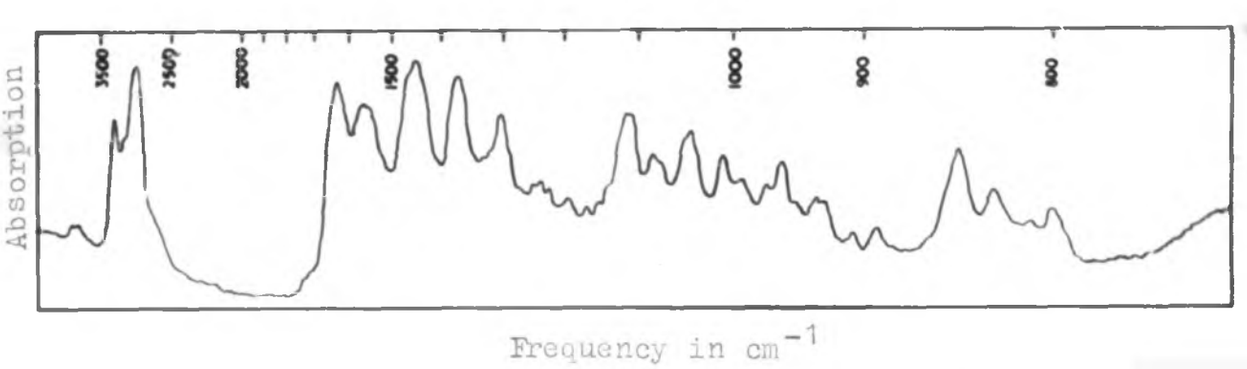

Fig.22. 3β-acetamido-18 — 20β-oxido-5-pregnene.

Nitrous acid reaction on 3-acetylholarrhimine

An opportunity for study of the course of nitrous acid reaction on a 20-amino-18-hydroxy system was provided by blocking the 3β-amino group as in 3-monoacetyl holarrhimine. From the reaction mixture the 18→20 oxido compound (23) was isolated as the sole product in substantial yields. The effectiveness of the anchimeric assistance from the hydroxyl position at position 18 in an intramolecular S_N2' displacement of the diazonium intermediate was demonstrated by the complete lack of side reactions in the nitrous acid deamination of 3-monoacetyl holarrhimine (23).

Oxidative deamination of 20α amino group

By Ruschig procedure the 20α-amino group of compound was smoothly deaminated to yield the 20-oxo compound (22a, R = H) which was isolated in the hemiketal form. The IR spectra of compound indicated the absence of an absorption due to the 20-carbonyl group. The structure of compound was confirmed by the formation of a methyl ketal (22b, R = Me) by the action of methanolic hydrochloric acid. The methyl ether showed a surprisingly high melting point.

Attempted synthesis of 20N methylholarrhimine

Methylation of 3-monoacetylholarrhimine with formaldehyde and formic acid gave in good yields the corresponding 20N-dimethyl compound. (21b, R = Me, R'= H). This

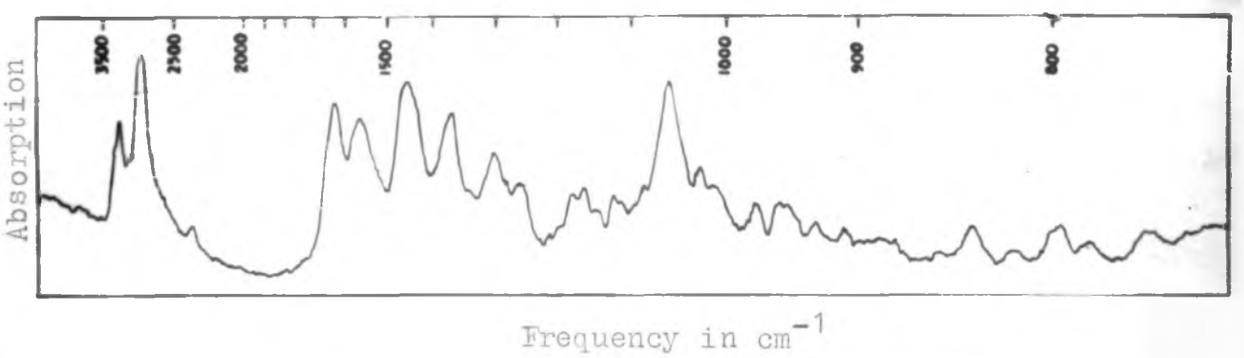

Fig. 23. 3β-acetamido-18-hydroxy-20α<u>N</u>-dimethyl-5-pregnene.

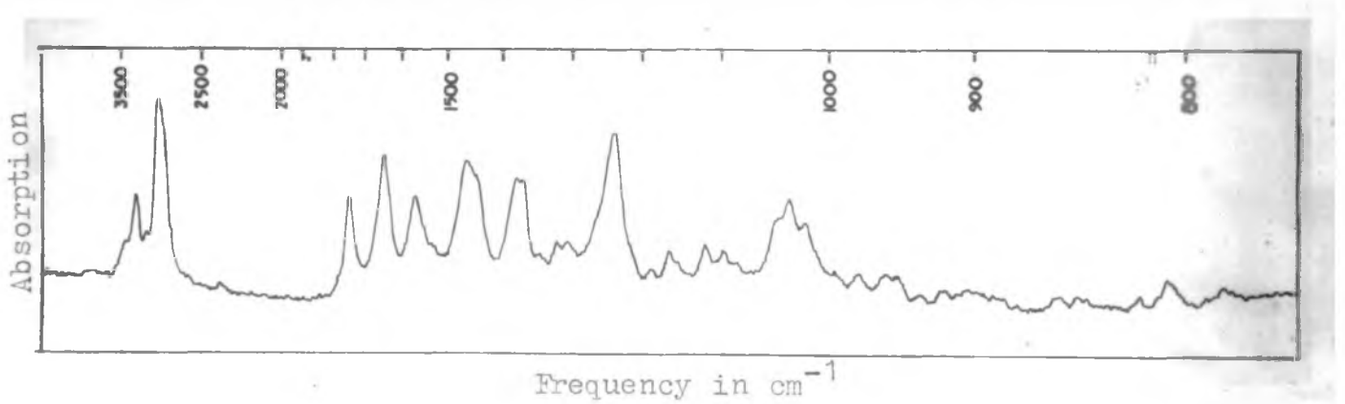

Fig. 24. 3β-acetamido-18-acetoxy-20α<u>N</u>-dimethyl-5-pregnene

compound was found to be insoluble in acetone, ether or benzene. The O-acetyl derivative (21c, R = Me, R'= Ac) was however soluble in benzene and was subjected to Von Braun demethylation with cyanogen bromide in this solvent. The 20N cyano compound exhibited the characteristic infrared band of a cyano-group and was subjected to alkaline hydrolysis. The product was a crude N-carboxy derivative which could not be decarboxylated by prolonged refluxing with acid or alkali. Presumably the proximity of the 18-hydroxyl group interfered with the decarboxylation reaction.

Triacetyl holarrhimine (17) : Holarrhimine (1.2 g) in pyridine (5.0 ml) was treated with acetic anhydride (3.0 ml) in cold. The mixture was allowed to stand overnight. Water (100 ml) was added and the solid separated was filtered. It was washed with water and crystallized from a mixture of ethyl acetate and ethanol. Recrystallized from methanol--acetone. m.p. 249°; lit.[5] 249°; [Found : C, 68.1; H, 9.2; $COCH_3$, 27.4. $C_{27}H_{42}O_4N_2,H_2O$ requires : C, 68.1; H, 9.24; $COCH_3$, 27.3]. Infrared bands at 1740, 1250 cm^{-1} ($-OCOCH_3$), 1635 ($-NHCOCH_3$), 3220 ($-NHCOCH_3$). (Fig. , page).

Diacetyl holarrhimine (18) : Triacetyl holarrhimine (17, 1.5 g) in absolute alcohol (80 ml) was refluxed with alcoholic potassium hydroxide solution (60 ml, 0.1N) for a period of 4 hr. Water (200 ml) was added to the reaction mixture and the precipitated solid was extracted in chloroform (3 x 100 ml). The chloroform extract was washed with dilute sodium chloride solution (5%, 3 x 50 ml) dried over sodium sulphate and concentrated to dryness. The substance (1.35 g) was crystallized from methanol-acetone. m.p. 285-286°; $[\alpha]_D$ - 37.0° (Ethanol C, 1.63); [Found : C, 72.6; H, 9.6; $-COCH_3$, 22.0. $C_{25}H_{40}O_3N_2$ requires : C, 72.2; H, 9.64; $-COCH_3$, 21.9%]. Infrared band at 1635 cm^{-1}. No bands at 1740 and 1250. (Fig. 18 , page 94).

Oxidation of diacetyl holarrhimine with chromic acid in acetic acid :

Diacetyl holarrhimine (0.11 g) in acetic acid (7 ml)

was treated with a solution of chromium trioxide (70 mg) in 98% acetic acid (2 ml) at room temperature with vigorous stirring. The reaction mixture was allowed to stand overnight, and then diluted with water (25 ml). The steroid was extracted with chloroform (3 x 10 ml). The chloroform layer was washed with water, aqueous sodium bicarbonate solution (3 x 8 ml, 5%) and finally with water. It was dried on sodium sulphate and evaporated to get the neutral material (92 mg) which was crystallized from methylene chloride-hexane. It was 3β,20α-diacetamido-18-aldo-5-pregnene (19). m.p. 189-190°; $[\alpha]_D$ - 16.5° (C, 4.66). [Found : C, 72.2; H, 9.6. $C_{25}H_{38}O_3N_2$ requires : C, 72.4; H, 9.18%]. Infrared bands at 2680, 1700 cm^{-1}. (Fig. 19 , page 97).

The bicarbonate washings were acidified with dilute hydrochloric acid and extracted with chloroform (3 x 10 ml). The chloroform extracts were washed with water, dried over sodium sulphate and evaporated to yield 3β-,20α-diacetamido--5-pregnene-18-oic acid (20; 10 mg) m.p. 220°. Infrared bands at 2700, 1710 cm^{-1}.

3β-,20α-diacetamido-18-mesyloxy-5-pregnene (18b)

Diacetyl holarrhimine (250 mg) was suspended in pyridine (5 ml) at 0° and methane sulphonyl chloride (0.3 ml) was added with vigorous agitation. After keeping it in cold for 5 minutes it was kept shaking on a shaker for 1 hr at room temperature. The reddish-brown mixture was poured on

a cold solution of sodium carbonate (5%, 20 ml) and extracted with chloroform containing 5% methanol (3 x 15 ml). The chloroform extract was washed with dilute hydrochloric acid, dilute sodium chloride solution, then water, dried over sodium sulphate and concentrated under vacuum. The residue (240 mg) was chromatographed over neutral grade II alumina (10 g). The chloroform eluate gave a colourless crystalline material (15 mg), recrystallized from methanol-acetone. m.p. 198-199°. [Found : S, 6.46. $C_{26}H_{42}O_5N_2S$ requires S, 6.47% Infrared bands at 1333, 1170 cm^{-1}. (Fig. 20 , page 97).

Oxidation of diacetyl holarrhimine (18) with lead tetraacetate :

Diacetyl holarrhimine (0.5 g) and vacuum dried calcium carbonate (0.3 g) were suspended in tetrahydrafuran (125 mg) and refluxed with lead-tetraacetate for 30 hr under completely anhydrous conditions. The product was filtered, concentrated to 10 ml and extracted in chloroform. The chloroform extract was washed with water (20 ml), potassium iodide solution (5%, 20 ml), sodium thiosulphate solution (10%, 20 ml), and water. It was dried over sodium sulphate and concentrated under vacuum. The product (485 mg) dissolved in benzene-chloroform (1:1, 10 ml) was chromatographed over alumina (neutral, grade II, 20 g). A non-polar compound (40 mg) (m.p.226-228°) eluted with benzene and chloroform analysed for $C_{25}H_{39}O_4N_2$.* The major fraction (395 mg) was eluted with chloroform. This was crystallized from methylene

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* Found : C, 69.8; H, 9.12; N, 6.1; $C_{25}H_{39}O_4N_2$ requires: C, 69.6; H, 9.05; N, 6.5.

chloride and hexane (m.p.236°) and recrystallized with methanol-acetone (m.p.245°). It showed no depression in melting point in mixture with triacetyl holarrhimine. The specific rotation and the infrared curves also were identical.

Monoacetyl Holarrhimine (21) : Diacetyl holarrhimine (18) (1.1 g) dissolved in methanol (100 ml) and refluxed with dilute sulphuric acid (2N, 80 ml) for 4 hr. Methanol was evaporated off on the water bath and the solution was cooled to 10°. The base was liberated by addition of liquor ammonia (20 ml) and extracted with chloroform (3 x 50 ml). The chloroform extract was washed with water, dried over sodium sulphate and evaporated to yield crystalline compound (21a) (0.98 g). It was recrystallized from tetrahydrofuran. m.p. 255-256°; $[\alpha]_D$ - 20.7°(C, 1.75). [Found : C, 74.1; H, 10.2. $C_{23}H_{38}O_2N_2$ requires : C, 73.4; H, 10.15%]. Infrared curve (Fig. 21 , page 100).

Compound (21a) can also be obtained from compound (17) without isolation of compound (18), in almost quantitative yields.

3β-acetamido-18-20β-oxido-5-pregnene (23) :

Compound (21) (175 mg) in dilute acetic acid (80 ml, 20%) was treated with a saturated solution of sodium nitrite (20 ml). The precipitate separated out was extracted in chloroform (3 x 40 ml). The chloroform extract was washed

with sodium bicarbonate solution, water, 2% sulphuric acid, water and dried over sodium sulphate. The neutral residue (147 mg) after evaporation of the solvent was crystallized from benzene or methanol. m.p. 230-231°; $[\alpha]_D$ - 48.7° (C, 1.54) [Found : C, 76.7; H, 9.8; N, 3.98. $C_{23}H_{35}O_2N$ requires : C, 77.3; H, 9.56; N, 3.93%]. Infrared curve (Fig. 22 , page 100)

3β-acetamido-18-hydro-20-keto-5-pregnene (18-20 hemiketal) (22):

To compound (21) (288 mg) in methylene chloride (25 ml) a methylene chloride solution of N-chlorosuccinimide (1%) was added with vigorous stirring. The end point (11.1 ml of N-chlorosuccinimide solution) was determined with bromo--thymol blue indicator paper. The reaction mixture was washed five times with water, dried over sodium sulphate and concentrated under vacuum at 35°. The residue (310 mg) was refluxed with sodium methoxide in alcohol (using 250 mg sodium and 15 ml absolute methanol) for 1 hr. The completion of the reaction was tested with acidified starch iodide indicator paper. The reaction mixture after addition of sulphuric acid (25 ml, 2N), was refluxed for another hour. It was diluted with water (100 ml) and the steroid was extracted with chloroform (3 x 50 ml). The chloroform extract was washed with dilute sulphuric acid, water, sodium bi-carbonate solution and finally with water. It was dried over sodium sulphate and concentrated to dryness under vacuum. The residue (260 mg) was recrystallized from methanol. m.p. 174°; $[\alpha]_D$ + 18.4° (C, 0.707).

[Found : C, 73.1; H, 9.9; N, 3.65. $C_{23}H_{36}O_3N$ requires : C, 74.0; H, 9.38; N, 3.76%] . ~~Infrared curve~~ : ~~Fig.~~ , ~~page~~ .

Methoxy derivative of compound (22)

By dissolving the compound (22) (50 mg) in absolute methanol and adding pure dry p-toluene sulphonic acid it gave a methoxy derivative. m.p. 288°. [Found : OCH_3 , 7.0. $C_{24}H_{38}O_3N$ requires : OCH_3 , 8.0%].

Hydrolysis of this derivative with dilute sulphuric acid gave back the compound (22).

18-Hydroxy-3β-acetamido-20α-dimethylamino-5-pregnene (21b):

Compound (21a) (300 mg) was heated on a water bath with a mixture of formic acid (1.5 ml) and formaldehyde (40%, 1.5 ml) for 4 hr. The product was cooled and added to water (50 ml). The base(21b) was liberated by the addition of liquor ammonia (2 ml) and extracted with chloroform (3 x 20 ml). The chloroform extract was washed with water, dried over sodium sulphate and concentrated to dryness. The residue (285 mg) was crystallized from methanol-acetone. m.p. 246-248°; $[\alpha]_D$ - 36.7° (C, 1.9). [Found : C, 74.64; H, 10.81. $C_{25}H_{42}O_2N_2$ requires : C, 74.60; H, 10.45%]. Infrared curve : (Fig. 23 , page 102).

18-acetoxy-3β-acetamido-20α-dimethylamino-5-pregnene (21c) :

Compound(21b, 250 mg) in pyridine (2 ml) was mixed

with acetic anhydride (1 ml) in cold. The mixture was kept overnight, added to water (50 ml) and extracted with chloroform (3 x 15 ml). The chloroform solution was washed with dilute sodium bicarbonate solution, water, dried over sodium sulphate and concentrated to dryness under vacuum. Traces of pyridine was removed by keeping the material in a vacuum desiccator containing concentrated sulphuric acid. The product (255 mg) was passed through a short column of grade II neutral alumina using benzene:chloroform as eluent. The colourless material recovered from the eluates crystallized by slow evaporation of a solution in acetone or benzene. m.p. 180-181°; $[\alpha]_D$ - 17.4° (C, 1.84). [Found : $COCH_3$, 15.92. $C_{27}H_{44}O_3N_2$ requires : $COCH_3$, 19.38%]. Infrared curve : (Fig. 24 , page 102). Infrared spectrum shows bands at 1740, 1248 cm^{-1} (for an acetoxy group). The compound (21c) unlike compound (21b) was soluble in acetone.

Attempted synthesis of 20N monomethyl holarrhimine : To compound (21c) (200 mg) in benzene (10 ml) was added slowly a solution of cyanogen bromide (0.4 g) in benzene (5 ml) with vigorous stirring keeping the temperature below 10°. The mixture was kept at room temperature for another 10 min and refluxed for 1 hr. It was cooled and kept for one day at 15°C. The insoluble quaternary methobromide was separated by filtration and the solvent layer neutral cyano compound was washed with water, dilute hydrochloric acid, water dried over sodium sulphate and concentrated to dryness. The crude

residue (125 mg) showed an infrared band at 2242 cm^{-1}, characteristic of a cyano compound. The residue was refluxed for 40 hrs with alcoholic potassium hydroxide (20%, 5 ml) using a potassium hydroxide guard tube. Water (100 ml) was added to the reaction mixture which was extracted with chloroform (4 x 10 ml). The chloroform extract was washed with dilute hydrochloric acid (3 x 10 ml, 1N), water dried with sodium sulphate and concentrated to dryness. The gummy residue weighed (12.4 mg).

The hydrochloric acid solution was basified with liquor ammonia (5 ml) to liberate any base which was extracted with chloroform (3 x 10 ml). The chloroform extract was washed with water, dried over sodium sulphate and evaporated to dryness. The residue (5.6 mg) could not be purified by chromatography or through its perchlorate salt.

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CHAPTER VII

S U M M A R Y

Ever since the discovery of aldosterone (I) and other 18-hydroxylated steroids from the adrenals there has been a widespread interest in the development of commercial syntheses of these compounds.

Development of partial syntheses of progesterone and corticosteroids starting from steroidal raw material available in an abundant supply from nature has been the greatest turning point in steroid industry. It was therefore, considered desirable to attempt some convenient partial synthesis of 18 substituted steroids from suitable naturally--occurring steroids. Kurchi, Holarrhena antidysenterica growing abundantly in India, contains 18-substituted steroidal alkaloids of a suitable type of which holarrhimine, 3β, 20α--diamino pregn5-ene, seemed to be an attractive starting material for these syntheses.

(1) Isolation of holarrhimine

A modified procedure for the isolation of holarrhimine was developed. The insoluble sulphates obtained according to the method of Siddiqui et al. were either distributed between chloroform and phosphate-citrate buffer of pH 8.0 and pH 7.2 to extract holarrhimine, or ~~of~~ purification of holarrhimine was accomplished through its insoluble p-nitrobenzylidine derivative according to the procedure of Sorm et al. From the mother liquors monomethyl holarrhimine was also isolated as the alcohol-insoluble sulphate.

(2) Conversion of holarrhimine to 18-substituted steroids

One of the major steps of conversion of holarrhimine to 18 substituted steroids is the replacement of the amino groups at 3β-and 20α-positions by oxygen.

(a) Nitrous acid reaction : The action of nitrous acid on holarrhimine resulted in the formation of several products which were separated by solvent-solvent distribution and partition chromatography over magnesium oxide columns. Six crystalline compounds were isolated by the above technique.

Two of these were identified as 3β-hydroxy-18-20--oxido (20β) pregn-5-ene and 3β,18-dihydroxy-20α-amino-pregn--5-ene by converting them to known compounds.

It was also possible to assign tentative structures for three more compounds from the nitrous acid reaction. Most of these products yielded the identical triacetoxy compound 3β, 18,20α-triacetoxy-pregn-5-ene, when they were treated with borontrifluoride and acetic anhydride.

(b) Ruchig's Method : Holarrhimine was converted to 18-hydroxyprogesterone in good yields by N-chlorination, dehydrochlorination and hydrolysis. 18-hydroxyprogesterone was converted to progesterone-18-carboxylic acid by chromic acid oxidation.

Attempted hydroxylation of progesterone-18--carboxylic acid by fermentation with moulds known to introduce

11α- or 11β- hydroxyl group in steroid nucleus did not succeed.

18-Hydroxyprogesterone was, however, hydroxylated in 11α-position by a strain of A.niger. A semicrystalline 11α-O-tosylate of this product was prepared.

An attempted synthesis of 3,20-dioxo-4-pregnene--11β → 18-lactone by chromic acid oxidation of the tosylate and a subsequent intramolecular displacement of the tosyloxy group by the 18-carboxylate anion did not succeed.

(c) Other transformations : Holarrhimine triacetate was converted to the neutral diacetamido compound and the basic 3-monoacetamido compound by progressive hydrolysis. The diacetate was further transformed to the corresponding 18-aldo- and 18-carboxy- compounds by chromic acid oxidation. The corresponding 18-O-mesylate from the diacetate was also obtained. Lead tetraacetate oxidation of the diacetate gave holarrhimine triacetate and a crystalline relatively non-polar steroid, $C_{25}H_{39}O_4N_2$.

3-Monoacetylholarrhimine was converted by Ruschig's procedure to 18-hydroxy-20-oxo-3β-acetamido-5-pregnene and to 3β-acetamido-18,20-oxido-5-pregnene by nitrous acid treatment.

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