A STUDY OF SOME NITROGEN-CONTAINING STEROIDS

A Thesis

by

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GENERAL INTRODUCTION

This thesis is a part of a project underway in this laboratory, the aim of which is the syntheses of various nitrogen-containing steroids.

Some amino steroids have been known to possess hypotensive or bacteriostatic properties. The syntheses of some other nitrogen-containing steroids have been prompted by their similarity to various alkaloids.

In the first part of the present work, the Mannich reaction of unsaturated and saturated ketosteroids derived from cholesterol was studied. The hydrogen atoms in rings A or/and B were activated by the adjacent keto group(s), and also by the double bond in the case of unsaturated ketones. It was established that the ketosteroids examined condensed preferentially with formaldehyde.

The potential pharmacological properties of the reduction products of steroidal oximes and the elucidation of their stereochemistry have attracted the attention of various workers.

In the second part of this research, the reduction of mono- and dioximinocholanic acids by means of sodium in alcohol and lithium aluminium hydride was investigated. Steroidal amino acids were synthesized using the former reducing agent. Their dipole structures were confirmed by infrared spectroscopy. The reaction of dioximinocholanic acids with lithium aluminium hydride resulted in the reduction of the carboxyl group to primary alcohol in all cases. The oxime groups, depending on their positions in the nucleus, were transformed either to a secondary alcohol or to a primary amine.

The infrared spectra of various keto- and oximino- cholanic acids prepared were determined and examined.

HISTORICAL INTRODUCTION

Part I - The Mannich reaction

1. Mechanism

The Mannich condensation is of very wide applicability, and was thoroughly reviewed by Blicke (1) in 1942. More recently, Matuszko (2), Bombardieri (3) and Fenyes (4), from this Laboratory, gave an account of the subsequent literature. Although the Mannich reaction has been investigated extensively, the course and the factors determining it have not been elucidated completely. However, all authors agree on a two stage process involving a condensation of formaldehyde with either a compound possessing one or more active hydrogens, or with an amine, and subsequent reaction of this intermediate with the third reactant.

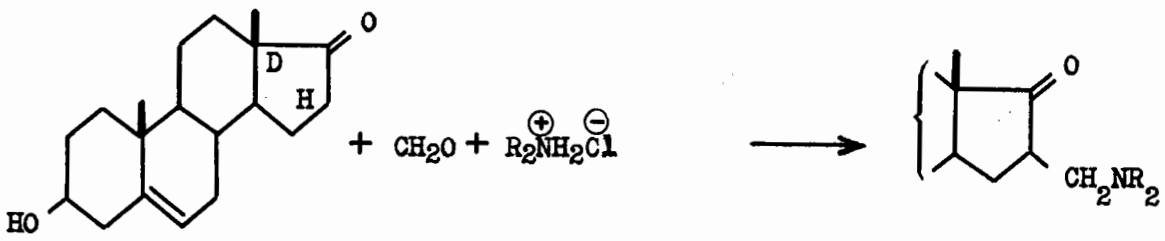
Bodendorf and Koralewski (5) suggested the formation, in the first step, of a β-ketomethylol, which would then react with a secondary amine. Philips (6) proposed that before condensing with the base, the methylol undergoes dehydration to an $\alpha\beta$-unsaturated ketone. Other hypotheses resulted from various experimental evidences. Some workers (7-11) considered a methylol-amine, R_2NCH_2OH, to be the first intermediate. Alexander and Underhill (12) supported this view on the basis of their kinetic study.

Lieberman and Wagner (13) postulated methylene-bis-amine, $R_2NCH_2NR_2$, as an intermediate. Their hypothesis was put forward to account for the preparation of several Mannich bases by the interaction of methylene-bis-amines with phenols (14) and other compounds containing an active hydrogen (13). By an accession of a proton,

methylene-bis-amine forms an ammonium ion, $R_2\overset{\oplus}{N}HCH_2NR_2$, which by loss of amine yields a carbonium ion $R_2N\overset{\oplus}{C}H_2$. The latter, according to Wagner (15), could also be obtained from methylolamine by protonation to give an ammonium-oxonium ion ($R_2\overset{\oplus}{N}HCH_2OH \rightleftharpoons R_2NCH_2\overset{\oplus}{O}H_2$), and subsequent loss of a molecule of water. Carbonium ion, the key intermediate in these schemes, would then combine with the carbanion, $R_2\overset{\ominus}{C}H_2$, resulting from an active hydrogen compound (ketone, aldehyde, phenol, nitroparaffin, etc.) by loss of a proton under the influence of a base. The above mechanism implies a dual acid-base catalysis. Hellman (16) adopted it as being in agreement with his extensive study of various condensation reactions involving Mannich bases.

2. Applications of the Mannich condensation in the steroid series

The application of Mannich reaction in the steroid series has hitherto been restricted to compounds containing an activated methylene group in the ring D, or in the aliphatic side chain attached to the carbon atom in 17-position. In 1948, Julian and his coworkers (17) were able to prepare 16-aminomethyl derivatives of dehydroisoandrosterone (I) by condensing dehydroisoandrosterone (II), paraformaldehyde and a secondary amine hydrochloride, such as dimethyl-, diethyl-amine, or piperidine hydrochlorides.



II I

However, a detailed description and the physical constants of one product only, 16-dimethylaminomethyldehydroisoandrosterone, were given. In a patent secured in 1951 (18), Julian reported several other steroidal Mannich bases, such as 16-(methylaminomethyl)dehydroisoandrosterone, 16-(dimethylaminomethyl)etioallocholan-3-ol-17-one, 16-(dimethylaminomethyl)estrone, and 21-(dimethylaminomethyl)-5-pregnen-3-ol-20-one.

When testosterone (androst-4-en-17-ol-3-one) and progesterone (pregn-4-ene-3,20-dione) were used in the Mannich reaction, no definite products could be isolated (18).

3. An abnormal Mannich condensation

Interesting results were reported recently by Bachman and Atwood (19) who prepared a series of γ-dinitroparaffins. The condensation between the active methylene group of primary nitroparaffins and formaldehyde gave good yields of products only in the presence of basic catalysts capable of forming Mannich base intermediates such as secondary amines. Low yields were obtained even with a tertiary amine and with sodium carbonate. The authors postulated a reaction mechanism involving Mannich-like base intermediates:

$$R_2NH + CH_2O + RCH_2NO_2 \longrightarrow RCH(NO_2)CH_2NR_2 \quad (A)$$

$$RCH(NO_2)CH_2NR \longrightarrow RC(NO_2)=CH_2 + R_2NH \quad (B)$$

$$RC(NO_2)=CH_2 + RCH_2NO_2 \longrightarrow RCH(NO_2)CH_2CH(NO_2)R \quad (C)$$

An alternate reaction sequence was indicated in view of the fact that pure tertiary amines and sodium carbonate also catalyzed the formation of γ-dinitroparaffins.

$$RCH_2NO_2 + CH_2O \longrightarrow RCH(NO_2)CH_2OH \qquad (D)$$

$$RCH(NO_2)CH_2OH \longrightarrow RC(NO_2)=CH_2 + H_2O \qquad (E)$$

The authors pointed out that the relative ease of reaction (B) as compared to (E) probably accounted for higher yields of products obtained with secondary amine catalysis.

In agreement with the above proposals, secondary and tertiary nitroparaffins do not undergo this reaction.

Part II - The reduction of steroidal oximes

The amino derivatives of steroids were expected to possess biological activity, due to the presence of a nitrogen function attached to the steroid nucleus. In some cases, the nitrogen containing steroids bore a close resemblance to natural alkaloids. These facts prompted the syntheses of a number of compounds of both types.

Partial syntheses of steroidal alkaloids have been reported by Uhle (20, 21).

Ruziczka and his coworkers (22) have prepared 2-(dialkylamino) ethyl ester and 2-(dialkylamino) ethyl amides of cholanic and desoxycholic acids in view of their similarity to Erythrophleum alkaloids such as cassaine, cassaidine and coumingine.

Heer and Hofmann (23) have synthesized very recently a series of steroid derivatives (C_{17}-pyridine, $_{17}$-picoline and C_{16}, $_{17}$-pyrrocoline) related to solanidine.

Certain amines have been synthesized because of their probable hypotensive properties (24, 25, 26).

A number of amino derivatives of cholesterol, cholestanol and bile acids have been prepared and found to be bacteriostatic agents (27-36).

The steroidal structure and the amebacidal properties of conessine prompted the syntheses of amino derivatives of allopregnane (36, 37), androstane (38) and trihydroxynorcholane (38).

1. Mechanism

When an oxime group located in the steroid nucleus is reduced, the resulting amino group may exist in either axial or equatorial conformation. It is now generally agreed (39) that reductions by alkali metals and proton donors, proceeding through long lived carbanions, give predominantly the most thermodynamically stable product. It has been suggested (40) that the catalytic hydrogenation of oximes follows the same course as that of ketones, and in highly acidic media (fast rate of reaction), axial substituents are produced. There is a good deal of experimental evidence supporting the above views, but the literature also contains several examples of reductions which gave mixtures of epimers in varying ratios (41, 42) or yielded anomalous products (30). In particular, the stereochemical course of reductions by lithium aluminium hydride does not seem to be well ascertained (42, 43, 44). Whenever various isomers can be produced in a reaction, the influencing factors are thermodynamic, kinetic and steric.

According to Corey's extensive work on the stereochemistry of brominated steroids (45), the conformation of the incoming substituent

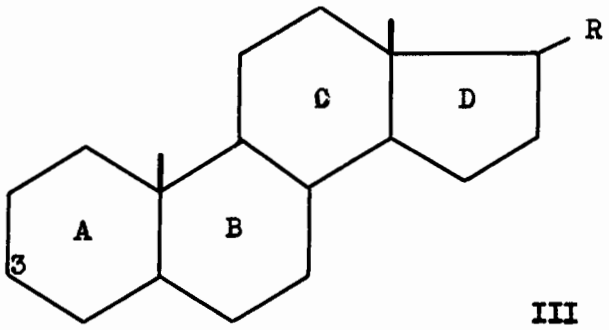
is governed by the thermodynamic and kinetic control of the reaction product. In the first stage of a reaction, the kinetic control is operating, and depending upon further conditions of the process and its duration, the product may or may not undergo thermodynamic equilibration into the more stable epimer.

Dauben and his coworkers studied the stereochemistry of hydride reductions of alicyclic (46) and steroidal (47) ketones. In order to interpret the distribution of isomers produced in these reactions, the authors postulated the concept of "steric approach control" and of "product development control". The former is related to the ease of formation of the initial organo-metallic complex, whereas the latter depends on the relative energetics of the formation of the product from the complex. If a reaction is product development controlled, a more stable isomer is obtained. When both factors are in operation, the relative proportions of isomers depend upon the predominance of one of the "controls", and also upon them being competitive or not.

2. Amino derivatives of the cholestane and coprostane series.

a) Aminocholestanes and aminocoprostanes

The chemistry of the reduction of steroidal oximes has been very much concerned with the 3-position in the ring A (III).



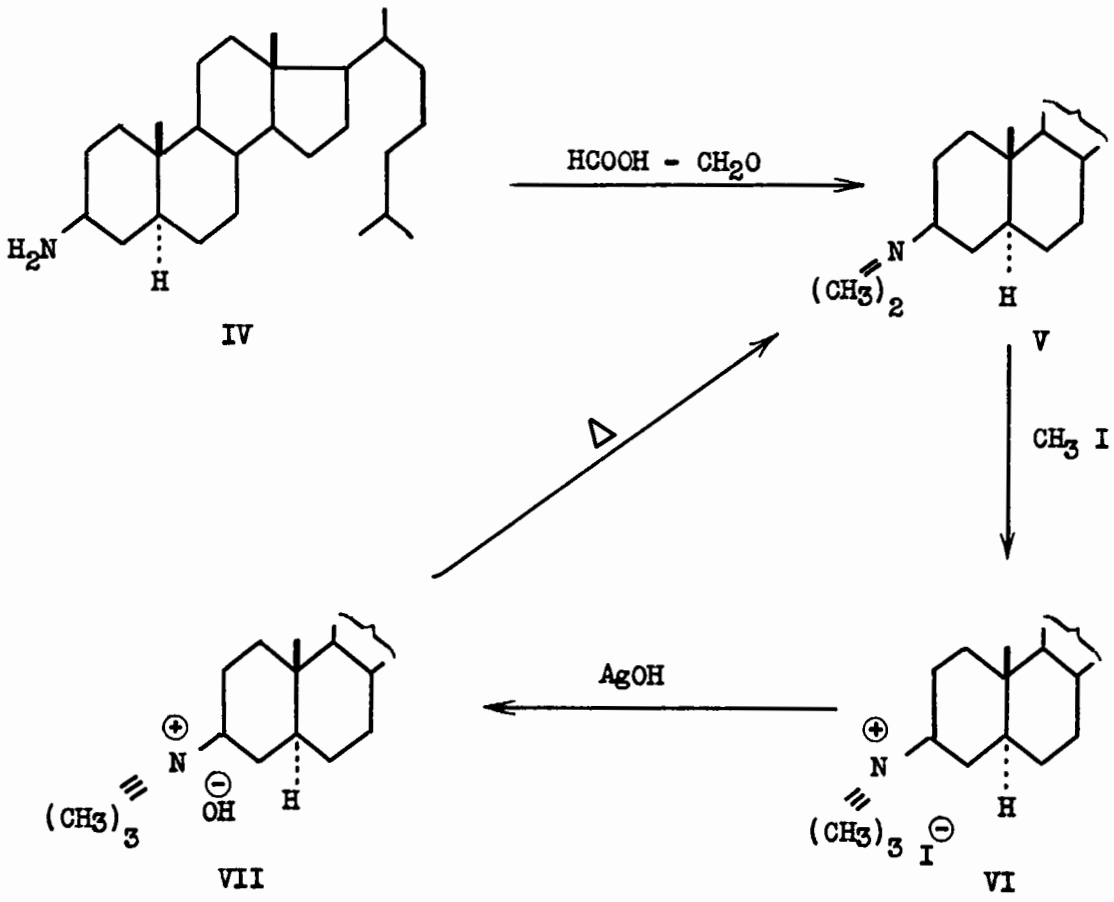
III

The first worker who should be mentioned in this connection is Loebisch who, in 1872, claimed to have prepared 3-aminocholestane when he treated cholestenyl chloride with an alcoholic solution of ammonia (48). His product melted at 104°. Later on, Walitzky (49), Diels and Abderhalden (50), and others tried unsuccessfully to obtain the above amine by Loebisch's procedure. In 1911, Windaus (41) became interested in possible physiological properties of aminosteroids. He reinvestigated Loebisch's work and succeeded in preparing, under special conditions, what was believed to be the hydrochloride of 3-aminocholestane. The corresponding base had a melting point of 98°. Only much later was it shown that Loebisch's and Windaus' products were mixtures of epimeric 3-aminocholest-5-enes ("cholesterylamine") (33, 38, 51, 52, 53). Searching for another way to prepare the amino compound, Windaus made use of 3-oximinocholest-4-ene which had been previously reported by Diels and Abderhalden (54). By reducing the oxime with sodium in ethanol, Windaus obtained an amino product, m. p. about 98°, from which he was unable to isolate any appreciable amounts of either epimer in a pure state. The acetylation and fractional crystallization of the reaction product yielded three different fractions which the author named α, β and γ.

The following year, Diels (55) obtained 3-aminocholestane by reduction of the corresponding oxime with sodium in amyl alcohol. The product melted at 110-120°. Diels also prepared the hydrochloride for which he did not report the melting point. In 1946,

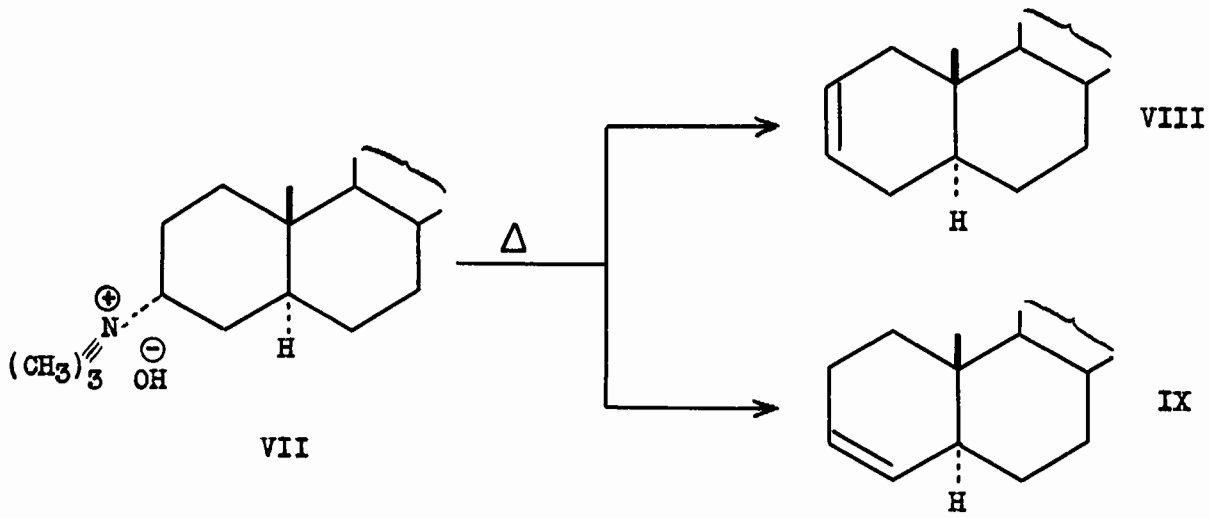
Barnett (29) found that the free base inhibited the growth of Gram-positive bacteria, but had scarcely any bacteriostatic activity against Gram-negative organisms.

In 1952, Dodgson and Haworth (38), in their search for new basic steroidal derivatives with pharmacological properties similar to the amebicidal properties of conessine, were led to prepare, among other compounds, 3β-dimethylaminocholestane. The base was obtained, in low yield, by reducing 3-oximinocholestane, previously reported by Fujii and Matsukawa (56), with sodium in amyl alcohol to 3β-aminocholestane. This intermediate compound was the major constituent of a mixture, m. p. 90-120°, which was acetylated and separated into two epimeric acetamido derivatives melting at 245-246°, $[\alpha]_D^{20}$ + 12°, and at 213° respectively; the former, on hydrolysis with acids, gave 3β-amino derivative, m.p. 106-120°. 3β-Aminocholestane (IV) was also found to give a digitonine complex quite readily, whereas the related acetyl derivative did not yield such adduct. Methylation of the 3β-amino group afforded 3β-dimethylaminocholestane (V). An attempted Hofmann degradation of the quaternary base, 3β-cholestanyltrimethylammonium iodide (VI), by the way of methohydroxide (VII), yielded the 3β-dimethyl compound (V), and provided additional proof for the conformation of the 3β-amino group.

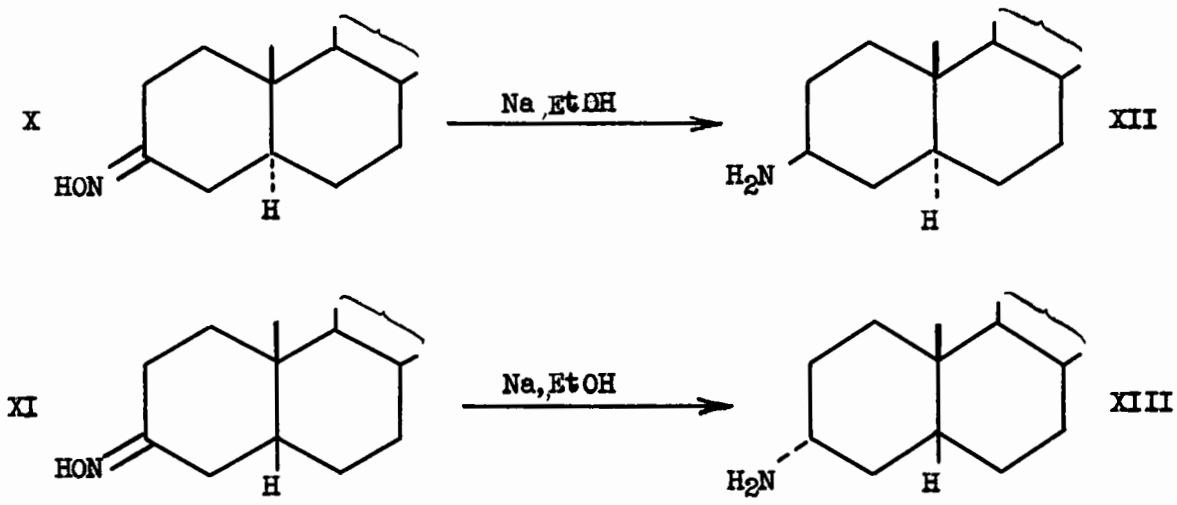


Hofmann decomposition, which is a bimolecular 1:2-elimination reaction (57) is favoured by a coplanar arrangement of the participating atoms. The highest degree of coplanarity exists in a trans-A/B configuration in a steroid molecule when a substituent in 3-position is α(axial). Consequently, 3α-substituents in compounds possessing trans-A/B rings should undergo bimolecular elimination more readily than 3β-epimers (58). Accordingly, 3β-dimethylaminocholestane was recovered in high yield when subjected to Hofmann degradation (see above). The condition is fulfilled

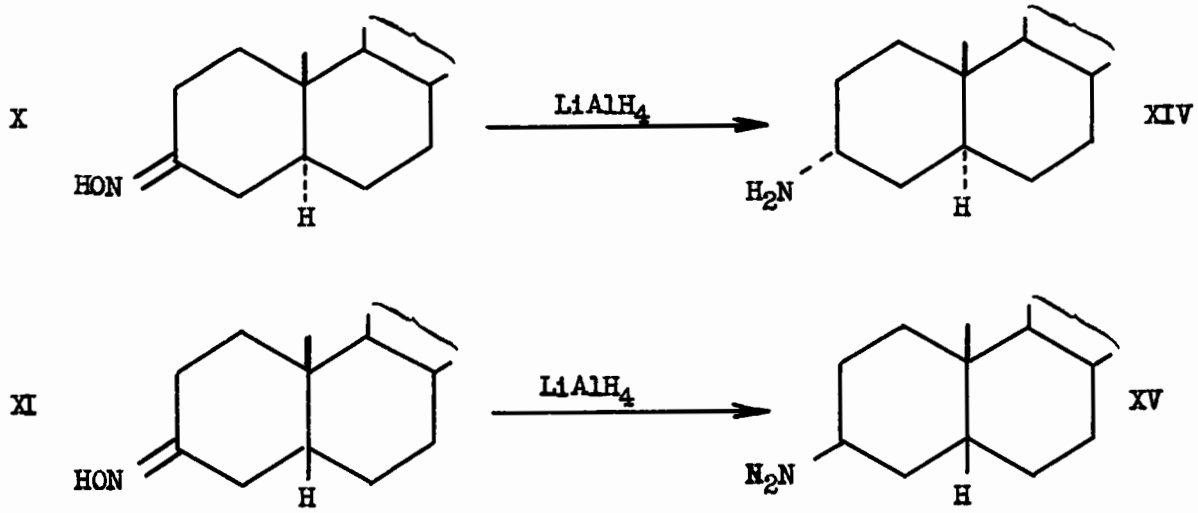
only in 3α-dimethylaminocholestane with its dimethylamino group in axial conformation. The 3α-epimer, prepared by reaction of cholestan-3βyl toluene-p-sulphonate with dimethylamine, underwent the degradation with great ease, and yielded a mixture of olefinic cholest-2-ene (VIII) and cholest-3-ene (IX).



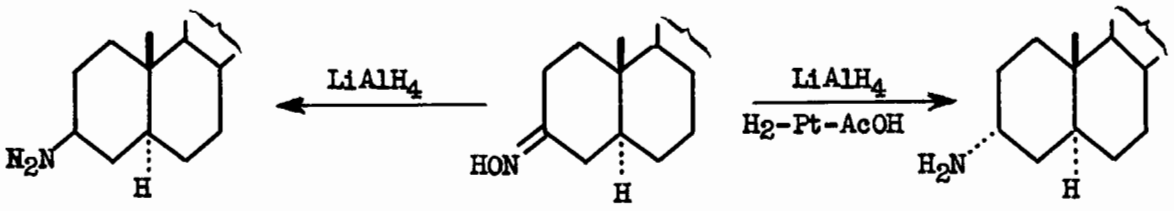
Shoppee and his coworkers reduced 3-oximinocholestane (X) and the isomeric 3-oximinocoprostane (XI) with sodium in ethanol, lithium aluminium hydride (59) and platinum in acetic acid (42). In accord with previous findings concerning sodium in alcohol reductions (40, 60, 61), equatorial amines were obtained, namely 3β-aminocholestane (XII) (m.p. 116-118°, $[\alpha]_D$ + 12°; acetyl derivative, m.p. 224°), and 3α-aminocoprostane (XIII) (M.p. 82-84°, $[\alpha]_D$ + 38°; acetyl derivative m.p., 218-219°).



However, conflicting results were obtained by the authors as to the other two modes of reduction. Shoppee's work in 1954 (59) indicated that lithium aluminium hydride yielded predominantly axial amines, and appeared, therefore, to possess a stereospecificity similar to that of catalytic reducing agents in acid medium. The two isomers obtained were 3α-aminocholestane (XIV) (m.p. 90°, $[\alpha]_D$ + 36°; acetyl derivative, m.p. 217-218°), and 3β-aminocoprostane (XV) (m.p. 139-141°, $[\alpha]_D$ - 20°; acetyl derivative, m.p. 100°).

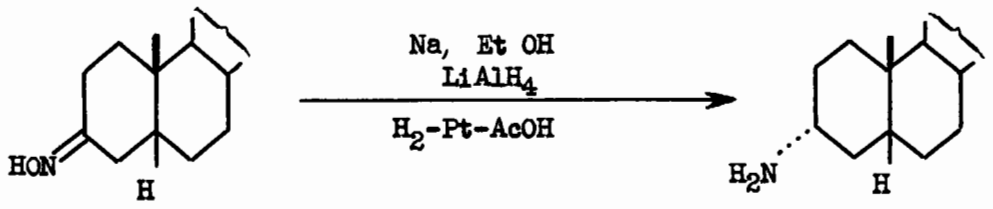


Similar work reported in 1956 by Shoppee and his group (42) indicated that lithium aluminium hydride reduction of 3-oximinocholestane led to the production of a mixture of amines composed of about equal amounts of both axial and equatorial epimers. Hydrogenation with platinum in acetic acid gave the axial epimer, 3α-aminocholestane (XIV), in agreement with expectation.



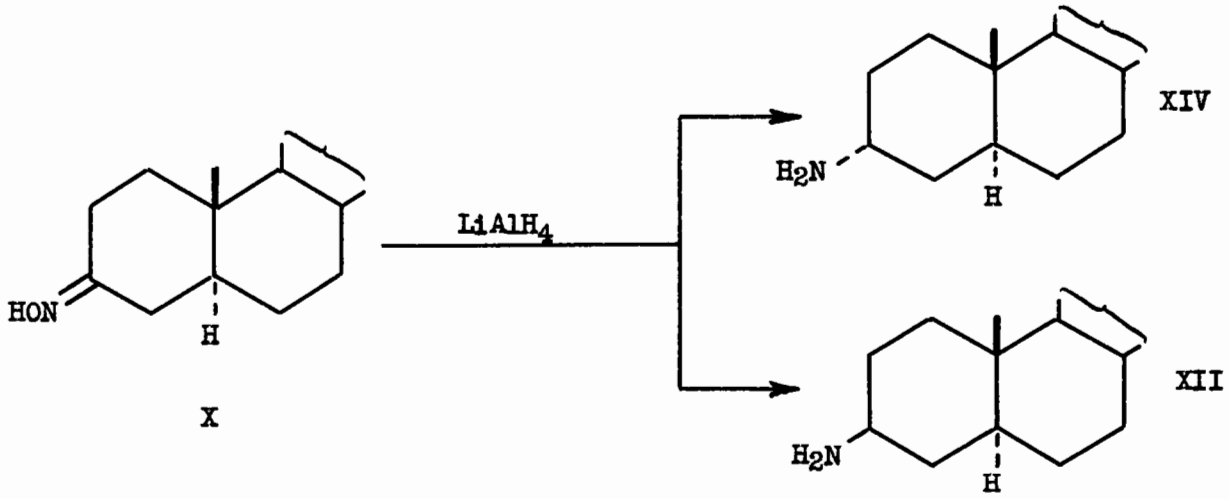
XII X XIV

The reduction of 3-oximinocoprostane (XI) with sodium in ethanol, platinum in acetic acid and lithium aluminium hydride took an unexpected course (42). Contrary to what Shoppee and his collaborators reported in 1954, all three reductions, followed by chromatography, yielded only the equatorial epimer, 3α-aminocoprostane (XIII).



XI XIII

In 1954, Šorm and his coworkers published the results of an extensive study of 3-amino derivatives of cholestane (63) which they considered of interest because of their relation to the conessine alkaloids. The problem of conformation of the basic group at $C_{(3)}$ was of particular importance. Šorm reduced 3-oximinocholestane (X) with lithium aluminium hydride in a reaction similar to that of Shoppee, and obtained a mixture of epimeric amines which he was able to separate and purify by chromatography and repeated conversions to hydrochlorides. From 9 g. of the oxime, 339 mg. of the axial 3α(XIV) (m.p. 88-89°) and 700 mg. of the equatorial 3β-(XII) (m.p. 116-117°) aminocholestanes were isolated.



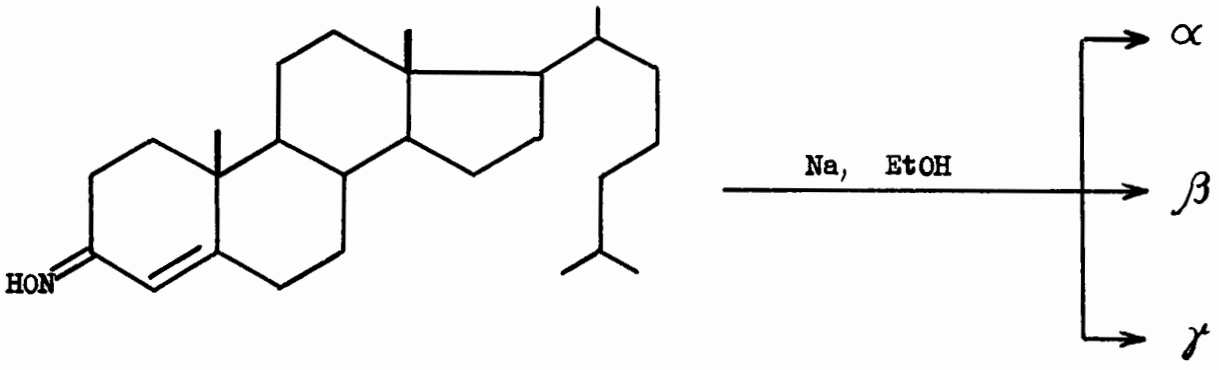
The stereochemical specificity of Hofmann reaction, proved already by Haworth (38), received further support from the work of Šorm on Curtius degradation of cholestane-3β-carboxylic acid, the configuration of which had been unequivocally established (64). The basic fraction from the Curtius degradation was directly methylated to yield the expected 3β-dimethylaminocholestane which was identical to the

compound prepared by Haworth.

Vanghelovici (65) synthesized 6-aminocholestane by reducing 3-chloro-6-oximinocholestane with sodium in ethanol. The hydrochloride and acetyl derivative were also reported.

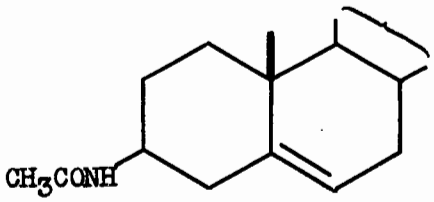
b) Aminocholestenes

In 1911, Windaus and Adamla (41) reduced 3-oximinocholest-4-ene (XVI) by using sodium in ethanol, and isolated, after acetylation, three different substances which they called α, β and γ.



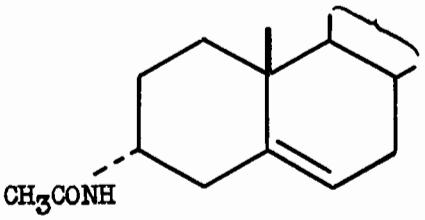
XVI

The fraction α, m.p. 243-244° (not the major product) was later proved by Julian and his coworkers (51) to be 3β-acetamidocholest-5-ene (XVII). The latter authors degraded 3β-benzylaminocholest-5-ene to 3β-aminocholest-5-ene which they converted to 3β-acetamidocholest-5-ene (m.p. 243°). This last compound has also been prepared by several other workers by various ways (42, 43, 66, 67).



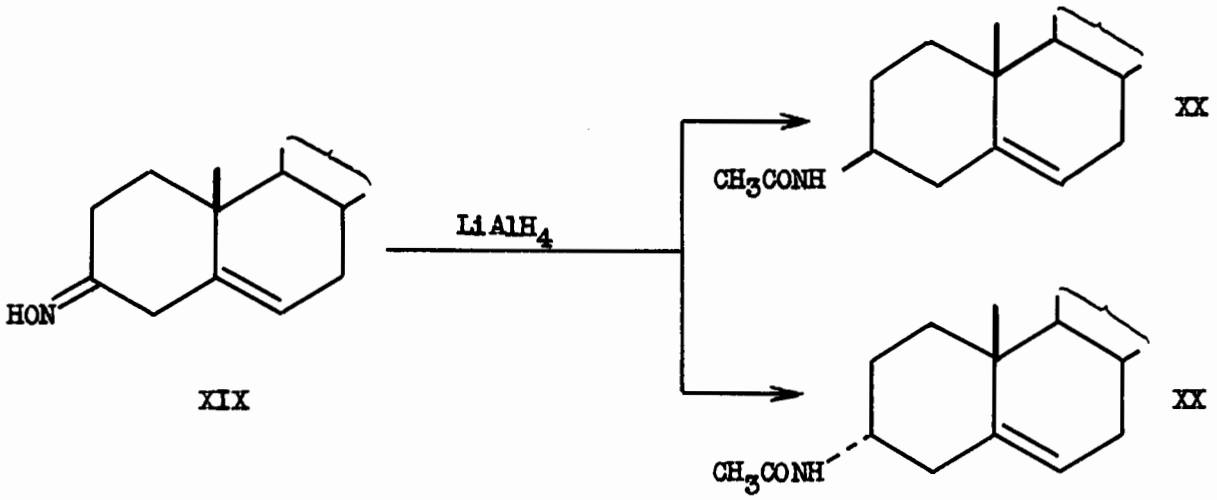
XVII

The identity of the fraction γ (m.p. 190°) was inferred from the work of McKay and his collaborators (53) who degraded 3α-benzylaminocholest-5-ene to the corresponding amine, which they converted to 3α-acetamidocholest-5-ene (m.p. 184.5°) (XVIII).



XVIII

Bannard and McKay (43) gave further confirmation by synthesizing directly the latter compound from 3-oximinocholest-5-ene (XIX). On reduction with lithium aluminium hydride, it gave a mixture of epimeric 3-aminocholest-5-enes, which on conversion to their N-acetyl derivatives and chromatographic separation yielded in a 2 to 1 ratio 3β-acetamidocholest-5-ene (XVII) (m.p. 243°, $[\alpha]_D$ - 45°, and 3α-acetamidocholest-5-ene (XX) (m.p. 184.5°, $[\alpha]_D$ - 59°.

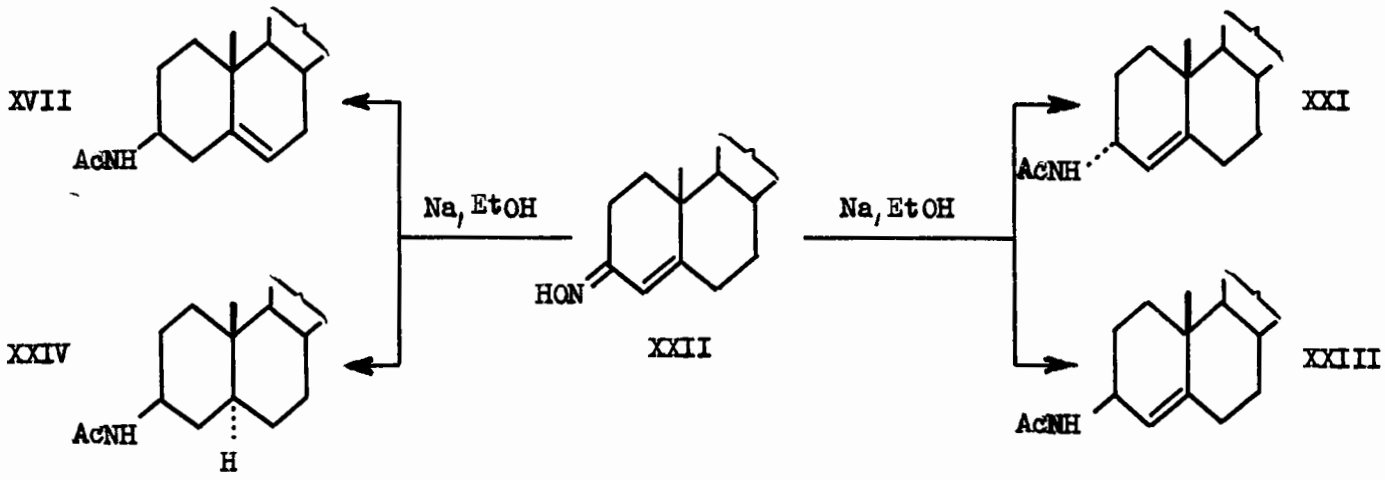


XIX XX XX

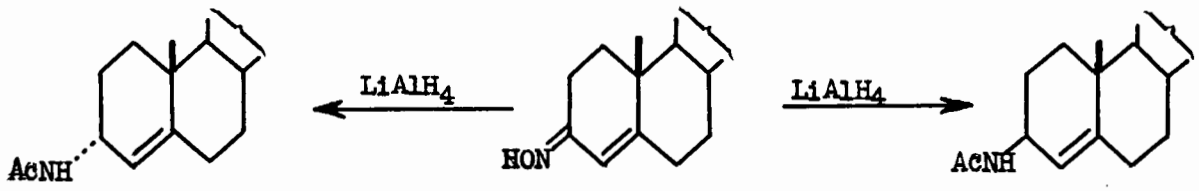
The more negative value for the specific rotation of the α-epimer stood in contrast to the generally more dextrorotatory character of 3α-substituted steroids (68). To explain this apparent discrepancy, Bannard and McKay envisaged the possibility of Walden inversion at $C_{(3)}$. Subsequently, Shoppee and his coworkers (42) reported the preparation of the above epimers in an analogous way; no yields were given. 3α-Acetamidocholest-5-ene had a melting point of 186-189° and $[\alpha]_D$ - 30°. This value for specific rotation was identical with the data obtained previously by two other groups of workers [-30° (67) and -31° (66)], but was very different from Bannard and McKay's value of -59°. If the specific rotation of -30° is correct, then the 3α-epimer is more dextrorotatory than the 3β, and the hypothesis of Walden inversion becomes unnecessary. As Shoppee pointed out, Bannard and McKay's preparation was probably contaminated with some difficultly separable and more levorotatory substance.

Shoppee (42) assigned to the γ fraction the structure

3α-acetamidocholest-4-ene (XXI) (M.p. 190°, $[\alpha]_D$ +97°) which he isolated as one of four products of the reduction of 3-oximinocholest-4-ene (XXII) with sodium and ethanol. The other three substances were the following: 3β-acetamidocholest-4-ene (XXIII) (m.p. 228-230°, $[\alpha]_D$ +7°), 3β-acetamidocholest-5-ene (XVII) (m.p. 239-243°) and 3β-acetamidocholestane (XXIV) (m.p. 240-244°, $[\alpha]_D$ +10°).



3α-acetamidocholest-4-ene (XXI) was also obtained, more conveniently, by reduction of 3-oximinocholest-4-ene (XXII) with lithium aluminium hydride, which yielded both epimers. Bannard and McKay (43) isolated, after acetylation and chromatography of the reduction product, equal amounts of 3β-acetamidocholest-4-ene (XXIII) (m.p. 223.5°, $[\alpha]_D$ + 6.2°), and 3α-acetamidocholest-4-ene (XXI) (m.p. 163-164°, $[\alpha]_D$ + 105.5°). Shoppee and his coworkers (42) separated from an analogous reaction small amounts of the 3α-epimer (XXI) (m.p. 190°, $[\alpha]_D$ + 94°), and large amounts of the 3β-epimer (XXIII) (m.p. 231°, $[\alpha]_D$ + 6°).

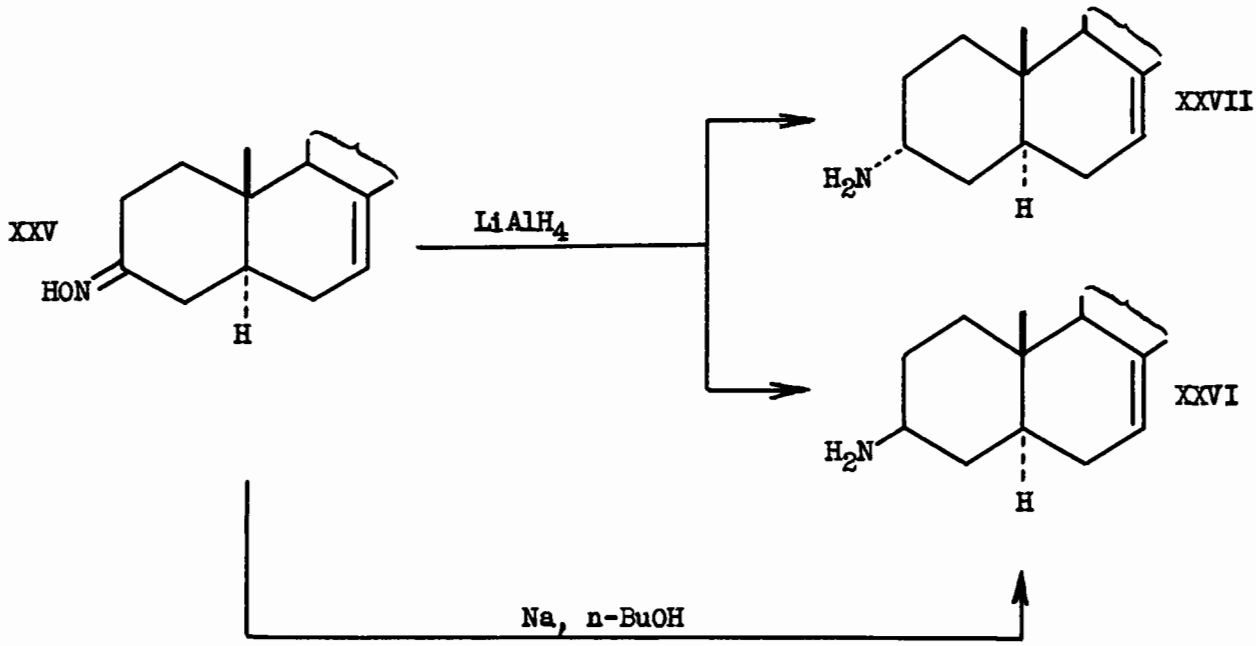


XXI XXII XXIII

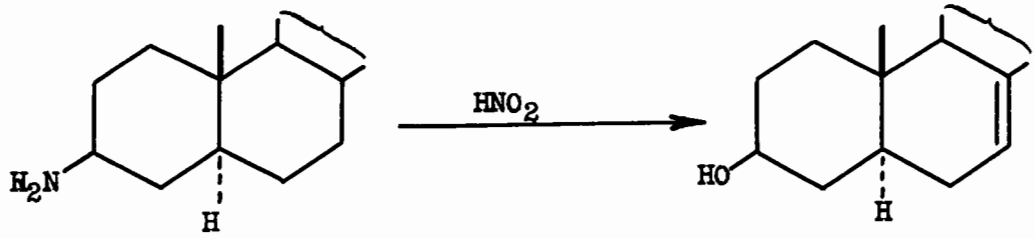
Catalytic hydrogenation of 3β-acetamidocholest-4-ene (XXIII) with platinum in acetic acid gave 3β-acetamidocholestane, and that of 3α-acetamidocholest-4-ene (XXI) yielded 3α-acetamidocoprostane.

Two structures have been tentatively assigned to Windaus' fraction β (major product, m.p. 216°). In 1955, Shoppee (67) expressed the view that it may be identical with 3α-acetamidocholestane previously prepared (m.p. 213°) by Dodgson and Haworth (38) as a derivative of a minor product in the reduction of 3-oximinocholestane with sodium in amyl alcohol. The following year, however, Shoppee (42) postulated that the fraction was 3β-acetamidocholest-4-ene (XXIII). He reported a melting point of 231°, whereas Bannard and McKay (43) gave 223.5°.

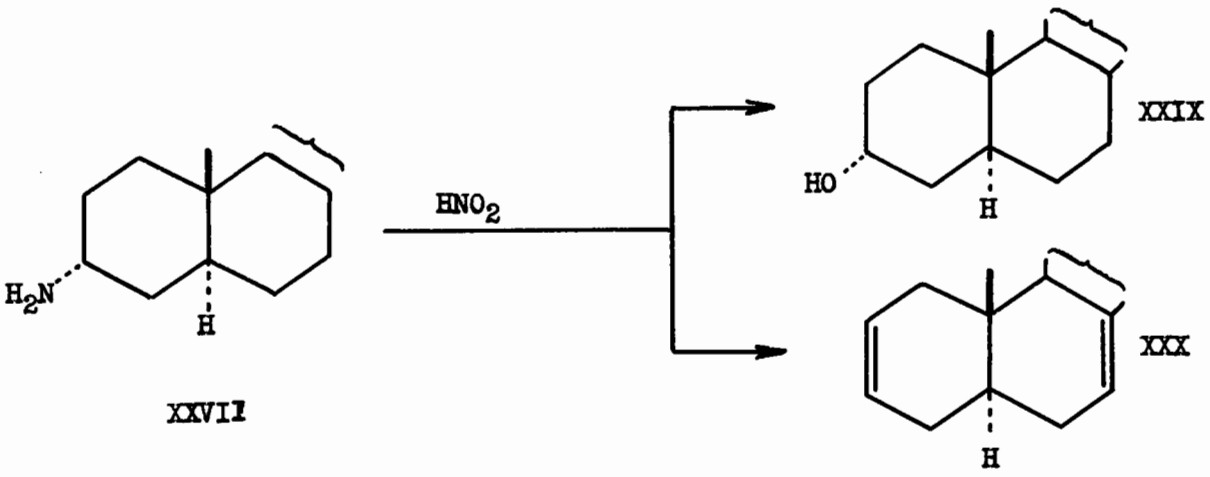
Very recently, Evans and Summers (44) reduced 3-oximinocholest-7-ene (XXV) with sodium in n-butanol, and obtained after chromatography, 3β-aminocholest-7-ene (XXVI) as an oil. Reduction of the oxime with lithium aluminium hydride gave, after chromatography, a 1 to 1 mixture of epimeric 3β- and 3α-aminocholest-7-ene (XXVII), both as oils.



The configuration assigned to the 3α- and 3β-epimers on the basis of the mode of preparation was confirmed by their reactions with nitrous acid. The equatorial amine (XXVI) was deaminated with retention of configuration to 3β-hydroxycholest-7-ene (XXVIII), whereas the axial amine (XXVII), by treatment with nitrous acid, gave 3α-hydroxycholest-7-ene (XXIX) and cholesta-2,7-diene (XXX).



XXVI XXVIII

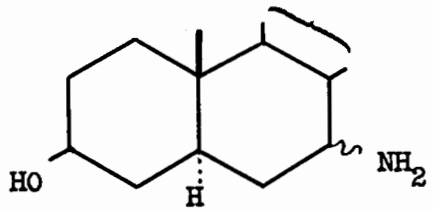


XXVII

In a later paper (69), the authors established that in the steroid series, equatorial amines are deaminated to secondary alcohols with retention of configuration. The axial amines react with retention of configuration and formation of secondary alcohols accompanied by elimination with production of olefinic compounds.

c) Aminocholesterol

In 1935, Eckhardt (70) prepared 7-aminocholesterol (XXXI) by reduction of 7-oximinocholesteryl acetate with sodium in ethanol. His final objective was 7-dehydrocholesterol (3β-hydroxycholest-7-ene) in view of its importance for the synthesis of vitamin D_3. Thermal decomposition of the amine salt or of the quaternary ammonium derivative was expected to yield the desired product. Instead, triple unsaturation resulted ($\Delta^{3,5,7}$).



XXXI

7-Aminocholesterol, as well as its salts, melted over a wide range of temperature, and was considered to consist of a mixture of epimers which could not be separated by crystallization. However, the differences in solubilities of the epimeric N-acetyl derivatives were large enough to allow their separation. N-Dimethyl derivative was also prepared by the author.

In 1941, Windaus and Eckhardt (71) took out a patent in which they described the preparation of 7-aminocholesterol (XXXI) (m.p. 167-170°) from 7-oximinocholesterol by reduction with sodium in ethanol. 7α- and 7β-Acetyl derivatives were reported, but no physical constants were given.

In 1946, Barnett (28) undertook the preparation of various aminosteroids to be tested as bacteriostatic agents. He repeated Eckhardt's work and found that the epimeric N-acetyl derivatives isolated by fractional crystallization melted somewhat higher than had been reported. However, Barnett was unable to obtain the pure epimeric bases from the related N-acetyl compounds. The alkaline treatment did not affect the N-acetyl groups, whereas acid hydrolysis

was either not effective or removed the amino group altogether. The free epimeric bases were eventually separated in about 1 to 1 ratio by direct fractional crystallization from methanol-acetone. 7-Aminocholesterol and its reduction product 7α-aminocholestanol showed appreciable antibacterial activity against Gram-positive organisms. Both epimers of 7-aminocholesterol exhibited equal activity. It should be noted that α and β notations were used arbitrarily by Eckhardt and Barnett.

d) Aminocholestanol

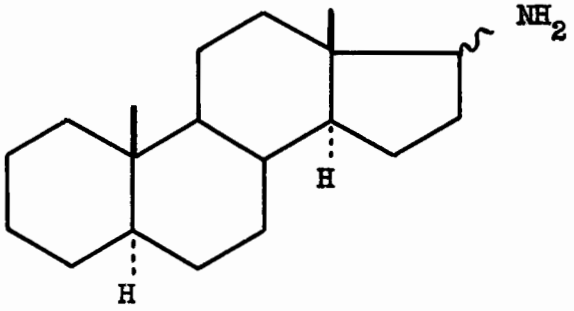
In the second part of his study of aminosteroids, Barnett (29) prepared 6-aminocholestanol by reduction of 3β-acetoxy-6-oximinocholestane with sodium in ethanol. Also a few diaminosteroids were produced in a similar way: 3,7-diaminocholest-5-ene, 3,6-diaminocholestane and 6,7-diaminocholestanol. The diamino compounds proved to be highly active in vitro against Streptococci and staphylococci, and appeared to possess higher bacteriostatic potency than the monoaminosteroids. They also showed considerable antibacterial activity against certain Gram-negative organisms. No physical constants were reported for any of the above mentioned compounds or their salts.

3. Amino derivatives of the androstane series

a) Aminoandrostanes and aminoandrostenes

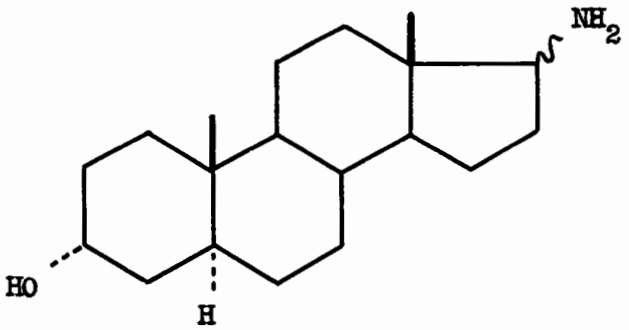
In an attempt to prepare water soluble androsterone derivatives for physiological testing, Marker (72) made two amino compounds. 17-Aminoandrostane (XXXII) was obtained as an oil from the reduction of 3-α-chloroandrostanone oxime with sodium and amyl alcohol. The oil could

be distilled under high vacuum at 110°, and formed the hydrochloride in dry ethereal solution. On diazotation, the base gave 17-hydroxyandrostane, identical to the product formed by the reduction of 3α-chloroandrostanone with sodium in amyl alcohol.



XXXII

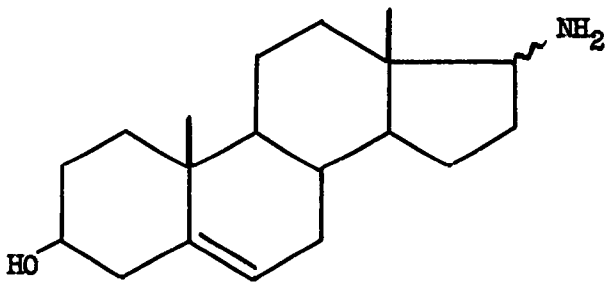
17-Amino-3α-hydroxyandrostane (XXXIII) was prepared in a similar way, from 3α-hydroxyandrostanone oxime. The base was obtained as an oil which distilled under high vacuum at 125°, and formed a crystalline hydrochloride. The base was transformed to the corresponding diazonium salt which, on further hydrolysis, gave 3,17-dione identical to the dione produced by the oxidation of 3β-hydroxyandrostanone.



XXXIII

In the same year, "Ciba" took out a patent (73) describing the preparation of 17-amino-3α-hydroxyandrostanone (XXXIII) from 3α-hydroxyandrostanone oxime by two different ways. Catalytic hydrogenation using platinum in dioxane containing small amounts of hydrochloric acid, gave the hydrochloride salt. The free base was obtained directly, in 73% yield, by reduction with sodium in ethanol. No physical constants of the products were given.

Ruzicka (74) also prepared 17-amino-3α-hydroxyandrostanone (XXXIII) by sodium in ethanol reduction, and succeeded in crystallizing the base, m.p. 187-188°. The hydrochloride melted at 365° (dec.). 3β-Hydroxy-17-aminoandrost-5-ene (XXXIV) and its hydrochloride were produced from 3β-hydroxy-17-oximinoandrost-5-ene in an analogous way. The hormonal activity of XXXIII and XXXIV was very low. Hence, the replacement of the hydroxyl group in 17-position by amino group markedly reduced the physiological properties (74).

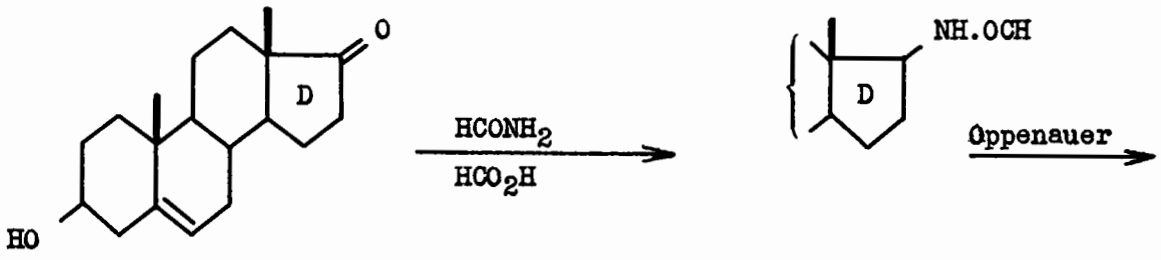


XXXIV

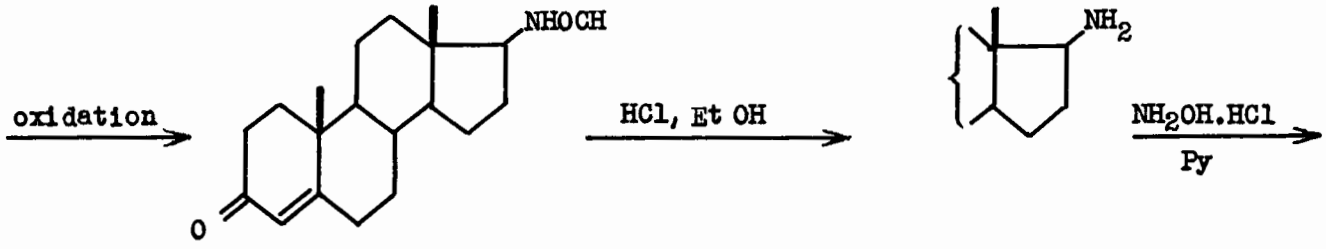
The latter compound was also described in two patents. "Ciba" (75) reported its preparation by the reduction of the corresponding oxime by alkali metal in alcohol. A therapeutical use for this substance was mentioned. "I. G. Farbenindustrie" (76) produced 3β-hydroxy-17-

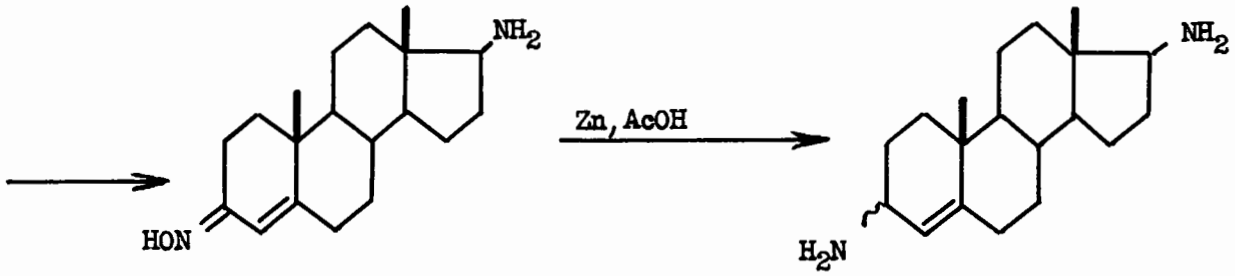
aminoandrost-5-ene (XXXIV) by Beckmann rearrangement of acetylpregnenolone oxime. (No physical constants).

Šorm and Joska carried out the syntheses of four nitrogen analogs of androgenic hormones (77) in connection with their work on the preparation and properties of basic derivatives of steroids (33, 37, 52, 63). 3ξ,17β-Diaminoandrost-4-ene (XXXV) was produced from 3β-hydroxy-17-ketoandrost-5-ene (XXXVI) by the Wallach modification of the Leuckart reaction, followed by reduction of the oxime (XXXVII) in 3-position with zinc shavings in acetic acid. Attempts to reduce the oxime with lithium aluminium hydride failed, and resulted in the recovery of the unchanged material.



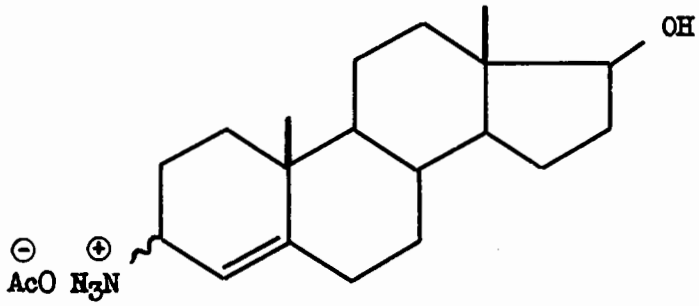
XXXVI





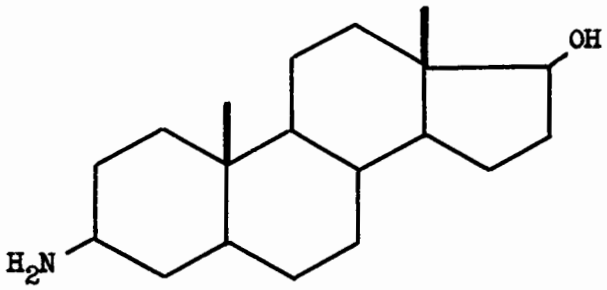
XXXVII XXXV

3ξ-Amino-17β-hydroxyandrost-4-ene was prepared from the related oxime by reduction with zinc in acetic acid. The free base could not be crystallized and was characterized as acetic acid salt (XXXVIII):

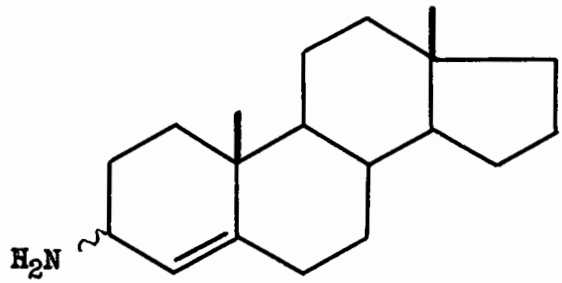


XXXVIII

3β-Amino-17β-hydroxyandrostane (XXXIX) was obtained from the corresponding oxime by reduction with sodium and ethanol, and 3ξ-amino-androst-4-ene (XL) was obtained, as an oil, from the related oxime with zinc and acetic acid. The 3β configuration in XXXIX was allotted by the authors on the basis of its mode of preparation.



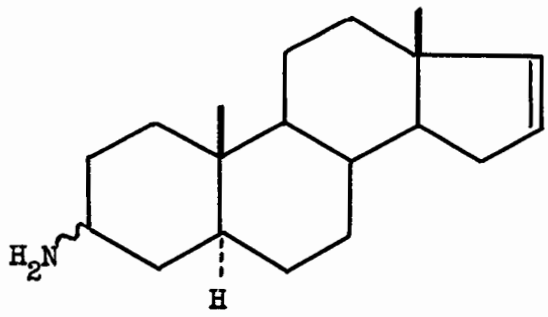
XXXIX



XL

All four amino compounds prepared by Šorm and Joska were devoid of biological activity.

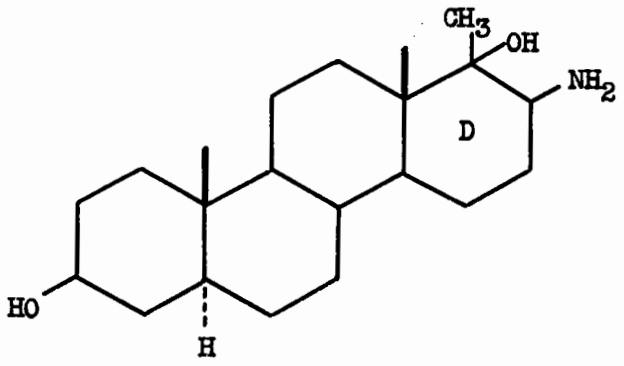
In the nineteen forties, Ruzicka and his collaborators carried out researches concerning the relationship between the odour and the constitution of steroids (78). In particular, the Swiss group studied several natural and synthetic hormonal compounds of the androstane series, and found some of them to exhibit "urea-like" odour. Among various substances, 3-aminoandrost-16-ene (XLI) was prepared by reduction of the corresponding oxime with sodium and ethanol, and was found to be odourless.



XLI

b) Amino-D-homoandrostanes

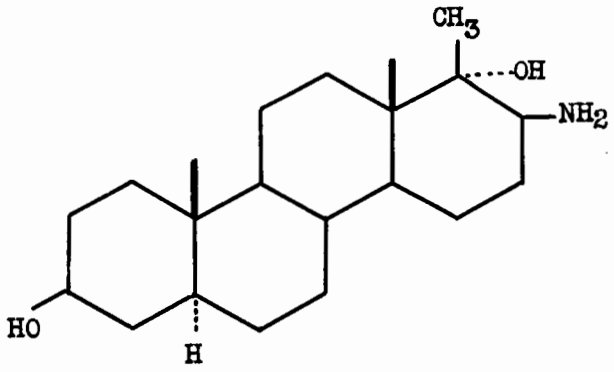
In the course of structural studies concerning the enlargement of ring D upon hydration of C_{17}-acetylenic derivative, Ruziczka (79) was led to reduce 3,17a-dihydroxy-17a-methyl-17-oximino-D-homoandrostane to the corresponding 17-amino compound (XLII) using platinum in acetic acid.



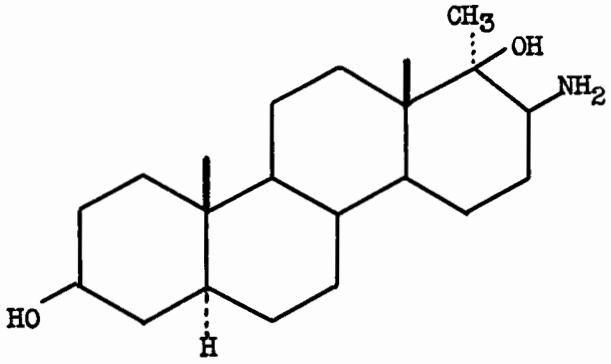
XLII

The axial conformation was assigned by Shoppee and Klyne (80) to the amino group in XLII on the basis of the work of McNiven and Read (61) pertaining to the behaviour of menthylammonium hydroxides on decomposition.

During a study of the methods for the conversion of D-homosteroids into steroids, Shoppee and his coworkers (60) prepared two series of epimeric hydroxyamines by reduction of the corresponding oximes. In the first series, 17β-amino-3β-17aα-dihydroxy-17a-methyl-D-homoandrostane (XLIII) and its 17aα-epimer (XLIV) were obtained by catalytic hydrogenation with platinum in acetic acid, according to Ruzicka's method (79).

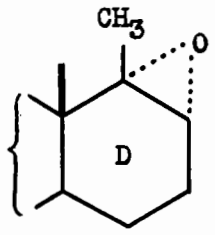


XLIII

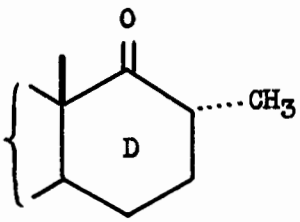


XLIV

The conformations of the substituents in ring D were confirmed by deamination of XLIII with nitrous acid to give an epoxide (XLV), the structure of which had been elucidated by several groups of workers (79,81,82,83). The conformations in XLIV were determined by Klyne (84) who identified the product of deamination of XLIV as 3β-hydroxy-17a-ketone (XLVI):

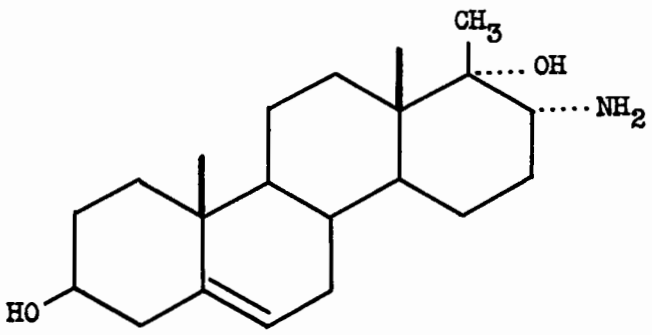


XLV

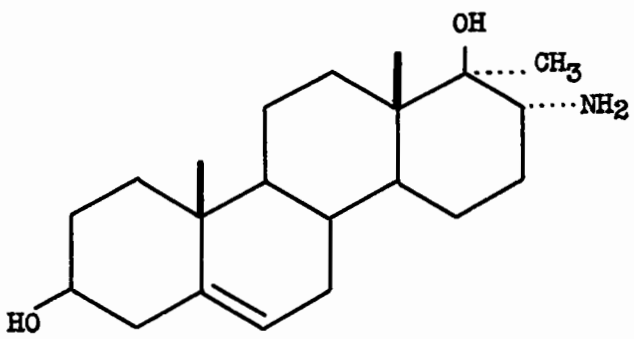


XLVI

The second series was produced by sodium and propanol reduction and gave 17α-amino-3β, 17aα-dihydroxy-17a-methyl-D-homoandrost-5-ene (XLVII) and its 17aβ-epimer (XLVIII):

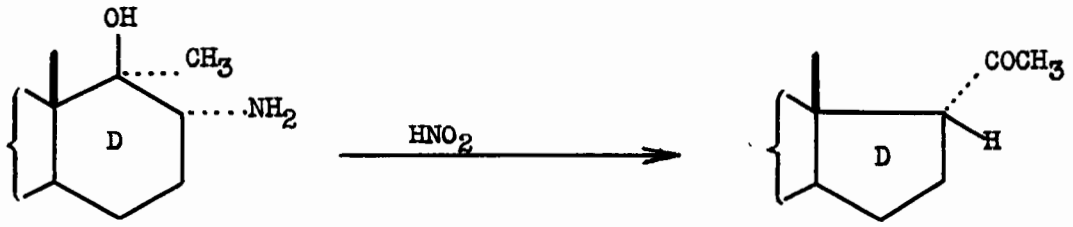


XLVII

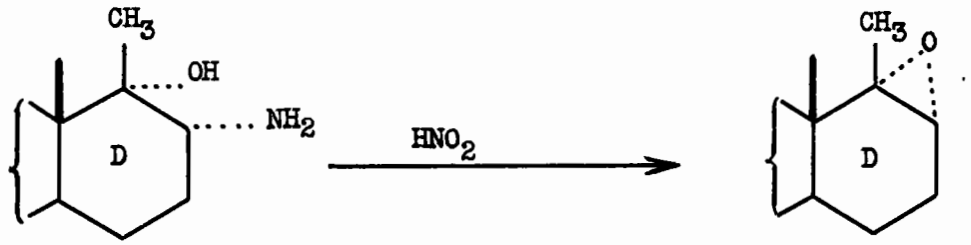


XLVIII

The equatorial conformation was assigned to the amino groups in XLVII and XLVIII on the basis of their mode of preparation (39, 40). If such were the case, the stereo-electronic considerations (transition state requiring coplanarity of the centres involved), suggested that both XLVII and XLVIII should undergo deamination with rearrangement accompanied by ring contraction to 17-isopregnenolone (IL). In fact, XLVIII gave IL in good yield upon treatment with nitrous acid, but, surprisingly, XLVII yielded mostly 17α, 17aα-epoxide (L).



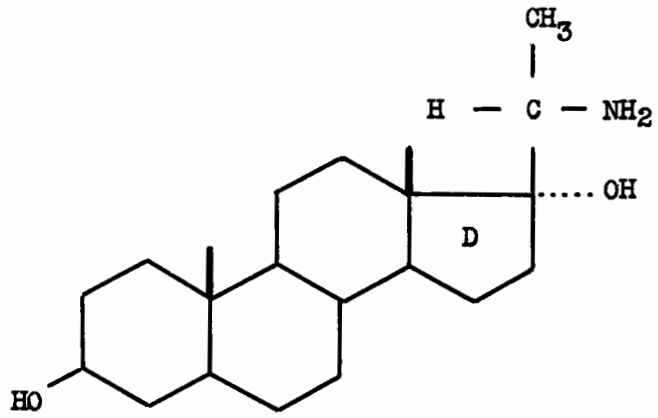
XLVIII IL



XLVII L

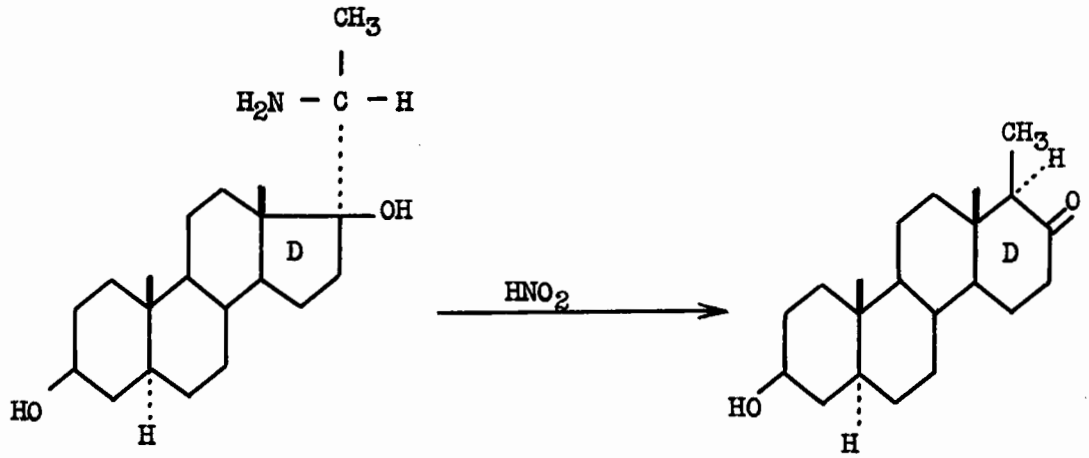
4. Aminopregnanes

Recently, Ramirez and Stafiej (85) studied the stereochemistry of the enlargement of the ring D by deamination with nitrous acid of a steroidal 20-amino alcohol having an assymmetrical centre at C_{20}. 3β,17α-Dihydroxy-20α-aminoallopregnane (LI), together with a small amount of 20β-epimer, was obtained by the catalytic reduction of the related 3-monoacetate-20-oxime using platinum in acetic acid.



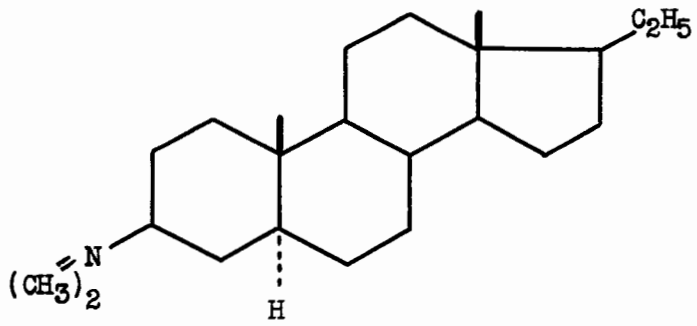
LI

The 20α-configuration was assigned to the predominant, higher melting isomer, on the basis of the more levorotatory character of its acetate as compared to that of the 20β-epimer. The authors also prepared, in an analogous way, a 20-amino alcohol having the 17β-hydroxy configuration (86). Catalytic hydrogenation of 3-monoacetate-20-oxime yielded stereochemically pure 3β,17β-dihydroxy-20β-amino-17-isoallopregnane (LII). The 20β-configuration was allotted on the basis of mechanistic considerations of the course of the nitrous acid deamination of (LII), the product of which was shown to be the more stable 3β-hydroxy-17aβ-methyl-D-homoandrostan-17-one (equatorial CH_3) (LIII):



LII LIII

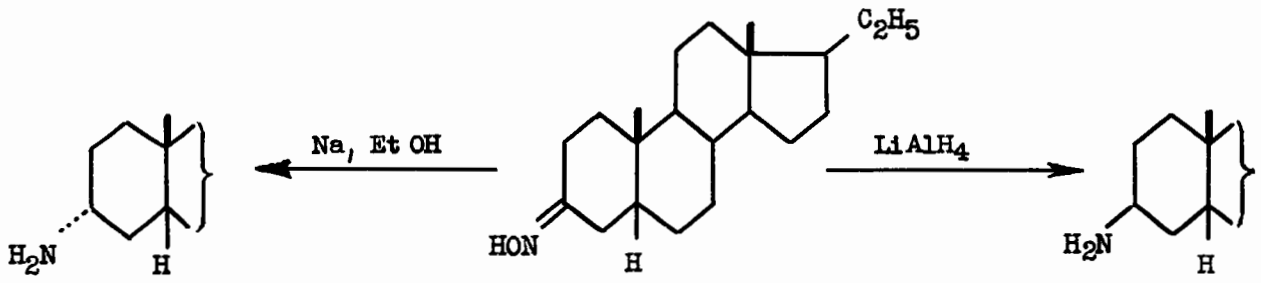
In the course of their studies on the constitution of conessine, Haworth and his coworkers (36) synthesized several amino derivatives of steroids and identified them with certain degradation products of the alkaloid. They prepared, among other substances, 3β-dimethylaminoallopregnane (LIV) from 3-oximinoallopregnane by reduction with sodium and amyl alcohol. No physical constants were reported for the intermediate 3β-aminoallopregnane which was methylated in crude state with formic acid and formaldehyde.



LIV

The conformation of the 3-dimethylamino group was confirmed by a Hofmann degradation. 3β-Dimethylaminoallopregnane was recovered unchanged from this reaction in high yield, whereas the 3α-epimer yielded allopregnene, as expected.

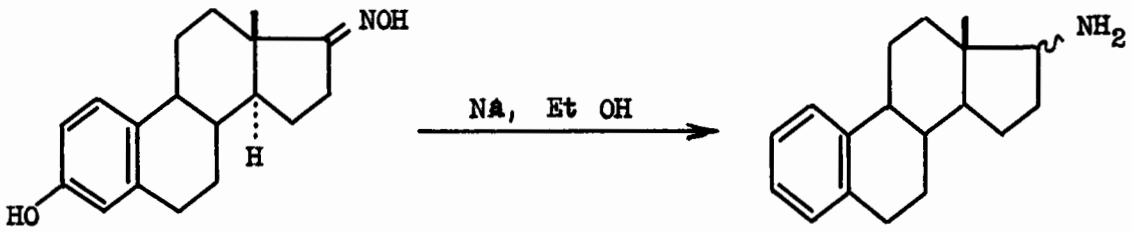
The isomeric bases of the normal pregnane series were synthesized by Shoppee and his collaborators (67) in connection with the studies of the replacement reactions at $C_{(3)}$ in Δ^5-steroids. 3α-Aminopregnane (LV) was prepared by reduction of the corresponding oxime (LVI) with sodium and ethanol. The epimeric 3β-aminopregnane (LVII), was the only product isolated from the lithium aluminium hydride reduction of the oxime. N,N-Dimethyl- and N-acetyl- derivatives of both epimers were also prepared by the authors. No yields were reported for the above products.



LV LVI LVII

5. Amino-oestra-1,2,5(10)-trienes

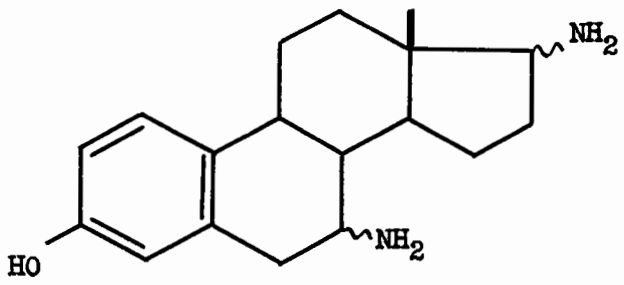
Lettré (87) carried out the reduction of equilin oxime (LVIII) with sodium in ethanol, and obtained 17ξ-amino-3β-hydroxyoestra-1,2,5(10)-triene (LIX) for which neither physical constants nor analysis were given.



LVIII LIX

The preparation of the same compound LIX was reported earlier (88) by sodium in ethanol reduction of oestrone oxime. Physical constants and analysis were not given.

Lettré also synthesized (87) 7ξ,17ξ-diamino-3β-hydroxyoestra-1,3,5(10)-triene (LX) by reducing 7-ketooestrone dioxime in an analogous way (no physical constants or analysis).

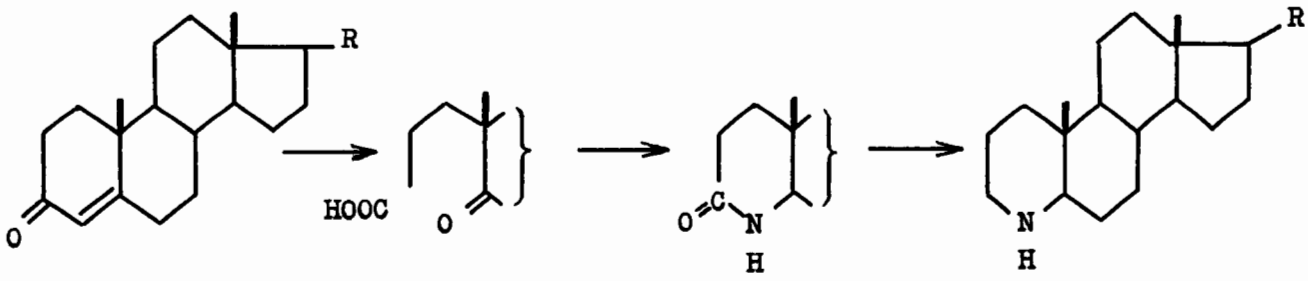


LX

3γ of LX per cc. was found to bring about vacuolization of chick heart fibroblast. When the hydroxyl group at $C_{(3)}$ was replaced by a methoxy group, the resulting derivative inhibited mitosis in doses of less than 1 γ per cc.

6. Steroids containing a nuclear nitrogen atom

Bolt (89) synthesized two steroidal compounds possessing a nuclear nitrogen atom in ring A. The base LXI was obtained from cholest-4-ene-3-one (LXII) which on ozonolysis, gave the ketocarboxylic acid LXIII in good yield. The oxime of the ketoacid was then reduced with sodium and ethanol to the lactam LXIV which was further reduced with sodium and amyl alcohol to LXI. An analogous series of reactions with testosterone acetate (androst-4-en-17-acetoxy-3-one) (LXV) yielded the base LXVI:



LXII: R = C_8H_{17} LXIII LXIV LXI: R = C_8H_{17}

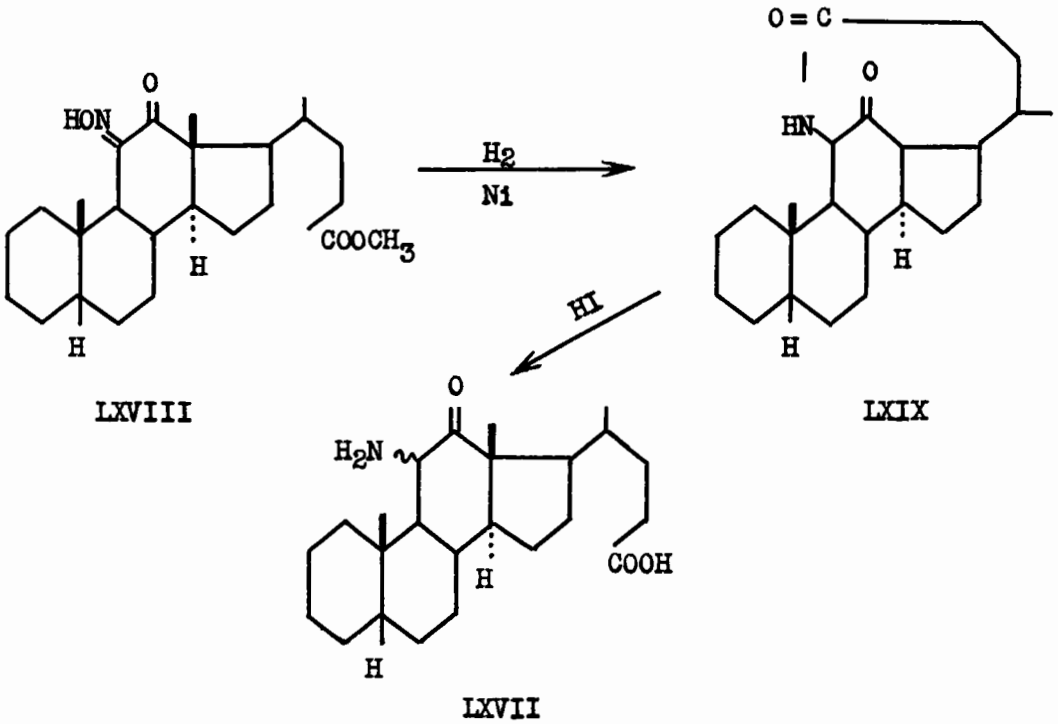
LXV: R = $OCOCH_3$ LXVI: R = OH

7. Amino derivatives of bile acids

It is well known that the bile acids (90) and their salts (91) exhibit bacteriostatic action in vitro, and this fact has motivated the syntheses of basic derivatives of these acids.

The introduction of amino groups into the nucleus has been chiefly accomplished by reduction of the corresponding oximes with sodium and alcohols (27, 30, 31, 92). A few basic derivatives of bile acids formed in this manner and some of their esters were found to exhibit antibacterial activity (31).

The first steroidal amino acid, 11-amino-12-ketocholanic acid (LXVII) was synthesized by Reichstein and Barnett (93,94), during their studies on the keto function in the 11-position of the steroidal nucleus. Those workers reduced methyl 11-oximino-12-ketocholanate (LXVIII) by Raney nickel under 80 atm. pressure at 100°, and obtained the related hydroxylactam (LXIX) which upon hydrolysis with hydroiodic acid, yielded the amino acid (LXVII):

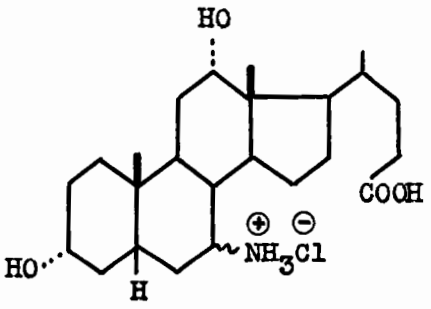


LXVIII LXIX

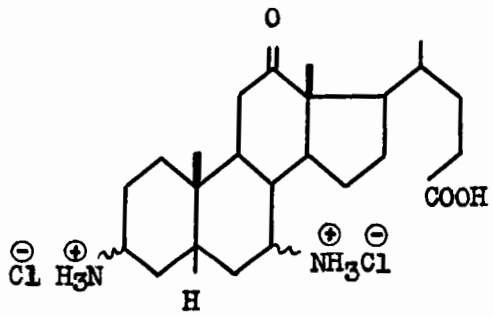
LXVII

The amino acid was identified by means of its methyl ester and N-acetyl derivative.

In 1946, Webb and his coworkers (27) began a series of investigations related to the syntheses of basic derivatives of various hydroxy cholamic acids. Two compounds containing amino groups in the nucleus were prepared. 7ξ-Amino-3,12-dihydroxycholamic acid hydrochloride (LXX) (m.p. 265-267°) was obtained from the corresponding oxime by reduction with sodium in amyl alcohol. A diamino-keto-acid, presumably 3ξ,7ξ-diamino-12-ketocholamic acid dihydrochloride (LXXI) (no melting point given), was prepared from the trioxime, derived from dehydrocholic acid, by an analogous process followed by the removal of the residual oximino group. The conformations of the amino groups were not ascertained.



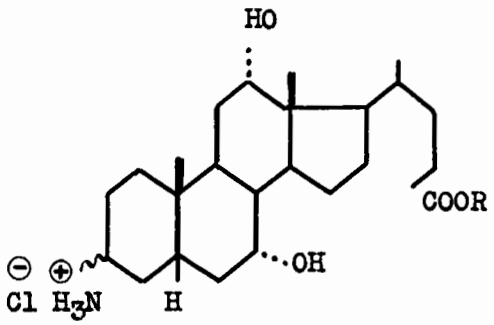
LXX



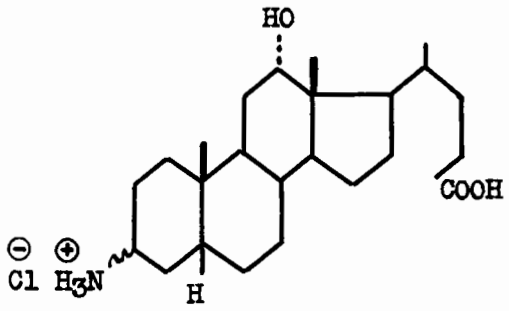
LXXI

The above amino acids were inactive in vitro against Staphylococcus aureus and Lactobacillus helveticus.

In 1949, Webb's group (31) prepared two amino derivatives of hydroxycholanic acids containing the basic group in 3-position. 3ξ-Amino-7,12-dihydroxycholanic acid hydrochloride (LXXII) (R = H), and 3ξ-amino-12-hydroxycholanic and hydrochloride (LXXIII) were prepared by reduction of the corresponding oximes with sodium and amyl alcohol. In the preparation of LXXII, a second crystalline product was obtained which contained no nitrogen and was not investigated further. The conformations of amino groups were not determined.



LXXII

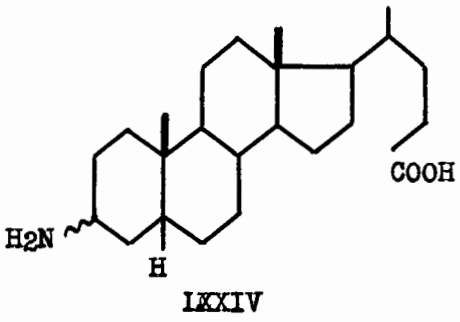


LXXIII

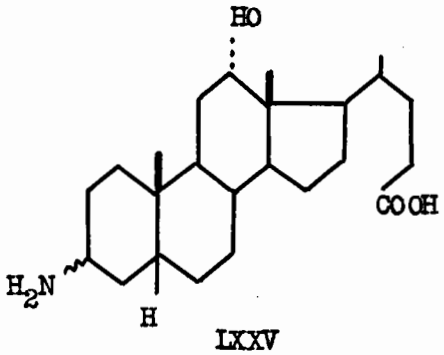
The isoamyl ester of 3ξ-amino-7,12-dihydroxycholanic acid (LXXII; R = iso-C_5H_{11}), showed marked activity against Staphylococcus aureus.

In the same year, a group of French industrial workers (30) described the preparation of five monoaminosteroids in the hydroxycholanic acid series by reduction of the related oximes. Two of those compounds had been previously synthesized by Webb and his coworkers (27, 31) and isolated as hydrochlorides. They were the following: 7ξ-amino-3α,12α-dihydroxycholanic acid hydrochloride (LXX), m.p.: Webb's 265-267°, French group's 275°, and 3ξ-amino-7α,12α-dihydroxycholanic acid hydrochloride (LXXII). The French workers were also

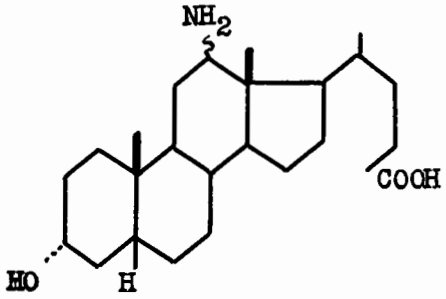
able to obtain the above two compounds as free amino acids. Other products prepared by the same group were 3ξ-aminocholanic acid (LXXIV), 3ξ-amino-12∝-hydroxycholanic acid (LXXV) and 12ξ-amino-3∝,7∝-dihydroxycholanic acid (LXXVI). The authors did not attempt to separate the∝and β epimers, nor did they ascertain the conformations of the amino groups:



LXXIV



LXXV

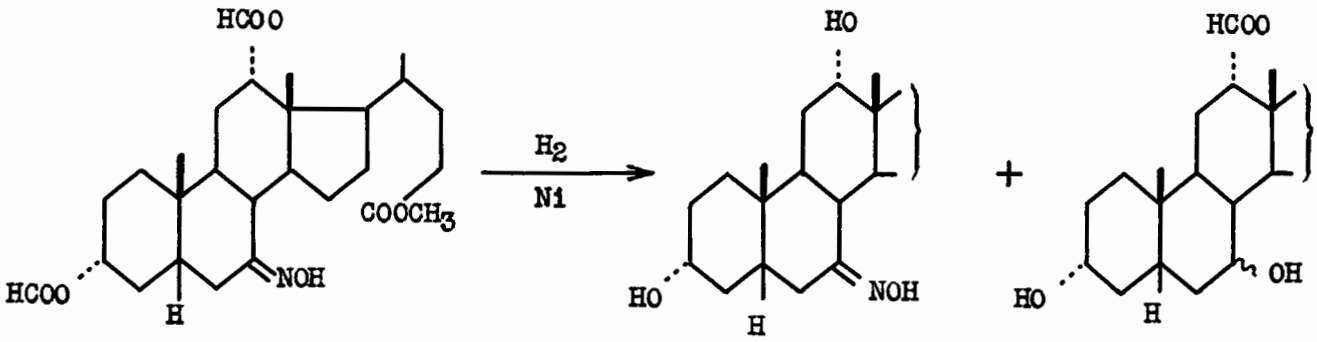


LXXVI

N-Acetyl derivative of LXXIV, and hydrochlorides of LXXV and LXXVI were also prepared.

The authors tried unsuccessfully to reduce the oximinoacids in ethanolic solution by catalytic hydrogenation under pressure using Raney nickel. When methyl 7-oximino-3∝,12∝-diformoxycholanate

(LXXVII) was subjected to this treatment, two pure compounds were obtained after chromatography; the major one was identified as methyl 7-eximino-3∝,12∝-dihydroxycholanate (LXXVIII), and the other was postulated to be methyl 3,7-dihydroxy-12-formoxycholanate (LXXIX) on the basis of the analytical result and absence of nitrogen.

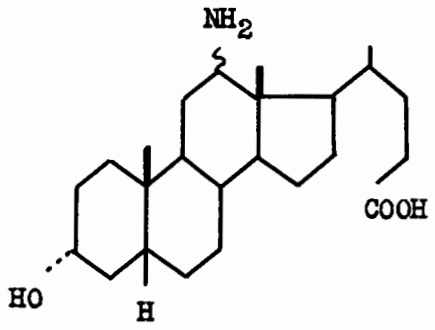


LXXVII LXXVIII LXXIX

In the first case, the reaction resulted in a complete deformylation with formation of LXXVIII. In the second case, partial deformylation occurred and the oxime group at $C_{(7)}$ was transformed to a secondary alcohol through the intermediate of ketimine and ketone. The mechanism of the formation of a secondary alcohol from an oxime was based on the results of Mignonac (95, 96), who had studied reductions of oximes very extensively. He had been able to isolate and identify certain products formed in the reductions of various ketoximes. Ketimines were found

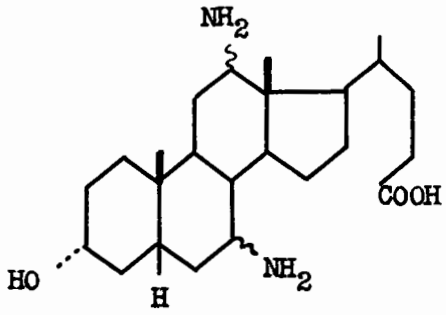
to be key intermediates in the reduction process, and the ratio of major products to secondary ones (e.g. secondary amines, ketones, secondary alcohols) depended upon their stabilities. Paul (97) isolated cyclohexanol as a side product in the catalytic reduction of cyclohexnone oxime to cyclohexylamine.

McPhillamy and Scholz (98) used the Hofmann degradation of an amino-compound as a means of introducing a double bond into steroids. In order to prepare a Δ^{4}-steroid, they synthetized 3α-hydroxy-12ξ-aminocholanic acid (LXXX) by reduction of the corresponding oxime with sodium and isoamyl alcohol.



LXXX

In 1954, the reduction of 3α-hydroxy-7,12-dioximinocholanic acid to 3α-hydroxy-7ξ,12ξ-diaminocholanic acid (LXXXI) was reported (92). The best yield was obtained with sodium amalgam; sodium in ethanol or Raney nickel gave poorer yields, and catalytic hydrogenation using platinum failed altogether.



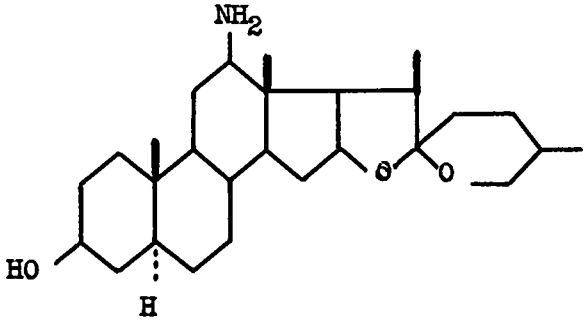
LXXXI

8. Amino derivatives of sapogenins

Recently, Heusser and his coworkers (99) studied the mechanism of Demjanow deamination.

Previously, Shoppee and his collaborators (60) had investigated semipinacolic deamination of several epimeric ∝-hydroxy amines in the D-homo-steroid series (cf. p. 29). The authors postulated, on the basis of stereo-electronic considerations, the operation of a process involving a diazonium ion rather than a carbonium ion intermediate.

The work of the Swiss group provided additional evidence supporting Shoppee's view. For their study, Heusser and his coworkers chose an amine which in case of rearrangement would yield a known product. This amine was prepared from hecogenin oxime by reduction with sodium and prepanol. The equatorial conformation of the amino group in the reduction product, 3β-hydroxy-12β-amino-22a, 5∝-spirostan (LXXXII), was assigned on the basis of the mode of preparation (40, 39).



LXXXII

Correlation of infrared spectra with the structure of some keto and nitrogen derivatives of steroids.

1. Infrared absorption spectra of ketosteroids, bile acids and their esters

A survey of the literature shows that a number of spectra of methyl and ethyl esters of bile acids have been recorded, but only a few spectra of free acids have been determined. The measurements have been carried out in solution. In this connection, it should be pointed out that carbonyl stretching vibrations being solvent sensitive, are displaced to lower frequencies in chloroform, as compared with carbon tetrachloride and carbon disulfide solutions.

Jones, Dobriner and their coworkers (100) recorded the spectra of several hydroxy- and polyhydroxy-cholanic acid esters. The authors studied particularly the C-O stretching vibrations of the carbonyl group of the carboxylic esters which were responsible for a band observed at 1742cm^{-1} in carbon disulfide and at 1732cm^{-1} in chloroform solutions. On the other hand, the position of the carbonyl maximum in the ketosteroids is known to be in the range 1754-1666 cm^{-1}, so that some overlapping can occur between the two bands, due to the keto function in esters and ketones. Therefore, the differentiation between a ketosteroid and a steroid ester is not always possible in this region of the spectrum. The carbonyl group of a carboxylic ester was found to be less sensitive to the position of substitution in the steroidal nucleus than the carbonyl group of the keto function.

The experimental evidence of the influence of the molecular environment on the frequency of the C-O stretching vibrations in dicarbonyl compounds showed that no interaction existed between carbonyl groups at positions 3 and 17, and 3 and 20. However, displacements of absorption maxima to higher frequencies were observed when the keto groups were separated by not more than two carbon atoms (101).

The authors (102) demonstrated the usefulness of correlations between carbonyl band intensities and molecular structure. In di- and poly-carbonyl compounds, the carbonyl intensities are essentially additive. Intensity measurements in solutions, therefore, permit the number of carbonyl groups in a compound to be determined even when the bands overlap completely.

The study of infrared absorption spectra of carbon disulfide solutions of a number of ketosteroids and steroid esters inferred the carbonyl band maxima for specific positions in the nucleus or in the side chain. The band at 1719-1717cm^{-1} was assigned to 3-ketone, at 1719cm^{-1} to 7-ketone, at 1716-1710cm^{-1} to 11-ketone, at 1710cm^{-1} to 12-ketone, at 1726cm^{-1} to 11,12-diketone, and at 1742-1735cm^{-1} to alkyl esters of cholanic, norcholanic, bisnorcholanic and related unsaturated acids (101).

In a later study, (103) four steroid carboxylic acids were examined in carbon disulfide solutions. Two of them were derivatives of oestra-1,3,5(10)-triene and showed maxima at 1704-1703cm^{-1}, and a weak band at 1750-1748cm^{-1}. Etioallocholanic acid had a maximum at 1703cm^{-1} and a weak band at 1754cm^{-1}; cholanic acid exhibited a strong

band at 1710cm^{-1}. No interpretation was given for the weak component bands observed in the spectra of three of the acids. It should be noted that multiple absorption bands had been recorded in this region of the spectrum in the vapour spectra of simple carboxylic acids (104).

Jones and his collaborators (105) have recently studied the infrared spectra of ketosteroids below 1350cm^{-1}, and were able to establish certain correlation between the location of functional substituents, unsaturation and position of the bands. However, the region 1800-1650cm^{-1} is still preferred for the most effective characterization of these compounds.

Wootton (106) examined the infrared spectra of a number of esters of bile acids, and some related carbinols which had markedly different side chains. He found that all spectra exhibited close resemblance in the range 1300-900cm^{-1}. Therefore, the value of the "fingerprint region" for unequivocal identification of compounds was rendered doubtful, especially for substances not highly purified. Investigated materials could be distinguished only in the 1500-1300cm^{-1} methylene and methyl C-H bending region.

2. The infrared spectra of oximes

The infrared spectra of several oximes have been reported, but a review of the literature seems to indicate that no such studies have been carried out in the steroid series. Most of the substances have been investigated as mulls in paraffin oil or in solution.

There are four spectral regions of interest in connection with oximes, and they are related to the OH stretching mode, C = N vibrations, OH deformation (bending) and N-O stretching vibrations.

REGION I -

The O-H stretching mode occurs in the range 3500-3200cm^{-1}, depending upon the degree of association through hydrogen bonding (107, 108, 109). The higher frequency values correspond to free OH valence vibrations. It is not known whether the hydrogen bonds are of N---H-O or H-O---H type.

Palm and Werbin (109) investigated several isomeric oximes in the solid state and in solution. They were able to differentiate between α and β isomers on the basis of the associated OH frequency which was lower in the β (near 3115cm^{-1}) than in the α (3250cm^{-1}) isomer.

REGION II -

The C = N stretching vibration occurs within the range 1690-1640cm^{-1} (110). Aliphatic oximes have an absorption band near 1670 cm^{-1} and a displacement of 30-40cm^{-1} towards lower frequency takes place in aromatic compounds. The C= N frequencies are independent of ring substitution (109). Cyclohexanoneoxime shows a strong band at 1669cm^{-1} (111). The C = N absorption bands are sometimes of low intensity. An explanation was offered by Goubeau and Fromme (112) who assumed the existance of the following resonance structures I and II:

I II

The form I must be predominant because of its lower dipole moment. However, the contributing nitrone form II reduces the double bond character of the C-N linkage, which results in the weakening of the absorption band due to C = N vibration.

REGION III -

The O-H bending (deformation) mode has been found between 1440-1020cm^{-1} by various workers (113,114,115). The work of Voter and his coworkers (116) using 1,2-cyclohexanedione dioxime and 1,2-cycloheptanedione dioxime as mulls, showed that deuteration of the above oximes caused the disappearance of a strong band around 1300cm^{-1}, which would then correspond to the O-H bending mode.

More recently, Palm and Werbin (117) studied the spectra of isomeric substituted benzaldoximes and their O-methyl and N-methyl ethers in the solid state and in solution. The solution spectra of α-isomers showed a band at 1265cm^{-1} which the authors assigned to the O-H bending mode, and tentatively to the in-plane O-H bending. If the above assignments are correct, it follows that there is a shift of the band in solution towards a lower frequency (from around 1300cm^{-1} to 1265cm^{-1}). This phenomenon had been observed by other workers (118, 119), in the O-H bending region where the effect of hydrogen bonding is opposite to that in the O-H stretching region.

REGION IV

The N-O stretching vibration has been assigned to 940-900cm^{-1} (112, 117, 120, 121, 122) and to 850-800cm^{-1} (117, 122, 123, 124, 125) regions. Bands in the latter region have been allotted by Hadži (126) also to the O-H out-of-plane deformation mode on the basis of their disappearance upon deuteration from the spectra of benzoquinone oxime and 1,4-naphtaquinone oxime.

Dioximes

Voter and his coworkers (116) reported the spectra of the dioximes of 1,2-cyclohexanedione, 1,2-cycloheptanedione and 2,3-butanedione taken as mulls in nujol and in perfluorokerosene. Absorption maxima corresponding to C = N stretching vibrations were of very low intensity and presented a single band in all cases.

Duyckaerts (127) recorded the spectrum of dimethylglyoxime in the solid state taken in paraffin oil. The absence of any bands due to C = N stretching vibrations was attributed to a strong hydrogen bonding.

3. The infrared spectra of amino acids and their hydrochlorides

The infrared studies of amino acids have been carried out quite extensively, mainly because of the stimulus provided by the biological importance of these compounds. The literature concerned has been reviewed during the past few years by several authors (128, 129, 130).

a) Monoamino acids

Monoamino monocarboxylic acids which normally exist as dipole ions ($^{-}OOC.....NH_3^{+}$) show characteristic absorption in five spectral

regions. Because of their generally low solubilities, they have been investigated mostly in the solid state in the form of suspensions in paraffin oil or as fused films.

REGION I

A broad band between 3180 and 3030cm^{-1} has been assigned to NH_3^+ stretching vibrations. This is below the usual N-H stretching region 3500-3300cm^{-1}. The primary aliphatic amino acids show absorption between 2630 and 2500cm^{-1} (131). A band at 2100 cm^{-1} has been observed in the spectra of the simple amino acids (132).

REGION II

Weak bands have been observed in the 3000-2000cm^{-1} region in the spectra of most amino acids. They seem to correspond to the NH_3^+ group since the hydrochlorides also absorb in this range.

REGION III

Two bands in the ranges 1640-1535 and 1520-1490cm^{-1} have been identified with N-H bending vibrations, which correspond to the NH_3^+ structure. Absorption at higher frequencies has been related to the symmetrical vibrations and those occurring at lower frequencies to unsymmetrical vibrations. The lower of the two bands is usually more intense. However, the overlapping strong absorption of the carboxylate ion between 1600 and 1550cm^{-1} may obscure the bands due to the NH_3^+ ion (128, 129, 134, 135, 136).

REGION IV

A strong band between 1600-1550cm^{-1} was found to correspond to the ionized carboxyl group. This band is suppressed upon formation

of a hydrochloride, whereas the acid carbonyl group absorbing in the range 1750-1700cm^{-1} is released.

A convenient method of confirmation of identification of the bands correlated to the NH_3^+ group in the regions 1640-1535, 1520-1490cm^{-1} is accessible in this manner.

REGION V

An unassigned band near 1300cm^{-1} has been found to be present in most spectra of amino acids. However, its value for the identification purposes seems to be doubtful.

b) Amino acid hydrochlorides

Besides the above mentioned bands of the NH_3^+ group, the hydrochlorides have appeared to exhibit specific absorption in the range 3050-2000cm^{-1}.

According to Randall and his coworkers (128) a series of bands between 3030 and 2500cm^{-1}, and also in the 2140-2080cm^{-1} region has been shown by most amino acid hydrochlorides.

In their study of aliphatic primary amines and their acetates and hydrochlorides, Depas, Khaladji and Vergoz (133) observed bands in the range 2565-2400cm^{-1}, and near 2000cm^{-1} in the spectra of the salts.

Sandorfy (130) reported that amine salts show two or more bands between 2800 and 2000cm^{-1} which may be quite characteristic of a given compound.

As already mentioned above, the carboxylate ion absorption (1600-1550cm^{-1}) is replaced in the hydrochlorides by the one due to the carbonyl group (1750-1700cm^{-1}).

c) Polyamino monocarboxylic acids

Since in polyamino monocarboxylic acids only one amino group participates in the formation of dipole ion, the spectra of these compounds would be expected to show the absorption characteristic of a free amino group in addition to that due to the NH_3^+ structure and to the carboxylate ion (COO-).

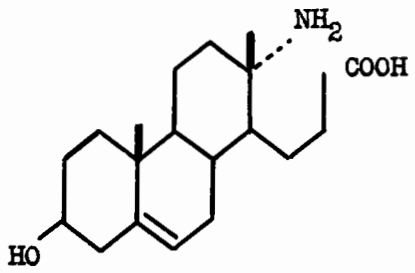
The primary amino group has been known to give rise to the N-H stretching vibration in the range 3500-3300cm^{-1} and to a band in the region 1650-1560cm^{-1} relative to N-H bending vibrations (128, 129).

Comparatively little is known at present about those complex compounds. Ornithine, which is a diaminocarboxylic acid, has been studied by Larsson (131) in the form of its hydrochloride. Surprisingly, the spectrum showed a band at 1550cm^{-1}, even though the carboxylic group was unchanged. Arginine, which contains=NH as well as NH_3^+ groups, showed stronger absorption in the range 1615-1590cm^{-1} than monoaminocarboxylic acids.

d) Steroidal amino acids

A survey of the literature shows that the infrared spectra of steroidal amino acids and amines have been recorded only in two instances.

Heusser and his collaborators (137) reported the spectrum of 3β-hydroxy-13α-amino-13,17-seco-androst-5-ene-17-acid (LXXXIII) prepared according to a German patent (138):



LXXXIII

The spectrum taken in mujol contained the following bands 3330cm^{-1} (m) (≥NH, ≥CH-OH), 2110 and 1557cm^{-1} (m) (betain), 1590cm^{-1} (s) (COO^-), 1557 and 1308cm^{-1} (s) (amino acid).

During a study of Demjanow deamination, Heusser and his coworkers (99) were led to synthesize an aminospirostan (LXXXII) whose infrared spectrum they obtained. The following bands were observed: 3380cm^{-1} (m) (C-OH, C-NH) and 1645cm^{-1} (m) (NH_2).

DISCUSSION

Part I - The Mannich reaction of ketosteroids

It is known that the activity of acidic hydrogens in substitution reactions, e.g. replacement by a halogen or by a metal, determines the ease with which a substance participates in the Mannich condensation.

The objective of this phase of the present research was to study the reactivity of hydrogen atoms in rings A and B of some steroidal ketones in the Mannich condensation. These hydrogen atoms were adjacent to the activating keto groups in positions 3 or 6, or both. The ketosteroids were derived from cholesterol and belonged to the unsaturated and saturated groups. The former group included cholest-5-en-3-one, 4,4-dimethylcholest-5-en-3-one and cholest-4-en-3-one. During the course of the work, it was felt that the high degree of reactivity of the methylene group in 4-position in cholest-5-en-3-one might have been responsible for rapid formation of oily materials under varied conditions. It was decided, therefore, to examine the reactivity of the methylene group in 2-position in a compound in which the position 4 would be rendered inactive. This was accomplished by methylation of cholest-5-en-3-one. The resulting 4,4-dimethylcholest-5-en-3-one proved to be totally unreactive in the Mannich reaction under conditions analogous to those used in the case of its precursor.

The investigation of cholest-4-en-3-one was undertaken in view of the importance of the "Δ^4-3-one" type of compounds in the classes of sex and adrenal hormones. The stability of the above structure is due to the conjugation present in the molecule. It is known that cholest-5-en-3-one easily isomerizes to cholest-4-en-3-one with the migration of the double

bond to the position of conjugation with the keto group. In the Mannich reaction, the latter ketone behaved similarly to testosterone (18) and progesterone (18), failing to give any definite condensation products.

The attention was then directed to saturated ketones. In this group, cholestan-3-one and cholestane-3,6-dione were studied. By analogy with acid-catalyzed halogenation of ketones (139,140), which is an electrophilic substitution, it was presumed that the Mannich condensation would proceed through the enolization of the compounds under investigation. In the unsaturated ketones, the enol form is stabilized by two conjugated double bonds. This effect is absent in the saturated ketones which were, therefore, expected to exhibit lesser reactivity with respect to their acidic hydrogen atoms. In fact, cholestan-3-one and cholestane-3,6-dione did participate in an abnormal Mannich reaction with formation of the corresponding methylene-bis-ketones. These reactions occurred in the presence of secondary amine hydrochlorides. Unreacted ketones were recovered when free amines were used instead of their salts. Analogous results were obtained when the ketones were allowed to react with paraformaldehyde in neutral or acidic media.

1. Mechanism of an abnormal Mannich reaction

A consideration of the experimental evidence leads to the postulation of two mechanisms by which cholestan-3-one and cholestane-3,6-dione may react with formaldehyde. The first scheme (Fig. 1) is presumed to represent an abnormal Mannich condensation (e.g. cholestan-3-one) in an acid-base catalyzed system. Although proof of an intermediate Mannich base is lacking, several similar reactions leading to methylene-bis-molecular

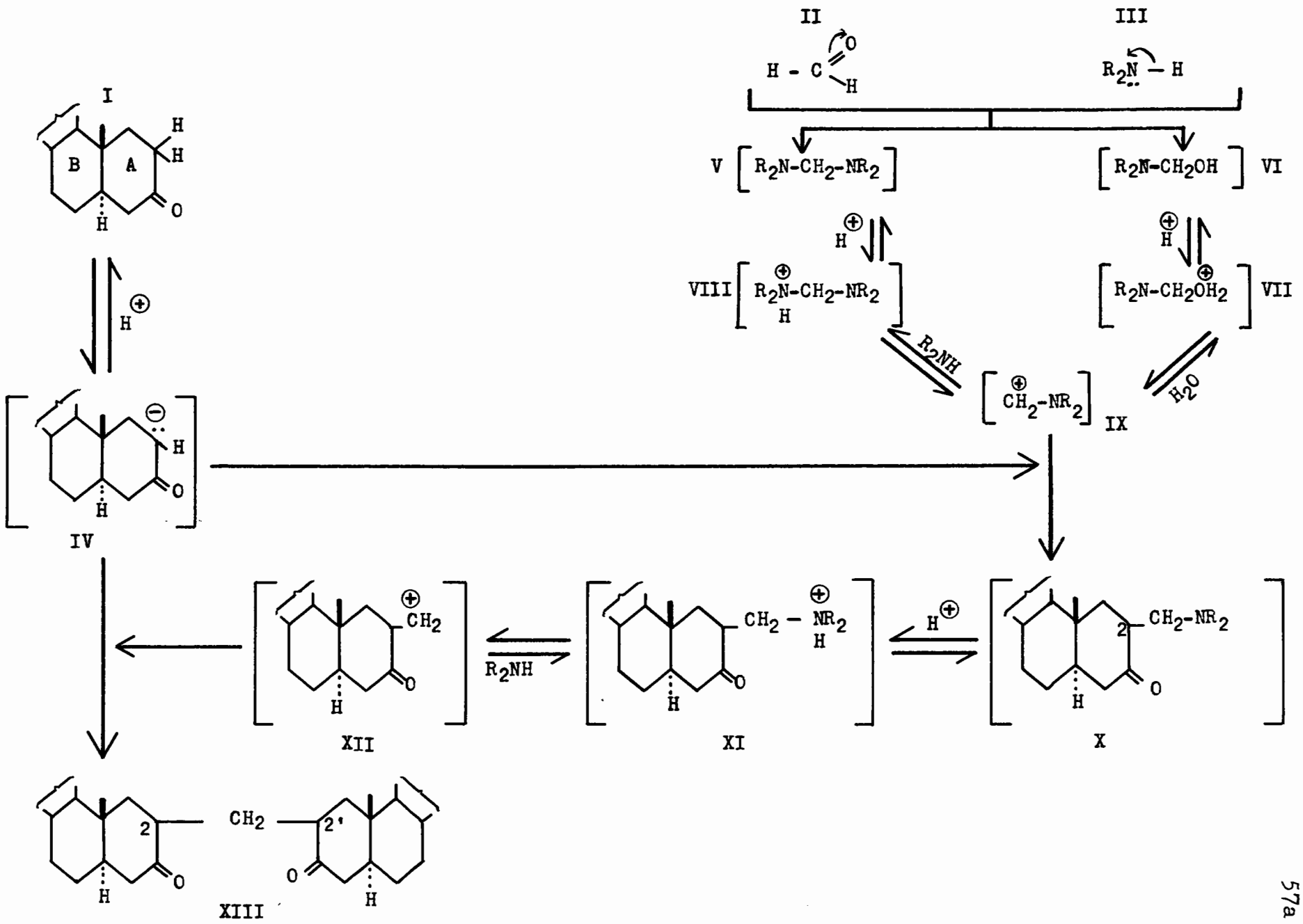
compounds are known to respond to alkaline and acidic catalysis in much the same manner as the Mannich reaction (14, 19, 141, 142, 143).

The outlined scheme suggests the formation of the carbonium ion X from formaldehyde and a secondary amine through the intermediate of either methylene-bis-amine V, or methylolamine VI. This cation combines then with the anion IV derived from the ketone, as in a normal Mannich reaction. Formation of the cation X is promoted by hydrochloric acid, and that of the anion IV by the amine present in the system. In the next step, the cleavage of the carbon-nitrogen bond is assumed with the loss of amine and formation of the carbonium ion XII. Such possibility had been clearly demonstrated by Hellman (16), who reviewed and studied extensively the use of Mannich bases for alkylations. In the final step, the cation XII condenses with the anion IV to give the methylene-bis-ketone XIII. The effective concentration of the acid required may be very low. In fact, in a non-aqueous medium, a salt of an added amine is only slightly ionized. The acid is distributed among all the bases present in the reaction system (reactants, intermediates, products), and may be strongly buffered. On the other hand, an excess of protons, e.g. when free acid is added, depresses the formation of the carbanion IV, and also that of the product. The effect of an added base, e.g. secondary amine, is to promote the production of the carbanion IV, but also to check the formation of all other intermediates which require the presence of hydrogen ions.

Fig. 1

Mechanism of an abnormal Mannich reaction

Scheme I


II
III
I
V
VI
VIII
VII
IX
IV
X
XI
XII
XIII
R2NH
H2O
R2NH

The scheme II (Fig. 2) is presented as an alternate mechanism in view of the uncertainty as to whether a secondary amine, derived from its hydrochloride, participates actively in the reaction, or acts only as a buffer towards the hydrochloric acid. This mechanism accounts for an acid-induced aldol condensation of formaldehyde with a ketosteroid. In the first step, a transition state complex II is formed which rearranges to a β-ketomethylol III. The latter, is unstable in acidic medium and gives upon protonation an oxonium ion IV which by loss of a molecule of water, affords a carbonium ion V. Finally, the carbonium ion combines with the carbanion VI derived from the ketone I, to give methylene-bis-ketone VII.

2. General Method and Conditions of reactions

Owing to the possible mechanisms outlined above, two schemes for the sequence of addition of the reactants seemed to be preferred:

1) amine and paraformaldehyde were allowed to react, then the keto compound was added either gradually or in one portion. This procedure favoured the formation of an aminomethanol, a methylene-bis-amine, or aminomethylene intermediates.

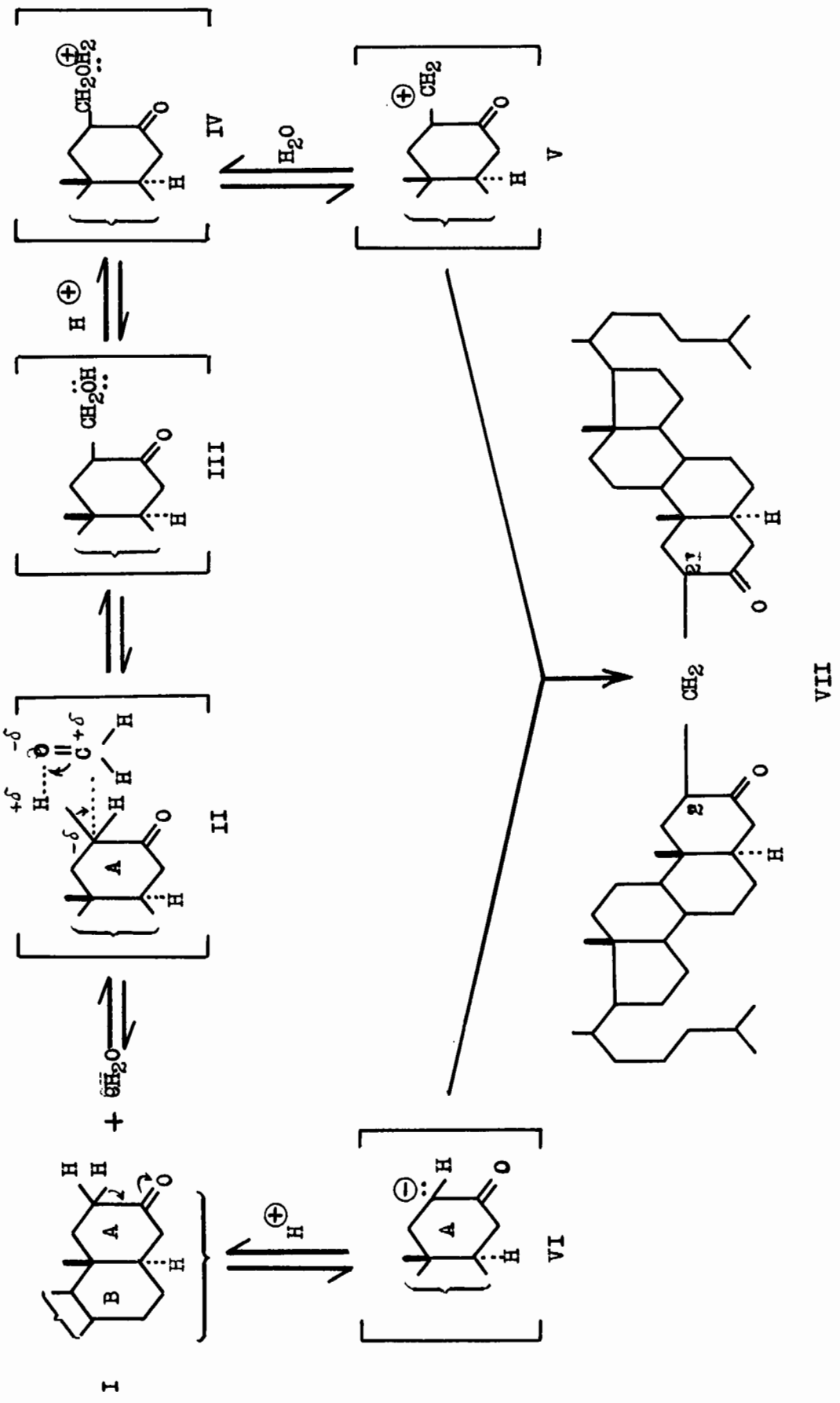
2) When the ketone and paraformaldehyde were first allowed to react, with subsequent addition of amine, a methylol derivative was the most probable intermediate.

During the course of this work, it was observed that keto compounds reacted preferentially with formaldehyde. Therefore, the procedure 2) was generally abandoned in favour of 1).

Fig. 2

Mechanism of an abnormal Mannich reaction

Scheme II



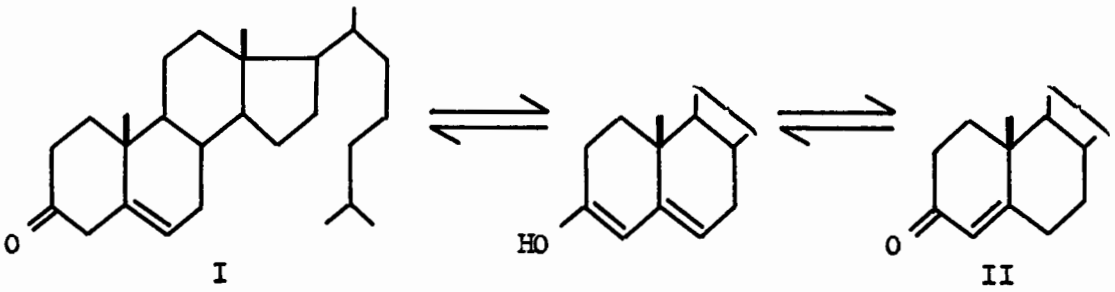
The use of paraformaldehyde was preferable to that of 40% aqueous solution of formaldehyde because of the very low solubility of ketosteroids in water.

Among secondary amines and their hydrochlorides, morpholine, piperidine and dimethylamine were chosen. These had been known to participate successfully in numerous Mannich condensations (1, 2, 3).

For the separation of the expected Mannich bases, the procedure described by Julian (17) was adopted. The reaction mixtures were diluted with 4% hydrochloric acid. When solvents non-miscible with water were used, the solutions were extracted with ether, the aqueous layers were separated, and neutralized with a saturated solution of sodium carbonate. The aqueous neutral solutions were extracted with ether again and the ethereal extracts were dried over anhydrous magnesium sulphate. Products were isolated by evaporation of the solvent under reduced pressure. In the cases of water miscible solvents, unreacted ketosteroids separated upon the addition of 4% hydrochloric acid and were filtered off. The remaining solutions were treated as above.

3. Reactions of cholest-5-en-3-one with paraformaldehyde and secondary amines or their hydrochlorides.

Cholest-5-en-3-one (I) isomerizes readily to its more stable isomer cholest-4-en-3-one (II) (144, 145).



I II

The stability of the latter ketone is due to the conjugation of the double bond at $C_{4,5}$ and of the keto group in 3-position. It was thought that, if this isomerization could be avoided, the hydrogen atom at C_4 should prove particularly active because of its activation by the adjacent double bond and keto group.

(A) Amines

(i) Morpholine

By using morpholine in equimolecular amounts with the ketone and paraformaldehyde, either in isoamyl alcohol or in dioxane as solvents, it was possible to obtain two products which contained nitrogen, had almost identical melting points (149° and 149.5°-150.5°)respectively, and formed hydrochlorides melting at the same temperature (227-232°).

The reaction was carried out successfully once in each solvent, but could not be reproduced in spite of very numerous attempts. Varying the molar ratios of the ketone, paraformaldehyde and amine from 1:1:1 to 1:2:3, to 1:5:7.5 did not contribute to the formation of the product. In all cases, brown visquous oils were obtained, and could not be

crystallized. They did not contain nitrogen and seemed to be derived from a condensation of the ketone with formaldehyde.

(ii) Piperidine

All attempts of condensation carried under similar conditions as with morpholine, using absolute ethanol, n-butanol and isoamyl alcohol as solvents, resulted in the formation of oily materials which could not be crystallized.

(B) Amine hydrochlorides

In the presence of morpholine, piperidine or dimethylamine hydrochlorides, the reaction of the ketone with formaldehyde gave brown viscuous oils which were darker than those obtained with free amines during the same refluxing time. It was assumed that formaldehyde was made available more readily for the condensation by the additional action of acid on depolymerization of the paraformaldehyde. Unreacted amine hydrochlorides were recovered in high yields in each case.

4. Attempted condensation of 4,4-dimethylcholest-5-en-3-one with paraformaldehyde and secondary amines or their hydrochlorides.

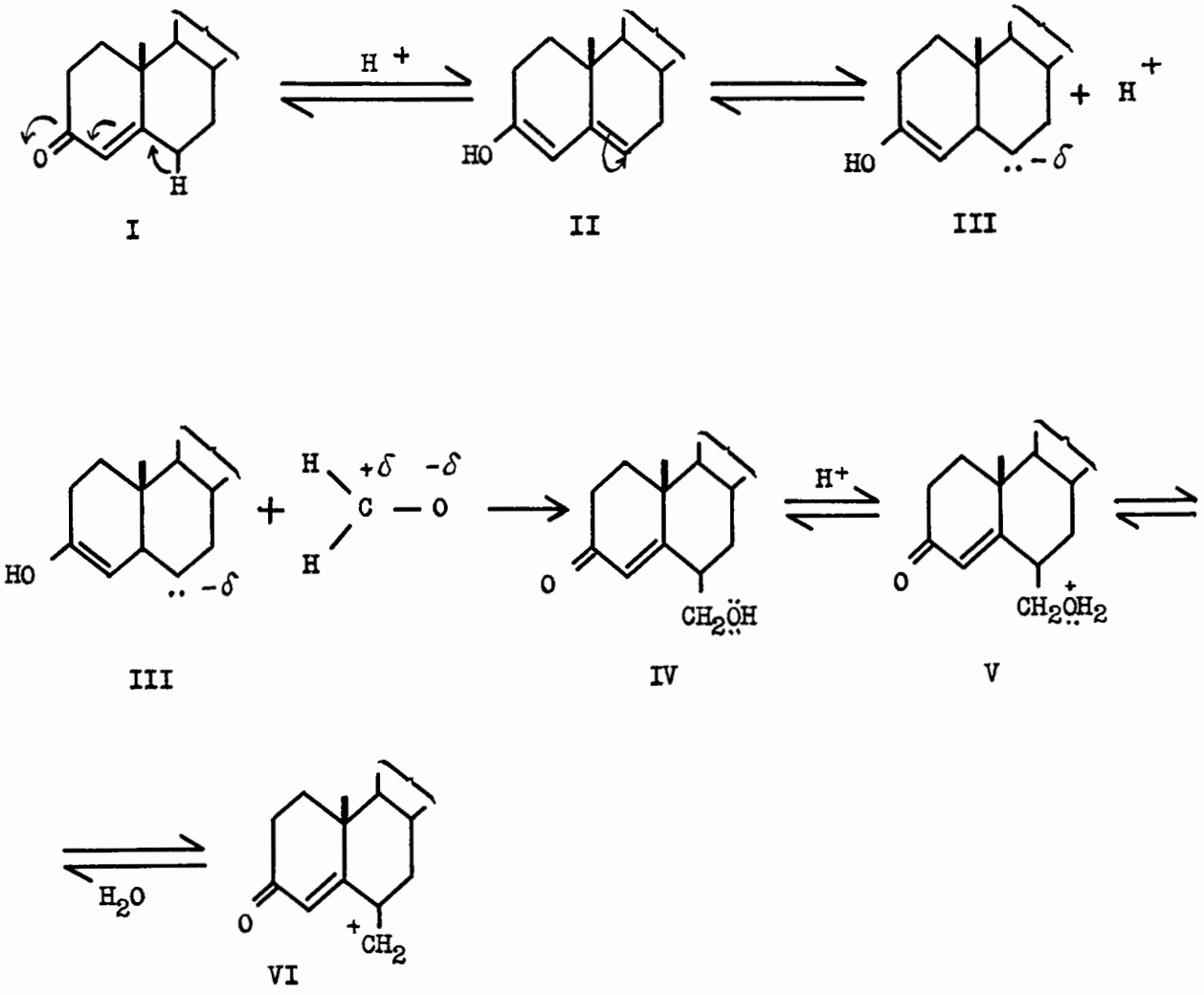
The ketone was recovered unchanged from reactions with morpholine, piperidine, their hydrochlorides or dimethylamine hydrochloride. Refluxing in isoamyl alcohol was maintained for four to four and a half hours. Under similar conditions, cholest-5-en-3-one had reacted with paraformaldehyde already after half an hour of refluxing.

5. Attempted condensation of cholest-4-en-3pone with paraformaldehyde and secondary amines

Bromination of cholest-4-en-3-one was known to take place only under enolizing conditions (e.g. hydrobromic acid or sodium acetate) (150). The unreacted ketone was recovered in high yields from reactions with paraformaldehyde and morpholine or piperidine. The refluxing time was four hours in isoamyl alcohol. From these results, it was concluded that the ketone was not sufficiently enolized by the secondary amines and therefore did not participate in the reaction. This view was confirmed by the behaviour of the ketone in the presence of amine hydrochlorides.

6. Reaction of cholest-4-en-3-one with paraformaldehyde and secondary amine hydrochlorides.

In a series of reactions with morpholine, piperidine and dimethylamine hydrochlorides, non-crystallizable oils were isolated after half an hour of refluxing in isoamyl alcohol, n-butanol or absolute ethanol. These oils seemed to be condensation products of the ketone with formaldehyde. This assumption was supported by the absence of the characteristic odour of the latter component in the reaction mixtures. The enolization of the ketone in acidic medium was a probable prerequisite in the condensation process which could have taken place in the following manner:



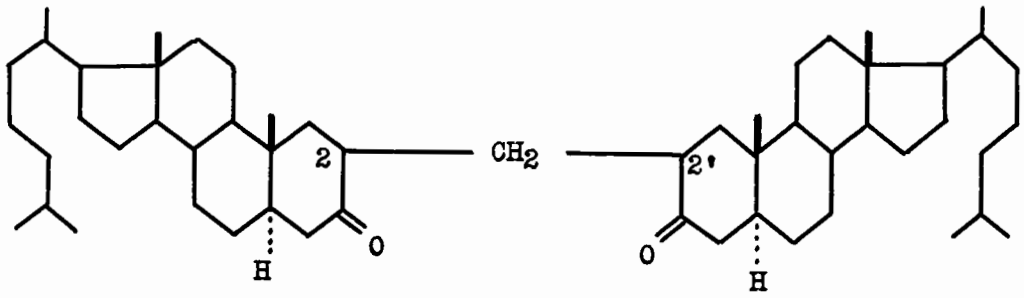
The apparently reactive carbonium ion VI may react further with the keto group of I or with the hydroxy group of IV.

7. Condensation of cholestan-3-one with paraformaldehyde in the presence of secondary amine hydrochlorides

Cholestan-3-one condensed with formaldehyde (20-30 minutes refluxing time) in the presence of morpholine or piperidine hydrochlorides. The product contained no nitrogen, melted at 208-210° and on the basis of analytical results and comparative study of infrared spectra, seemed

to be methylene-bis-ketone. The yield was 35.4% based on cholestan-3-one used, in reactions carried out in isoamyl alcohol. When dioxane was used as solvent, the crude product melted over a wide range of temperature, and only a small amount of a material melting at 205-207° could be isolated with difficulty.

The infrared absorption spectrum of cholestan-3-one showed a carbonyl band at 1710cm^{-1}, and a band of medium intensity at 1425cm^{-1}. The latter one had been assigned to C-H scissoring vibrations in an unsubstituted methylene group at C_2 or C_4 in 3-ketosteroids (146, 147). The spectrum of the reaction product showed two carbonyl bands at 1725cm^{-1} (S) and at 1700cm^{-1} (m). A weak band due to methylene groups adjacent to the keto function appeared at 1413cm^{-1}. This spectroscopic evidence inferred that the new compound contained two residues of cholestan-3-one joined by a methylene bridge in either 2 or 4 position. In this connection, it should be noted that the bromination of cholestan-3-one with bromine in acidic medium takes place in 2-position (148). Since an aldol condensation in basic or acidic medium proceeds through enolization, it is reasonable to postulate that the linkage in the methylene-bis-ketone is between the carbon atoms in 2-position. If such were the case, the structure of the compound should be represented by LXXXIV which is di[2,2'-3-ketocholestanyl] methane (M.W. 784).



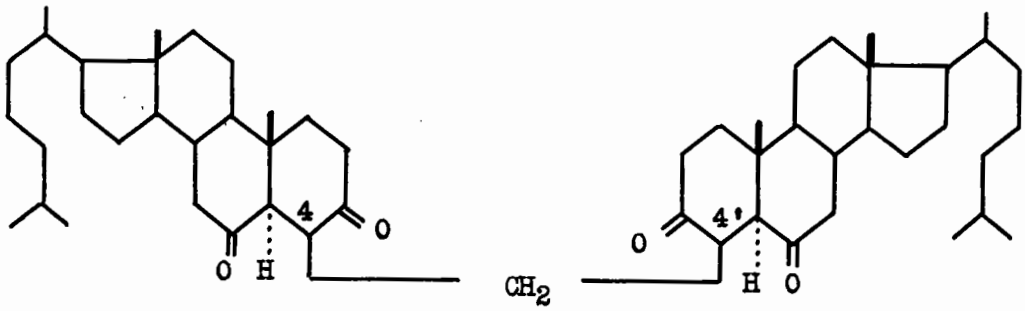
LXXXIV

Cholestan-3-one was recovered unchanged when the corresponding free amines, morpholine or piperidine, were used. An explanation can be given by considering the competitive effect of two nucleophilic reactants, the ketone and an amine, with regard to an electrophilic reagent which is formaldehyde. When the amine is replaced by the related hydrochloride, the ketone remains the only nucleophilic species, and the condensation with formaldehyde takes place.

8. Condensation of cholestane-3,6-dione with paraformaldehyde in the presence of secondary amine hydrochlorides

In a reaction similar to that of cholestan-3-one, cholestane-3,6-dione was refluxed with paraformaldehyde and morpholine or piperidine hydrochloride for 45 minutes in isoamyl alcohol. The product was obtained in 21.2% yield based on cholestane-3,6-dione used and contained no nitrogen. It was only slightly soluble in organic solvents, could be purified by repeated recrystallizations from boiling acetone and melted at 242-242.5°. The analytical results and the infrared absorption spectrum inferred a methylene-bis-ketone structure. Cholestane-

3,6-dione showed a single very strong carbonyl band at 1710cm^{-1}, and a band of low intensity at 1420cm^{-1}. The latter one corresponds to unsubstituted methylene groups adjacent to the keto function at C_3 (see above). The spectrum of the condensation product exhibited two bands in the carbonyl region at 1727cm^{-1} (s) and at 1710cm^{-1} (vs). The band due to methylene groups was shifted to 1425cm^{-1}, and became a shoulder on the more intense band at 1465cm^{-1}. There are in cholestane-3,6-dione three methylene groups in positions 2, 4 and 7 adjacent to keto functions. The reactivities of hydrogen atoms belonging to these methylene groups can be evaluated in terms of certain substitution reactions. Bromination of cholestane-3,6-dione with 3 moles of bromine in acidic medium leads to 4,7-dibromocholest-4-en-3,6-dione (149). According to the probable mechanism, the position 4 would be the first point of an electrophilic attack, followed by dehydrobromination and formation of α,β -unsaturation at $C_{4,5}$. A subsequent bromination yields the unsaturated 4,7-dibromo compound. It is therefore postulated that the linkage in the methylene-bis-ketone is between carbon atoms in 4-position. The formula LXXXV represents di [4,4'-di-3,6-ketocholestanyl] methane (M.W.812).



LXXXV

Part II - Reductions of oximinocholanic acids

9. General methods and conditions of the reactions

In the second part of the present investigation, the reduction of several oximinocholanic acids using sodium in alcohol and lithium aluminium hydride was studied. These oximinocholanic acids were prepared from desoxycholic and dehydrocholic acids. The infrared absorption spectra of various keto- and oximino-cholanic acids were recorded and examined. The reductions of oximes in various positions of the steroid nucleus were expected to give steroidal amino acids or amino alcohols. Reducing agents included sodium in alcohol and lithium aluminium hydride. Reductions of oximinocholanic acids with the latter reagent have not been reported in the literature. Oximinocholanic acids, rather than their esters, were used in order to avoid the reduction of ester groups by lithium aluminium hydride and by Bouveault-Blanc effect. n-Propanol and n-butanol gave the best yields of purest products in reductions with sodium. The colour of reaction mixtures in isoamyl alcohol was darker and crude products contained appreciable amounts of tarry impurities. Reductions of oximes with alkali metals and proton donors are known to give predominantly the most thermodynamically stable product (39, 40) in which the equatorial conformation of the amino substituent is preferred. Accordingly, the conformations of amino groups in the steroidal amino acids synthesized by reductions with sodium in alcohol were assigned on the basis of their mode of preparation.

A series of preliminary experiments was carried out in order to establish appropriate conditions for reductions with lithium aluminium

hydride. Oximinocholanic acids and their methyl esters, which were prepared using diazomethane, proved to be slightly soluble in ether. Using the Soxhlet technique only 10-15% of the sample of oximinoacid or ester could be transferred by extraction into the reaction vessel over a period of 48 hours. Since the oximinoacids were quite soluble in tetrahydrofuran, the latter was adopted as solvent.

The reduction of oximes to primary amines consumes one half mole of lithium aluminium hydride according to the following partial equation:

$$2R_2C = NOH + LiAlH_4 \longrightarrow 2R_2CHNH_2$$

The reduction of carboxylic acids to primary alcohols consumes three-quarters of a mole of lithium aluminium hydride as indicated in the following equation:

$$4RCOOH + 3LiAlH_4 \longrightarrow (RCH_2O)_4LiAl + 2LiAlO_2 + 4H_2$$

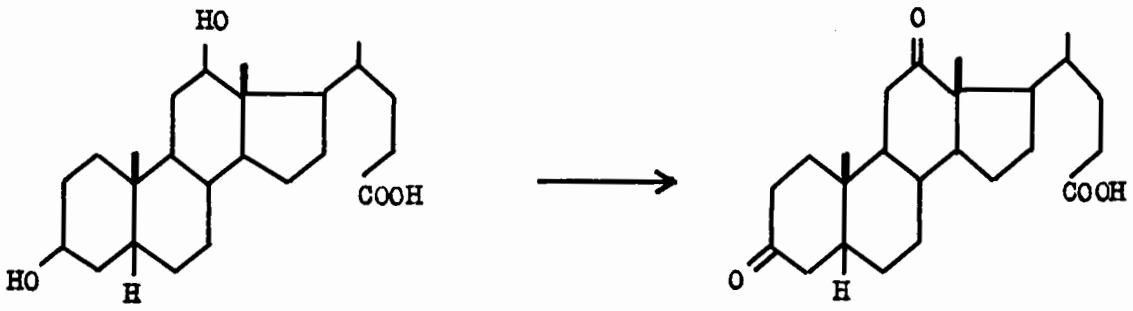
The acidic hydrogen consumes one-quarter mole of the hydride. The preliminary experiments showed that two-fold to five-fold molar excess of lithium aluminium hydride led to the formation of mixtures of products which exhibited hydroxyl and carbonyl infrared absorption bands. When about a ten-fold excess of the reagent was used, definite reaction products could be isolated in all cases.

The relative order of reactivities of oxime groups in various positions of the nucleus was established visually by observing the rate of formation of organo-metallic complexes during the course of the reactions. This order was analogous to the one which had been reported for the corresponding ketones (47), and went decreasingly from 3- to 7- and 12-positions.

The infrared absorption spectra of the reaction products were determined in potassium bromide because of their low solubilities in the appropriate organic solvents.

10. Preparation of 3,12-diketocholanic acid by oxidation of 3,12-dihydroxycholanic acid

The oxidation of desoxycholic acid (LXXXVI) to dehydrodesoxycholic acid (LXXXVII) was carried out using the procedure described by Heilbron (150) for the oxidation of acetylenic carbinols.



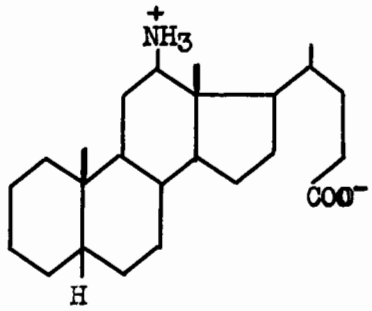
LXXXVI LXXXVII

The method consists in using the oxidizing reagent composed of chromium trioxide and sulphuric acid in acetone solution. The advantage of the method resides in a gradual addition of the reagent which is never present in excess, and in an easy separation of the inorganic residue, insoluble in an organic solvent. The applicability of this method is limited only by the solubility of the substance to be oxidized. The above procedure gave consistent yields of above 90 per cent of the pure product, as compared to about 70 per cent yield obtained by Wieland's

method of oxidation in acetic acid (151). The purification of the product in the latter case was also more tedious.

11. Reduction of 12-oximinocholanic acid with sodium in alcohol.

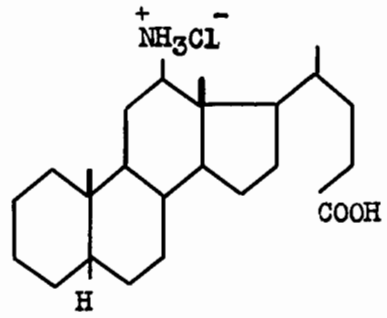
The reduction was expected to yield mainly 12β-aminocholanic acid (LXXXVIII). The highest yields of purest product were obtained in n-propanol and n-butanol.



LXXXVIII

The product existed as a dipole ion and was precipitated at its isoelectric point (pH 5.6-5.8) with 1% sulphuric acid. The existence of ionic structure was confirmed by the infrared absorption spectrum which showed bands characteristic of carboxylate ion at 1550cm^{-1} (S) and of $\overset{+}{N}H_3$ group at 1625cm^{-1} (m). The C-O stretching vibrations were absent. The product melting at 115-116^{o} was obtained after several recrystallizations from acetone-water. It was assumed, in accord with the rule concerning reductions by alkali metals and proton donors (39, 40), that this product contained the more thermodynamically stable equatorial (12β) amino substituent.

Several treatments of 12β-aminocholanic acid with concentrated hydrochloric acid resulted in the formation of the corresponding hydrochloride (LXXXIX). The salt did not possess the dipole structure since the carboxylic group was present in the non-ionic form.



LXXXIX

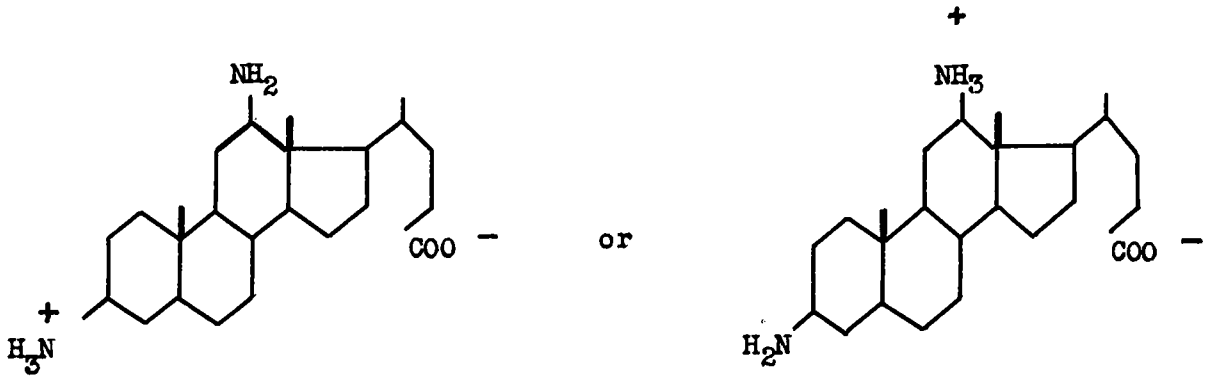
The infrared absorption spectrum showed suppression of the band due to the carboxylate ion (1550cm^{-1}), and release of the carboxyl band at 1705cm^{-1} (VS). The maximum due to the $\overset{+}{N}H_3$ group was shifted towards lower frequencies (1610cm^{-1}).

12. Reaction of 12-oximinocholanic acid with sodium amalgam in ethanol

The reaction of 12-oximinocholanic acid with 3% sodium amalgam resulted in the transformation of the oxime group to the keto group. The corresponding 12-ketocholanic acid was identified by mixed melting point determination with an authentic sample. For further confirmation, the oxime was prepared from the reaction product and showed no depression of the melting point with an original sample of 12-oximinocholanic acid.

13. Reduction of 3,12-dioximinocholanic acid with sodium in alcohol

This reaction was expected to form 3α,12β-diaminocholanic (XC) acid (3α and 12β equatorial) as the major product.



XC

Possessing one carboxyl and two amino groups this diaminocarboxylic acidbehaves as a base, and it can form a dihydrochloride. Owing to this basic character, the isoelectric point should be at a pH above 7. The product was obtained in good yields from reactions using n-propanol and n-butanol. It was precipitated with 1% sulphuric acid at pH 8.6-8.8.

The infrared spectrum showed bands characteristic of NH_2 (1660cm^{-1} sh), $\overset{+}{N}H_3$ (1623cm^{-1} s), and COO^- (1575cm^{-1} vs). The band due to the carboxylate ion absorption was shifted by 25cm^{-1} towards higher frequencies as compared with the corresponding band in 12β-aminocholanic acid (1550cm^{-1}).

14. Reaction of 3,12-dioximinocholanic acid with lithium aluminium hydride

A product containing no nitrogen and insoluble in either acids or bases was formed in this reaction. The reaction mixture was hydrolysed with a saturated Rochelle salt solution, and tetrahydrofuran was evaporated under reduced pressure. The product could then be extracted satisfactorily with n-butanol. When tetrahydrofuran was not removed completely, the solution tended to emulsify during the extraction.

The infrared absorption spectrum showed a very strong band at 3340cm^{-1} due to associated hydroxyl group, and also a series of high intensity bands in the region 1075-1011cm^{-1}. The latter range had been tentatively assigned to C-O stretching vibrations in steroid alcohols. The benzoate derivative of the reaction product showed carbonyl absorption at 1780 and 1715cm^{-1}, and aromatic ring vibrations at 3060, 1600 and 705cm^{-1}.

On the basis of the above data and analytical results, the compound was postulated to be 3ξ,12ξ,24-trihydroxycholane. The steric course of reductions with lithium aluminium hydride being uncertain, the conformations of the hydroxyl groups in 3- and 12-positions were not assigned. Lithium aluminium hydride was postulated as a complex salt (152).

$$Li^{+} \qquad AlH_4^{-}$$

An evidence for the necessity for a donor solvent and for monoetherate formation in lithium hydride reductions was obtained. It was suggested, therefore, that there is the following equilibrium in solution:

$$AlH_4^{-} \rightleftharpoons H^{-} + AlH_3$$

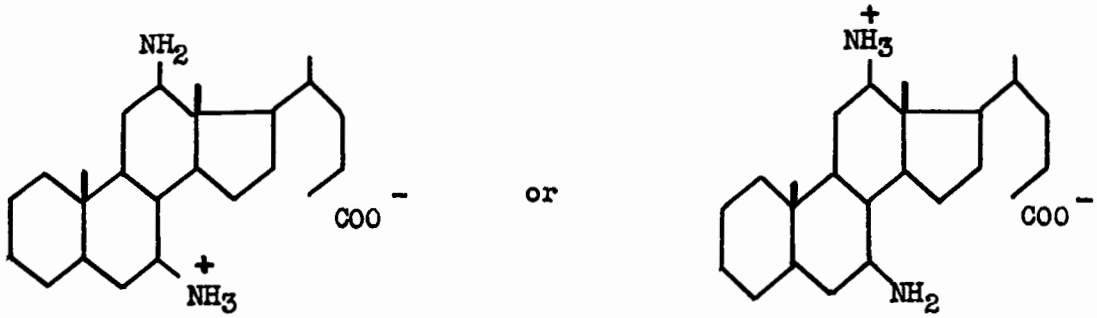
The ether coordinates with the AlH_3, and the hydride ion is the active entity in lithium aluminium reactions (153). Tetrahydrofuran, being more basic than ether (154) can be substituted for the latter one in these reactions.

A mechanism was postulated (fig. 3), which explains the reaction of 3,12-dioximinocholanic acid with lithium aluminium hydride. In Fig. 3, only the oxime group in 12-position (I) is illustrated, as an example.

The electron pair on the nitrogen atom in I is available for coordination with AlH_3 to give the complex II. The tendency for the electron pair on the oxygen to take part in hydrogen bonding results in the formation of a quasi-ring such as has been postulated in numerous Grignard reactions (155). The hydride ion present in the solution attacks the electron deficient nitrogen, and upon alkaline or acidic hydrolysis, an unstable imine is formed (IV). One molecule of imine (IV) condenses with one molecule of the isomeric enamine (V) to give a Schiff's base (VI). Under hydrolytic donditions, the latter yields the corresponding ketone (VII) which is further reduced to the secondary alcohol VIII, by the lithium aluminium hydride still present in the reaction mixture.

15. <u>Reduction of 7,12-dioximinocholanic acid with sodium in alchhol</u>

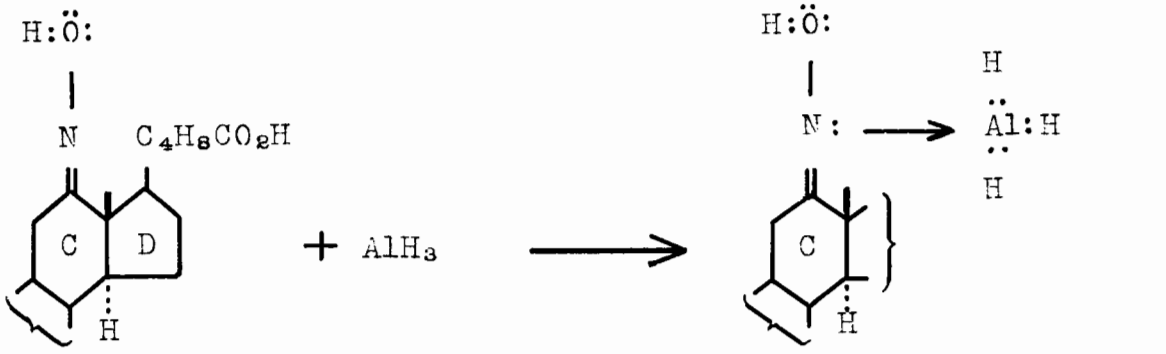
This reaction was expected to form 7β,12β-diaminocholanic acid (XCI) (7$\beta$and 12β equatorial) as the major product.



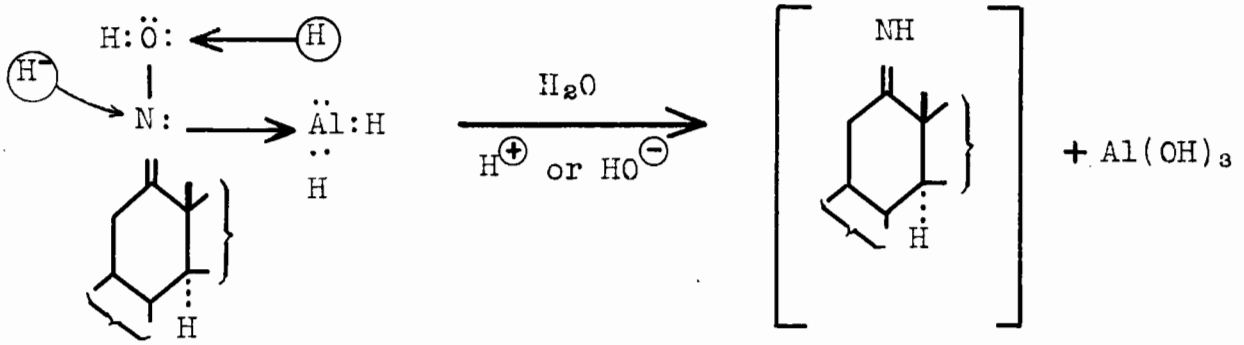
XCI

Fig. 3

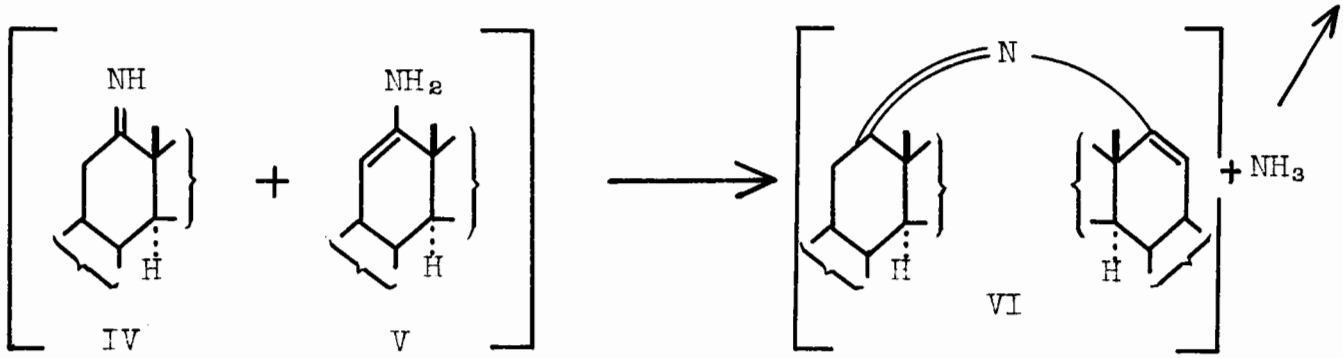
Schematic representation of the reaction of 3,12-dioximinocholanic acid with lithium aluminium hydride

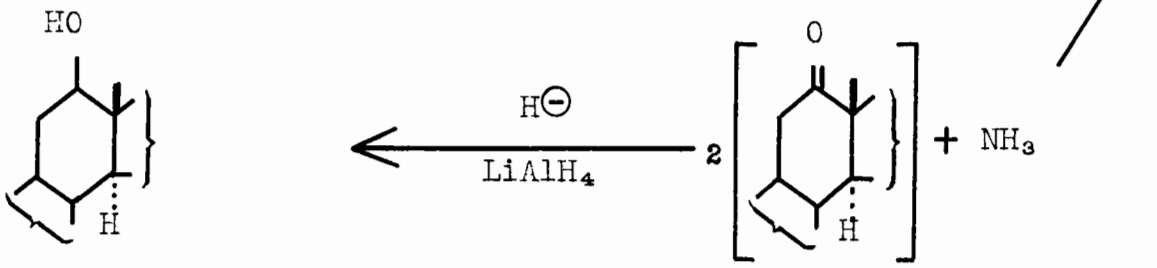


I II



III IV





VIII VII

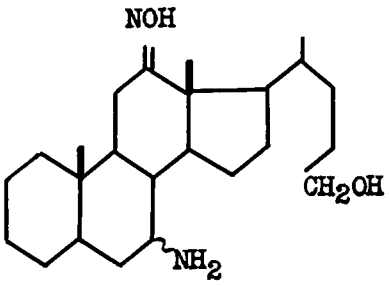
The diaminoacid was obtained in similar (over 80 per cent) yields when the reduction was carried out in n-propanol or n-butanol. Apparently, the product was appreciably soluble in water. The precipitation of the amino acid from the reaction mixture at its isoelectric point (pH 8.3-8.6) was brought about by addition of dilute sulphuric acid. A 4% solution of the acid was used first in order to keep the total volume of the solution to a minimum. In order to approach the isoelectric point gradually, 2% and 1% sulphuric acids were used consecutively.

The infrared absorption spectrum showed two bands in the N-H stretching region due to NH_2 and $\overset{+}{N}H_3$ groups (1635, 1630cm^{-1}). The shoulder which appeared at 1560cm^{-1} on the main carboxylate ion band (1550cm^{-1}) could be assigned to the N-H bending vibrations in NH_2, $\overset{+}{N}H_3$ or in both.

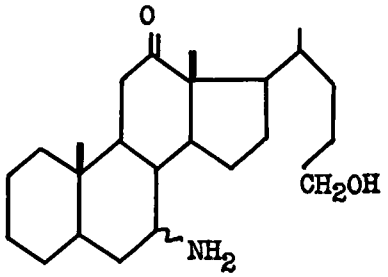
16. Reduction of 7,12-dioximinocholanic acid with lithium aluminium hydride.

From a reaction similar to that of 3,12-dioximinocholanic acid, a product was extracted with n-butanol after two and a half hours of refluxing. In contrast, this product did contain nitrogen. Its infrared spectrum showed the absence of carbonyl absorption, which meant that the carboxyl group had been reduced to a primary alcohol. This assumption was supported by a band of very high intensity at 3300cm^{-1}. Another band at 1660cm^{-1} (m) could have been due to an amino, oximino, or both groups. Three compounds were taken into consideration in view of the above data: dioximino-, diamino-, or amino, oximino-carbinols. Acidic hydrolysis of the reaction product gave a material which also contained nitrogen, exhibited a carbonyl band at 1700cm^{-1}, and bands at 3300cm^{-1} (vs) and 1655cm^{-1} (m). The hypothesis of an amino, oximino carbinol fully agreed with analytical results.

The 12-position being more sterically hindered (methyl group at C_{13}) than 7-position, the reaction product was postulated to be 7ξ-amino-12-oximino-24-hydroxycholane (XCII); consequently, its hydrolysis product was 7ξ-amino-12-keto-24-hydroxycholane (XCIII), also in agreement with elementary analysis.



XCII



XCIII

It appears from the above results that a preferential reduction of only one oxime group had taken place.

17. <u>The correlation between the infrared absorption spectra and the structure of ketocholanic acids</u>

The infrared spectra of the four following ketocholanic acids in potassium bromide were recorded: 12-keto-, 3,12-diketo-, 7,12-diketo- and 3,7,12-triketo-cholanic acids.

<u>The O-H stretching vibrations</u>

Carboxylic acids exist normally in dimeric form with very strong intermolecular hydrogen bridges between the carbonyl and hydroxyl groups. The compounds examined showed a very weak or no absorption in the normal free O-H or associated O-H stretching regions. Instead, there was evidence for a series of weak or very weak bands between 2850 and 2500cm^{-1}

which was indicative of strongly hydrogen-bonded hydroxyl groups of the type -OH....O = C. This finding was in accord with the extensive data available on relatively simple molecules of carboxylic acids (156).

The C = O stretching vibrations

All of the ketocarboxylic acids studied showed a single very strong maximum in the carbonyl frequency region between 1705 and 1695cm^{-1}. 7,12-Diketocholanic acid exhibited another band of high intensity at 1730cm^{-1}, and 3,7,12-triketocholanic acid showed a shoulder at 1735cm^{-1} on the main wide carbonyl absorption. Since the other two ketoacids did not contain a keto group in 7-position, the maximum at 1735-1730cm^{-1} was tentatively assigned to 7-ketone. It appeared from the above data that some overlapping occurred between the bands due to the C = O stretching vibrations in the keto and carboxyl groups. Therefore, the differentiation of the two carbonyl containing groups was not possible in this region of the spectrum. The comparison of the range of the keto absorption in the steroidal ketoacids and ketosteroids examined in the present work indicated that the hydrogen bonding did not cause any appreciable frequency shift of the C = O stretching vibrations.

The C-O stretching vibrations

All ketoacids exhibited two bands of high or medium intensity in the range 1280-1245cm^{-1}. No specific assignment of these bands was contemplated because of the lack of general agreement as to the nature of vibrations in this region of the spectrum. It is pointed out, however, that saturated ketosteroids examined in this work also showed bands in the above mentioned range: cholestan-3-one at 1230cm^{-1} (m) and cholestane-3,6-dione at 1262 and 1240cm^{-1} (m).

The O-H deformation vibrations

The ketoacids showed one or two bands of medium intensity in the region 955-900cm^{-1}. The band at about 955cm^{-1} was present in both diketocholanic acids and in the triketocholanic acid. The latter exhibited an additional maximum at 925cm^{-1}. 12-Ketocholanic acid showed a medium band at 902cm^{-1}. These bands were absent in the spectra of mono- and di-ketosteroids, and were assigned to the O-H deformation.

18. The correlation between the infrared absorption spectra and the structure of oximinocholanic acids.

The spectra of the following compounds were determined: 12-oximino-, 3,12-dioximino-, 7,12-dioximino- and 3,7,12-trioximino-cholanic acids. The last substance (m.p. 273° dec.) was prepared by oximation of 3,7,12-triketocholanic acid (dehydrocholic acid) according to the procedure outlined by Schenck and Kirchhof (157).

The OH stretching vibrations

All acids exhibited strong absorption bands in the range 3560-324-cm^{-1} due to associated and non-associated hydroxyl groups. These bands were absent in the spectra of previously discussed ketocholanic acids. It was concluded that they corresponded to the stretching vibrations of OH structure belonging to the oxime groups (= N-OH), rather than to those of carboxyl groups. The broadest bands in this region were shown by 12-oximino- and 3,7,12-trioximinocholanic acids at 3320 and 3270cm^{-1}, respectively. 7,12-Dioximinocholanic acid exhibited a very strong narrow band at 3560cm^{-1}, characteristic of a free hydroxyl group, and a strong broad absorption with

a maximum at 3240cm^{-1} which was indicative of bonded OH. The hydroxyl groups in 12-oximinocholanic acid appeared to be associated, since the related band was at 3320cm^{-1}. The above data inferred varying degrees of hydrogen bonding in the four oximinocholanic acids studied. These differences might be due to the presence of intramolecular and intermolecular bonding between hydroxyl groups, as it is the case in polyhydric alcohols. The intramolecular association is largely independent of concentration. Its presumed effect, however, could not be studied because of the slight solubility of the compounds involved in organic solvents.

All oximinoacids showed one or two bands at 2440-2520cm^{-1} of variable intensity which was thought to be related to the intermolecular hydrogen bonding of carboxyl group.

Two of the compounds, 3,12-dioximino- and 3,7,12-trioximino-cholanic acids exhibited a very broad band (2000-1800cm^{-1}) whose maximum was at 1900cm^{-1} in the former, and at 1935 cm^{-1} in the latter. No explanation was offered for this band.

The C = O stretching vibrations

All acids, except 3,12-dioximinocholanic acid, showed a band of medium intensity or a shoulder on the main carbonyl absorption between 1760 and 1720cm^{-1}. In the latter compound, this band was possibly masked by a broad absorption due to the C = O stretching mode. Since it was improbable that, under the experimental conditions, the acids were present in monomeric form in any proportion, no explanation was given for the component bands mentioned above. It is noted that similar bands had been observed in the solution spectra of other steroidal acids (103) and in

the vapor spectra of simple carboxylic acids (104). The main carbonyl absorption showed a maximum between 1700 and 1695cm^{-1}.

The C = N stretching vibrations

The C = N absorption appeared in the range 1670-1637cm^{-1}. In the mono- and di-oximinoacids, the number of maxima corresponded to the number of oxime groups present in the molecule. The trioximinocholanic acid showed the main absorption at 1660cm^{-1} containing two shoulders on the high frequency side at 1665 and 1670cm^{-1}. This evidence did not permit any assignment of bands to oxime groups in particular positions in the nucleus.

The N-O stretching vibrations and O-H deformation mode

A series of characteristic bands appeared in the spectra of all oximinoacids below 1000cm^{-1}. There was a maximum of high or medium intensity at 990-985cm^{-1}, except 12-oximinocholanic acid which showed a weak band at 980cm^{-1}. The di-and tri-oximinocholanic acids exhibited strong absorption between 965 and 955cm^{-1}. 12-Oximinoacid had a strong band at 940cm^{-1}. Another series of strong bands appeared at 915-905cm^{-1} in all but the monooximinoacid. The region below 900cm^{-1} contained a medium or strong absorption between 865 and 855cm^{-1} in all compounds. Various other bands of medium intensity were present in the range 800-700cm^{-1}. No specific assignments were attempted in the region below 1000cm^{-1} because of the crowded conditions of bands due to N-O stretching and O-H bending vibrations. It is believed, however, that the higher frequency absorption (1000-900cm^{-1}) corresponded to N-O stretching mode, whereas the lower frequency bands (900-800cm^{-1}) resulted from O-H deformation vibrations.

Fig. 4

Infrared absorption spectra of cholestan-3-one, di-[x,x' 3-ketocholestanyl] methane, cholestane-3,6-dione, and di-[x,x'-di-3,6-ketocholestanyl] methane.

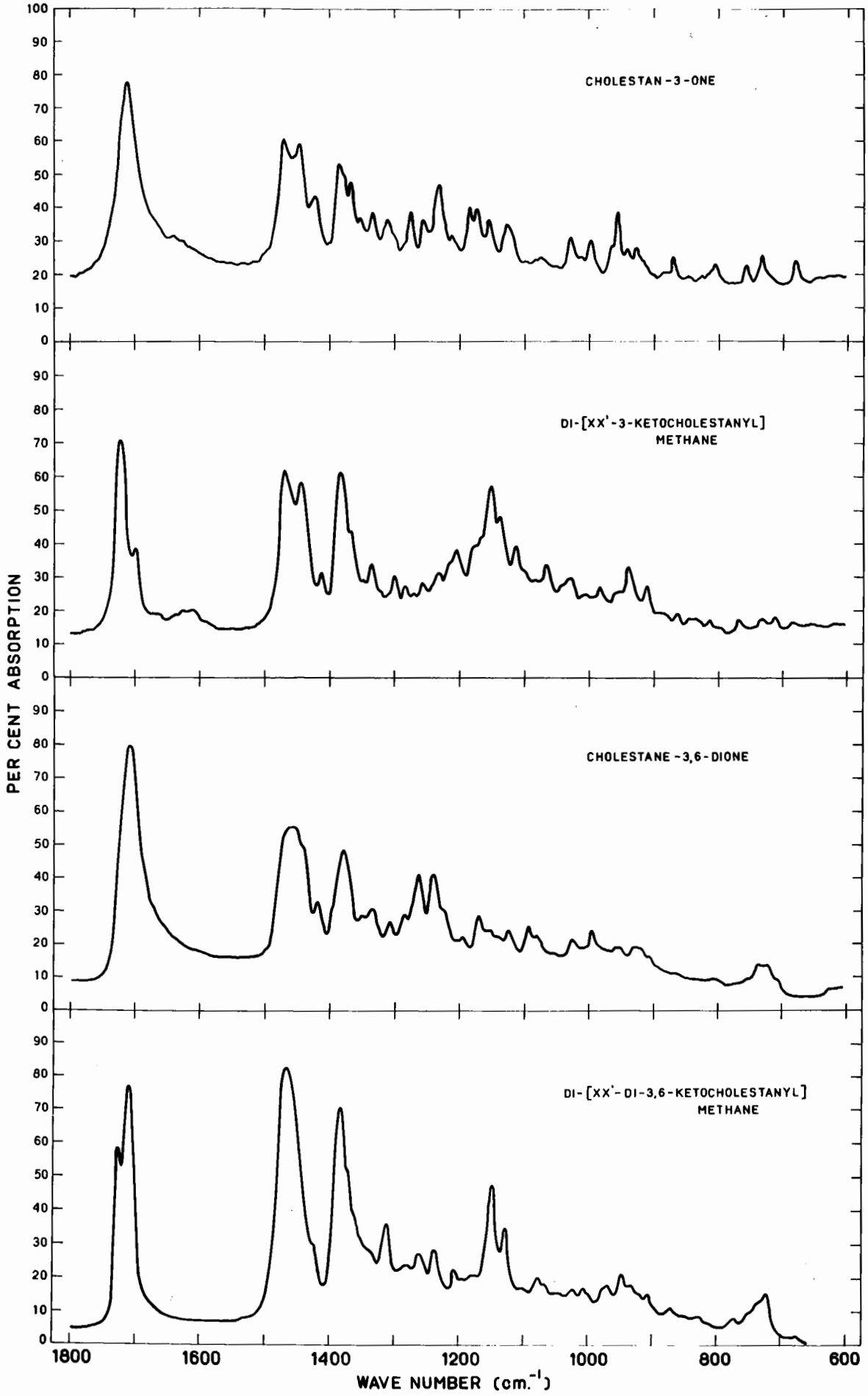

CHOLESTAN-3-ONE
DI-[XX'-3-KETOCHOLESTANYL] METHANE
CHOLESTANE-3,6-DIONE
DI-[XX'-DI-3,6-KETOCHOLESTANYL] METHANE
PER CENT ABSORPTION
WAVE NUMBER (cm.⁻¹)
1800
1600
1400
1200
1000
800
600

Fig. 5

Infrared absorption spectra of 12β-aminocholanic acid and 12β-aminocholanic acid hydrochloride.

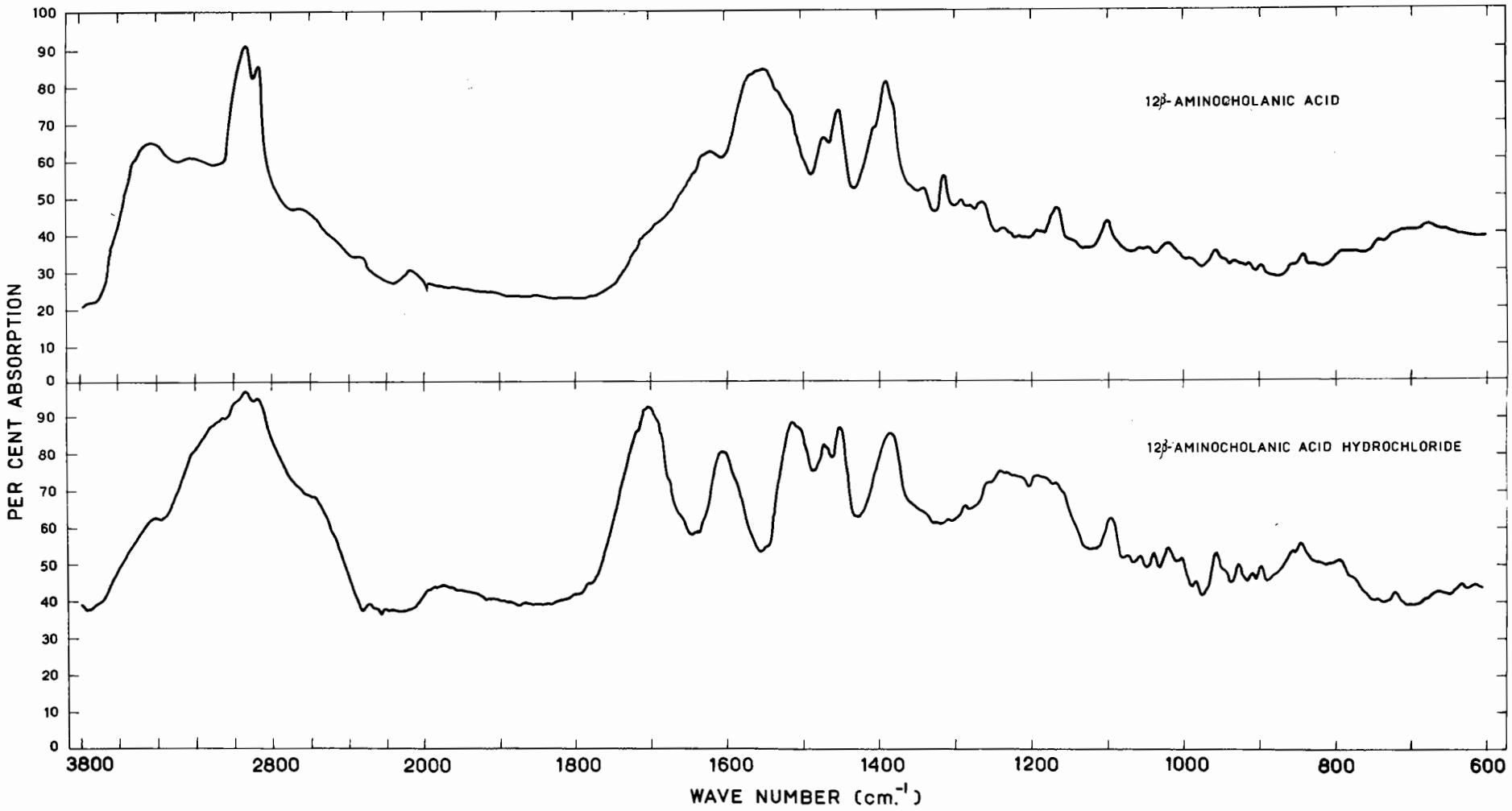

12β-AMINOCHOLANIC ACID
12β-AMINOCHOLANIC ACID HYDROCHLORIDE
PER CENT ABSORPTION
WAVE NUMBER (cm.⁻¹)
100
90
80
70
60
50
40
30
20
10
0
3800
2800
2000
1800
1600
1400
1200
1000
800
600

Fig. 6

Infrared absorption spectra of 3ξ,12ξ,24-trihydroxycholane and 3ξ-amino-24-oximino-24-hydroxycholane

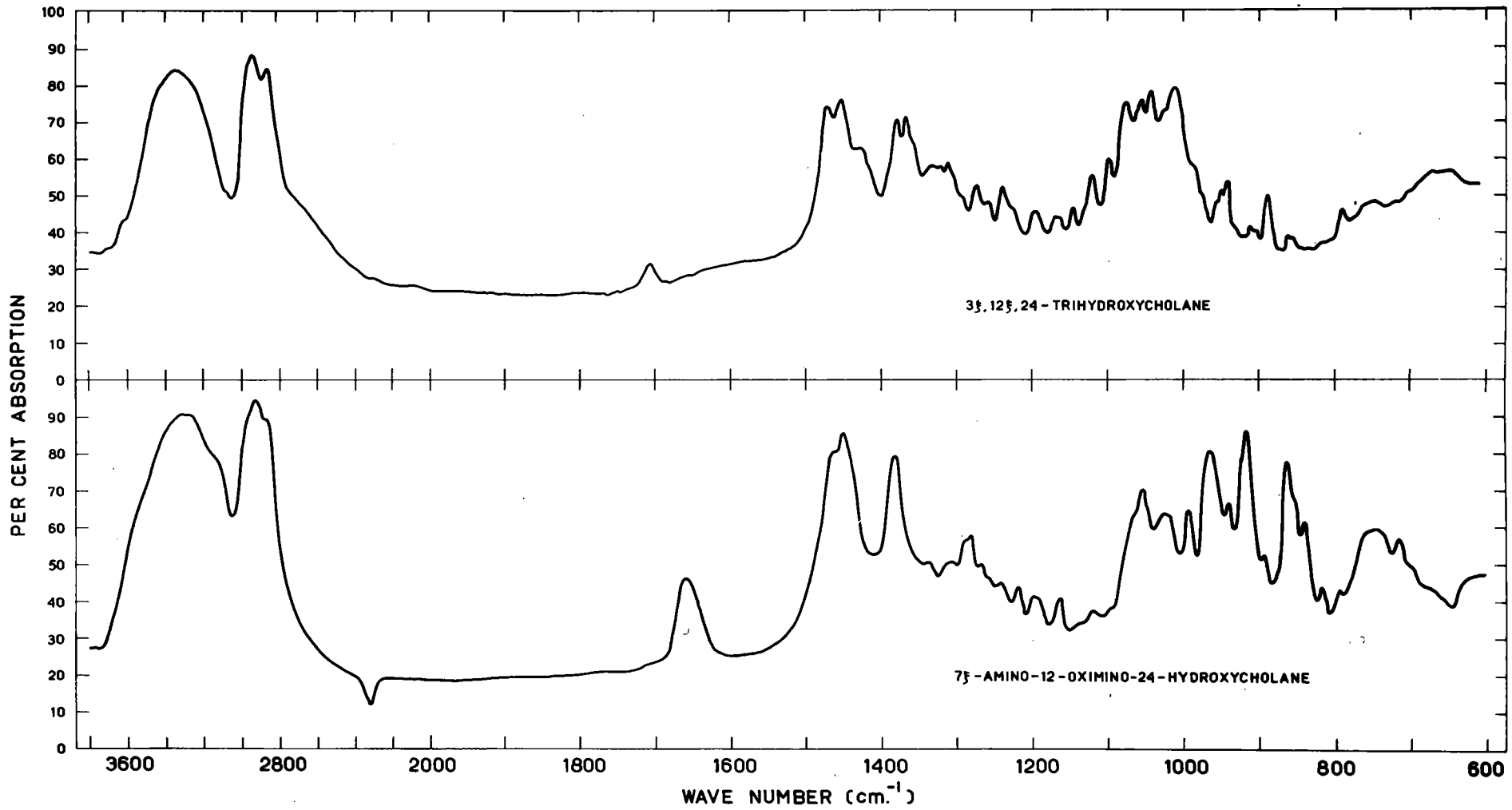

PER CENT ABSORPTION
100
90
80
70
60
50
40
30
20
10
0
3β, 12β, 24 - TRIHYDROXYCHOLANE
7β - AMINO - 12 - OXIMINO - 24 - HYDROXYCHOLANE
3600
2800
2000
1800
1600
1400
1200
1000
800
600
WAVE NUMBER (cm.⁻¹)

Fig. 7

Infrared absorption spectra of 12-mono-, 3,12-di-, 7,12-di- and 3,7,12-tri-ketocholanic acids.

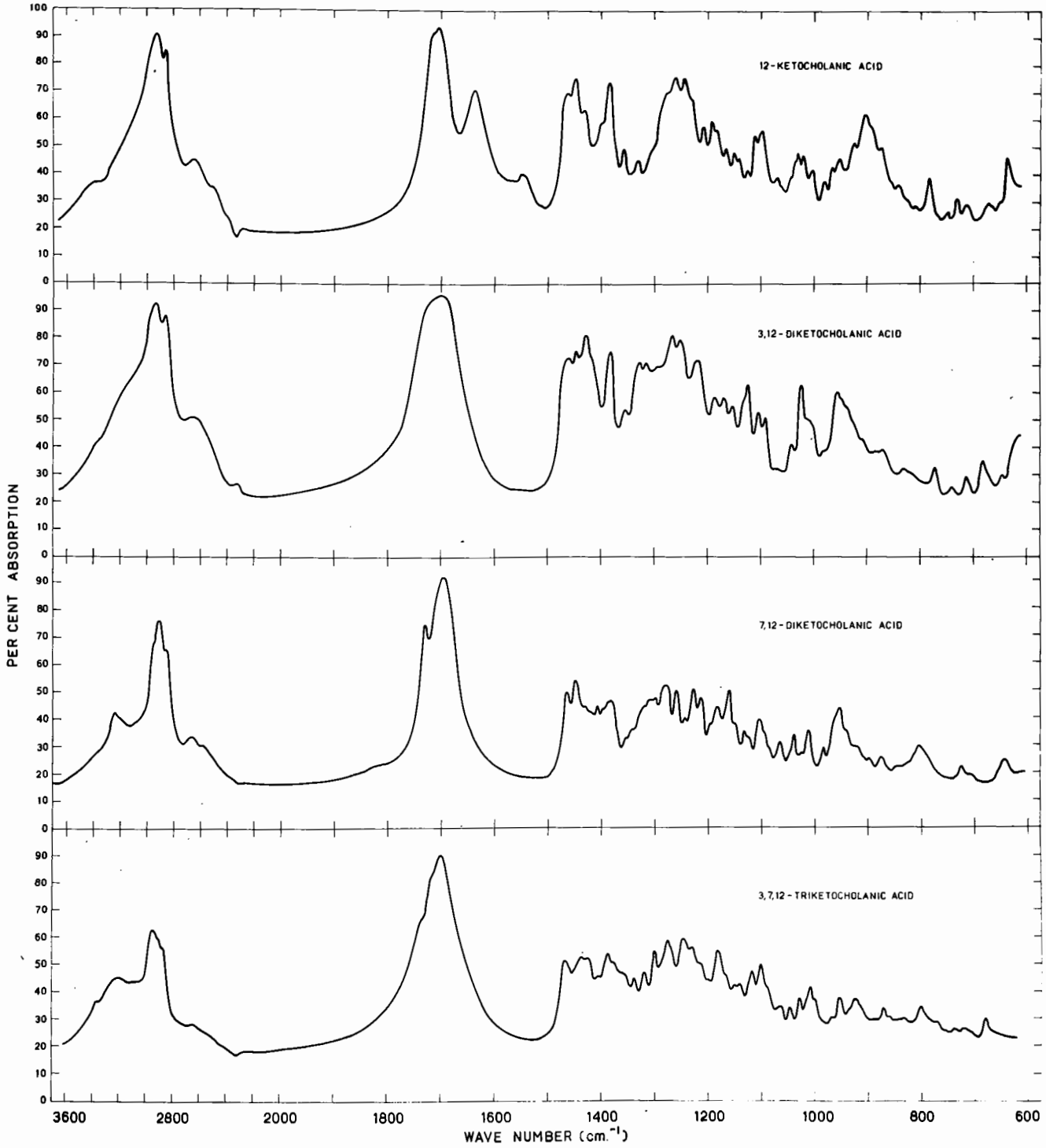

12-KETOCHOLANIC ACID
3,12-DIKETOCHOLANIC ACID
7,12-DIKETOCHOLANIC ACID
3,7,12-TRIKETOCHOLANIC ACID
PER CENT ABSORPTION
WAVE NUMBER (cm.⁻¹)
3600
2800
2000
1800
1600
1400
1200
1000
800
600

Fig. 8

Infrared absorption spectra of 12-mono-, 3,12-di-, 7,12-di- and 3,7,12-tri-oximinocholanic acids.

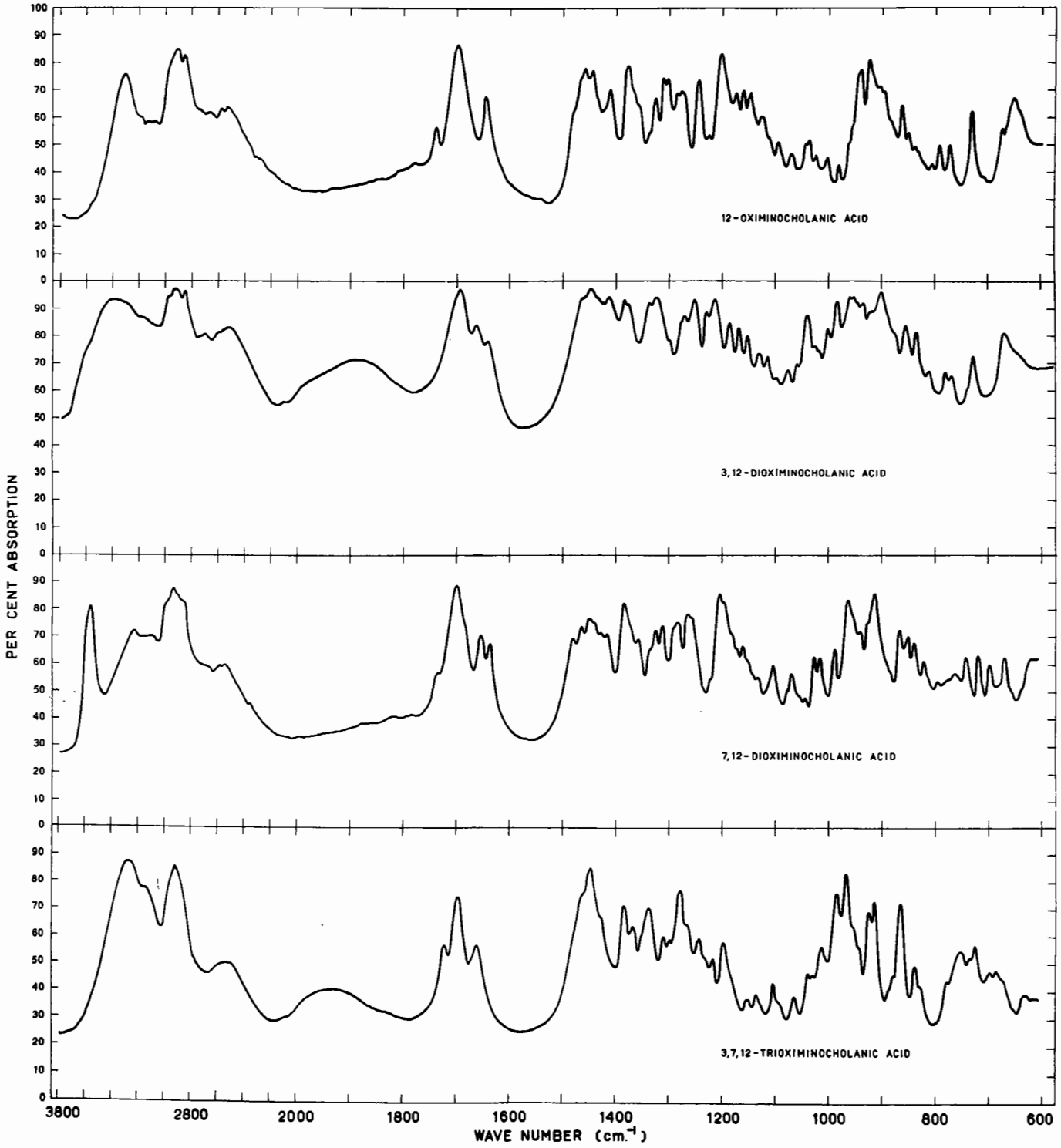

12-OXIMINOCHOLANIC ACID
3,12-DIOXIMINOCHOLANIC ACID
7,12-DIOXIMINOCHOLANIC ACID
3,7,12-TRIOXIMINOCHOLANIC ACID
PER CENT ABSORPTION
WAVE NUMBER (cm.⁻¹)
3800
2800
2000
1800
1600
1400
1200
1000
800
600

TABLE I

Positions of absorption maxima in the infrared spectra of:

Cholestan-3-one I	Di-[x,x' 3-keto-cholestanyl] methane II	Cholestane-3,6-dione III	Di-[x,x'-di-3,6-cholestanyl] methane IV
2940 vs	2940 vs		2960 vs
2860 vs	2880 sh	2840 m (bd)	2860 vs
	1725 s		1727 s
1710 vs	1700 m	1710 vs	1710 vs
1470 s	1470 s	1460 s	1465 vs
1445 s	1445 s		
1425 m	1413 w	1420 w	1425 sh
1385 m	1385 s	1380 m	1382 s
1380 sh			1375 sh
1367 w	1367 sh		1365 sh
1350 vw		1350 vw	
1338 w	1335 w	1335 w	
1310 w	1300 w	1310 w	1310 m
1275 w	1285 w	1285 w	1280 vw
1255 w	1257 w	1262 m	1260 w
1230 m	1235 w	1240 m	1237 w
1215 vw	1205 m	1225 sh	1207 vw
1185 w	1180 sh	1195 vw	
1172 w	1170 sh	1170 w	
1155 w	1150 s		1150 m
1127 w	1137 m	1125 w	1127 m
	1113 w		1100 vw
	1100 sh		
1070 vw	1065 w		1077 w
	1045 sh		1065 sh
	1035 sh		
1027 w	1025 w		1025 vw
	1005 vw		1007 vw
995 w	985 w	992 w	
965 sh		980 vw	970 w
955 m			
940 vw	940 vw		947 w
925 w		925 vw	925 sh
	910 w		905 vw
870 w	865 w		870 vw
805 w	810 vw		825 vw
	770 vw		775 vw
755 w			750
735 w	730 vw		725 w

TABLE II

Positions of absorption maxima in the infrared spectra of:

12β-Aminocholanic acid hydrochloride I	12β-Aminocholanic acid II	3α,12β-Aminocholanic acid III	7β,12β-Aminocholanic acid IV
3400 sh	3440 m	3380 s	3400 m
		3310 sh	
3200 sh			
3060 sh	3040 sh		
2940 vs	2940 vs	2920 vs	2930 vs
2880 sh	2870 s	2860 sh	2860 s
			2660 vw
2600 sh		2610 sh	2630 vw
			2480 vw
		2300 vw	
	2080 sh	2180 w	
1705 vs			
		1660 sh	
	1625 m	1623 s	1635 sh
			1630 sh
1610 m			
	1565 sh	1575 vs	1560 sh
	1550 s	1550 sh	1550 m
1515 s			
1505 sh			
1473 m	1473 m	1465 sh	1467 sh
1452 s	1452 s	1452 s	1450 m
		1430 vw	
1385 s	1390 s	1395 vs	
		1382 vs	1380 vs
	1340 vw	1348 w	
	1315 m	1322 w	
1290 vw	1295 vw	1295 w	
	1280 vw	1280 vw	
1260 sh	1265 vw	1260 w	1260 w
1170 vw	1165 m	1175 vw	1185 vw
1100 m	1100 m	1100 vw	1100 sh
			1095 w
1075 w		1070 w	1075 vw
1060 w	1060 vw	1060 vw	1055 vw
1040 w	1045 vw	1045 vw	
1020 w	1020 w	1030 w	
		1012 w	1015 w
		970 m	
955 m	960 w	945 m	955 w
927 w		920 m	
910 vw		905 m	
845 m	845 w	855 m	860 vw

TABLE III

Positions of absorption maxima in the infrared spectra of:

3ξ,12ξ,24-Trihydroxycholane	3ξ-Amino-12-oximino-24-hydroxycholane	3ξ-Amino-12-keto-24-hydroxycholane
	3500 vs (bd)	
3440 sh		
3340 vs		3300 vs (bd)
2940 vs	2940 vs	2940 vs
2870 vs	2860 vs	2860 vs
2300 vw		
2080 vw		
		1700 m
	1660 m	1655 m
1470 s	1465 sh	1465 sh
1450 s	1450 vs	1450 vs
1377 s	1380 s	1380 vs
1365 s		
1238 m	1240 vw	1235 vw
1120 m	1120 vw	1135 vw
1092 m		1080 sh
1075 s		
1055 s	1055 s	1055 s
1042 s		
	1025 s	1020 s
1011 vs		
	995 s	990 s
950 m	965 vs	965 vs
942 m	915 vs	915 vs
890 m		
	865 s	865 s
855 w	845 m	840 w
	820 w	820 w
	750 m (bd)	

TABLE IV

Positions of absorption maxima in the infrared spectra of:

12-Ketocholanic acid I	3,12-Diketocholanic acid II	7,12-Diketocholanic acid III	3,7,12-Triketocholanic acid IV
			3380 sh
		3240 m	3200 m
2940 vs	2940 vs	2960 sh	2950 s
		2910 s	2920 sh
2860 vs	2860 vs	2840 sh	2880 sh
2640 sh	2680 sh	2660 w	2650 vw
2515 sh		2590 vw	
		1730 s	1735 sh
1715 sh			
1705 vs	1705 vs (bd)		
	1695 sh	1697 vs	1700 vs (bd)
1637 m			
1545 vw			
1510 vw			
1462 s	1460 s	1465 m	1470 m
1447 s	1445 s	1450 m	
1435 m	1430 s	1430 vw	1435 m
		1420 vw	1423 m
1400 sh		1408 w	1405 vw
		1395 sh	
1385 s	1383 s	1387 w	1385 m
		1380 w	1375 sh
			1370 sh
			1365 sh
1360 w	1355 w	1355 sh	1360 sh
1335 w	1335 sh	1345 sh	1335 sh
	1327 m	1320 sh	
	1315 m	1315 sh	1317 w
		1300 w	1298 m
		1280 m	1275 m
1265 s	1265 s	1260 m	
1245 s	1250 s	1230 m	1245 m
1210 m	1215 m	1215 m	1212 sh
1192 m			
	1187 w	1185 w	1180 m
1165 w	1170 w	1160 m	1165 sh
	1124 m		
1110 m			1115 m
1100 m	1105 w	1105 m	1100 m
	1090 w	1095 sh	1090 sh
1070 vw		1065 w	1060 w
1045 sh	1042 vw	1040 w	1045 w
1020 w	1023 m		1027 w
1002 w	1005 sh	1010 w	1005 m
952 w	955 m	953 m	952 m
925 sh	925 sh	930 sh	925 m
902 m		898 vw	
872 sh	870 vw	875 m	870 w
782 m	772 w		767 vw

TABLE V

Positions of absorption maxima in the infrared spectra of:

12-Oximinocholanic acid I	3,12-Dioximinocholanic acid II	7,12-Dioximinocholanic acid III	3,7,12-Trioximinocholanic acid IV
		3560 vs	
	3400 s		
3320 s			
3190 sh	3200 sh	3240 s (bd)	3270 vs
3090 vs			3150 sh
2920 vs	2980 sh	2940 vs	2920 vs
2860 vs	2850 vs	2920 sh	
	2700 vw		
2580 vw			
2540 vw	2520 w	2540 w	2520 m
2280 sh	1900 w (bd)		1935 w (bd)
1740 m		1760 sh	
			1723 m
1700 vs	1695 vs	1700 vs	1698 s
			1670 sh
	1665 m		1665 sh
		1655 m	1660 m
1647 m	1640 m	1637 m	
	1470 sh	1480 sh	
1460 s	1465 sh	1467 sh	1460 sh
1445 s	1447 vs	1450 m	1449 vs
1412 m	1415 s	1417 vw	1430 sh
1378 s	1385 s	1385 s	1385 m
1325 m	1325 s	1325 m	1340 m
1315 s		1315 m	1310 w
1305 s	1300 sh		1300 vw
1290 m	1280 sh	1285 m	1280 s
1277 m	1272 m	1270 m	
1245 s	1252 s	1255 m	1245 w
	1230 m		
1205 vs	1215 s	1205 vs	1220 w
	1185 m	1185 sh	1195 m
1175 m	1170 m	1175 sh	
1162 m		1165 w	1160 sh
1150 m	1155 m		1150 w

TABLE V (cont'd)

I	II	III	IV
1115 sh	1115 sh	1105 m	1105 w
1070 w	1075 w	1070 m	1065 w
1035 w	1040 s	1030 m	1040 w
	1002 m	1017 m	
980 w	985 s	990 m	985 s
	955 s	965 vs	965 vs
940 s	935 s	930 sh	945 sh
			925 m
905 sh	905 vs	915 vs	915 s
862 m	855 m	867 s	865 s
850 w		850 m	
835 vw	835 m	840 m	840 w
		745 m	755 m
730 m	730 m	720 m	725 m
705 sh		700 m	700 vw
650 m	670 m	670 m	685 vw

EXPERIMENTAL

Infrared absorption spectra determinations

a) Apparatus and Materials

A Perkin-Elmer Model 21 double beam spectrophotometer equipped with a sodium chloride prism was used.

The spectra, unless otherwise stated, were determined in potassium bromide infrared quality, obtained from the Harshaw Chemical Co., Cleveland, Ohio, U.S.A. Potassium bromide was kept in an anhydrous condition at all times; when the bottle had been opened a few times, the material was redried in an Abderhalden over boiling water for twenty-four hours.

b) Spectroscopic technique

The procedure for the preparation of potassium promide discs was as follows: adequate amounts of a substance (0.5-1.0 mg.) and of potassium bromide (approx. 0.4 g.) were placed together with three small steel balls in a 1 ml. glass tube equipped with a ground glass stopper. The mixture was ground for three minutes, using a Perkin-Elmer vibrator. Subsequently, it was pressed under 20,000 lbs./sq.in. for two minutes in the Perkin-Elmer pellet die, and the resulting disc was placed in the spectrophotometer.

The determinations of spectra were carried out using the following settings of the instrument:

resolution 927, response 1:1, gain 5.5, speed 5.5, and suppression 0.

The scale of recordings was 100 cm^{-1}/cm for the range 3800-2000cm^{-1}, and 100cm^{-1}/4 cm. for the range 2000-600cm^{-1}.

The following scale of intensities was adopted; very weak (vw), weak (w), medium (m), strong (s), very strong (vs), broad band (bd) and shoulder (sh).

Part I - Mannich Condensation of Ketosteroids

Preparation of secondary amine hydrochlorides used in Mannich reaction

The hydrochlorides of the secondary amines were prepared by the addition of a calculated amount of dilute hydrochloric acid to the amine. The mixture was kept cool during the addition of the acid by placing the three-necked flask equipped with a mechanical stirrer, condenser and dropping funnel in an ice bath. The solution was then evaporated to dryness under reduction pressure, and the remaining salt was recrystallized from absolute ethanol and dried in a vacuum dessicator over sodium hydroxide pellets.

Secondary amines

They were dried by refluxing over sodium hydroxide pellets for six hours and were then distilled, and stored over solid sodium hydroxide.

Cholest-5-en-3-one

The procedure described by Fieser (145) was followed, with a few modifications. Cholesterol was obtained from Brickman and Company in Montreal, and was used without further purification.

(i) 5α,6β-Dibromocholestane-3β-ol

Seventy-five grams of cholesterol was dissolved in 500 ml. of absolute ether by short boiling and stirring in a 2-l. beaker. The solution was cooled to 25°, and a solution of 2.5 g. of anhydrous sodium acetate and 11.3 ml. of bromine in 300 ml. of acetic acid was added with stirring. A stiff white paste resulted. The mixture was cooled to 20°, the product separated by suction filtration, and washed with

acetic acid (300 ml.) until the filtrate was colourless. A second crop of material was obtained by adding 300 ml. of water to the combined filtrate and washings. The precipitate was filtered and washed with acetic acid till the filtrate was colourless. The product was air-dried on a large filter paper in a fume-cupboard at room temperature overnight. Yields in the first and second crops were 87.7 g. and 4.1 g., respectively. Total yield was 85 g. (82 per cent of the theoretical on the basis of 1 to 1 acetic acid complex).

(ii) 5α,6β-Dibromocholestan-3-one

The acetic acid-moist 5α, 6β-dibromocholestane-3β-ol from 75 g. of cholesterol was dispersed in 850 ml. of acetic acid in a 2-l, three-necked, round-bottomed flask equipped with a mechanical stirrer and a thermometer, the third neck being provided with a ground glass stopper. The flask was mounted over an ice-water bath. A solution of 34 g. of sodium dichromate dihydrate in 850 ml. of glacial acetic acid, preheated to 90°, was poured into the stirred dispersion at room temperature. All solid material dissolved in a few minutes, and the temperature rose to 58°. After another two minutes, the stirring was stopped and the flask was completely immersed in the ice-water bath for ten minutes. The stirring was resumed, and the temperature lowered to 25°. Then 200 ml. of water was added and the temperature further decreased to 15°. The product was filtered with suction using a large Büchner funnel, and washed with cold methanol until the filtrate was colourless. The white crystalline material was air-dried in the dark in a fume-cupboard at room temperature overnight. It melted at 71-73° (dec.) and the yield was 78.9 g. (92.5 per cent of the theoretical). The melting points reported in the literature were 73-75° (145), 80° (144) and 68-69° (158).

(iii) Cholest-5-en-3-one

The methanol-moist 5α, 6β-dibromocholestan-3-one from 75 g. of cholesterol was suspended in 1-l. of ether in a 2-l. three-necked, round-bottomed flask equipped with a mechanical stirrer and thermometer. The flask was mounted over an ice-bath. Acetic acid (12 ml.) was added to the stirred suspension and the temperature was lowered to 15°. Then, 45 g. of zinc dust was added portionwise over a period of 5 minutes, the temperature being kept between 15 and 20° by cooling. When the exothermic reaction was over, the ice-bath was removed and stirring continued for 20 minutes. Then, 20 ml. of pyridine was added, and the resulting white suspension stirred briefly. The solution was filtered with suction, and the cake washed several times with ether. The filtrate was almost colourless. It was washed three times with water, once with 300 ml. of 5% sodium bicarbonate solution, and once again with water. Then, it was dried over anhydrous magnesium sulphate, filtered and concentrated to 400 ml. After addition of 200 ml. of methanol, the concentration was continued to a volume of 450 ml., and the product allowed to crystallize first at room temperature, and then in an ice-box for a few hours. The white prisms separated, were collected on the filter, and dried in a vacuum dessicator. The yield from the first crop was 38.1 g., m.p. 123-126°. Concentration of the mother liquor afforded 6.05 g. of the product which melted at 117-122°. The total yield was 85 per cent of the theoretical (64.6% per cent of the theoretical based on cholesterol used). Recrystallization from ether-methanol raised the melting point of each drop to 127-129°. The melting points of 127° (144) and 126-129° (145) were reported in the literature.

Attempted condensation of cholest-5-en-3-one with paraformaldehyde and secondary amine hydrochlorides

a) Using morpholine hydrochloride

A 25 ml. three-necked conical flask was equipped with a mechanical stirrer, a condenser fitted with a calcium chloride tube, and a nitrogen inlet tube. Morpholine hydrochloride (0.185 g.; 0.15 mmoles), paraformaldehyde (0.04 g; 0.15 mmoles) and 6 ml. of isoamyl alcohol were placed in the flask. The mixture was stirred for 20 minutes at room temperature, then heated to reflux in an oil bath in a slow stream of dry nitrogen. All solids dissolved, and 0.580 g. (0.15 mmole) of cholest-5-en-3-one was added portionwise in the course of one hour. Refluxing was continued for another hour, at which time the solution was brown. It was allowed to cool to room temperature and long needles separated immediately. They were filtered off and dried. The material weighed 0.150 g. and melted at 171-172°. The mixed melting point with an authentic sample of morpholine hydrochloride was 173-174° (undepressed). The remaining filtrate was treated as follows: it was shaken with 10 ml. of 4% hydrochloric acid, diluted with water and extracted with ether. The organic layer was brown. It was separated and dried over anhydrous magnesium sulphate, then allowed to stand with decolourizing carbon Nuchar-C190-N for a few hours at room temperature with occasional shaking. After filtration of the solution, the solvents were evaporated off, first on the steam bath under reduced pressure (water aspirator), and then in vacuo. The residual tarry dark brown oil was thoroughly dried over phosphorus pentoxide. This oil contained no nitrogen, did not crystallize, and possessed a tendency to form films.

The aqueous layer was made alkaline with a saturated sodium carbonate solution, and extracted again with ether (five portions of 10 ml. each). The ethereal extract was washed with water, dried, and evaporated to dryness under reduced pressure, leaving no residue.

Similar results were obtained when the molar ratios of the ketone, paraformaldehyde and morphaline hydrochloride, were varied from 1:1:1 to 1:2:3 and 1:5:7.5. The total refluxing time was also varied from 15 minutes to 2 hours in each case. The results were not affected when absolute ethanol or n-butanol was substituted for isoamyl alcohol under the above mentioned conditions.

b) Using piperidine hydrochloride

The procedure and various conditions outlined in the case of morpholine hydrochloride were followed. Film-forming oils which could not be crystallized resulted in all cases. They contained no nitrogen. The major part of the amine salt was recovered.

c) Using dimethylamine hydrochloride

Same results were obtained as in a) and b).

Condensation of cholest-5-en-3-one with paraformaldehyde and morpholine

(i) In dioxane

A similar apparatus was used as in the previous reaction. Dry morpholine (0.43 g.; 0.005 mole) and paraformaldehyde (0.15 g.; 0.005 mole) were placed in the flask and stirred together for one hour at room temperature, a stream of dry nitrogen being passed through the system. The mixture became slightly yellow. A solution of 1.93 g. (0.005 mole) of the ketone in 6 ml. of dry, warm dioxane was added, and stirred for 20

minutes at room temperature. The solution did not show any change of colour. It was refluxed in an oil bath for one hour and became yellow. After cooling to room temperature, a solid material precipitated out. The mixture was shaken with 15 ml. of 4% hydrochloric acid and diluted with water. The yellow solid material remained undissolved. The mixture was extracted with two portions of ether 20 ml. each, and the ethereal extract was dried over anhydrous magnesium sulphate. After filtration, the solution was concentrated to 8 ml. under reduced pressure, and warm methanol was added dropwise to turbidity. A slightly yellowish precipitate separated upon cooling to room temperature. The latter was filtered with suction, washed on the filter with a small amount of cold methanol, and dried. The crude product weighed 0.20 g., and melted at 142-145°. One recrystallization from acetone gave white needles, m.p. 149.5-150.5°. The product contained nitrogen.

The hydrochloride was formed by dissolving a small sample of the compound in absolute ether and by passing dry hydrogen chloride gas through the solution for a few minutes. The salt, in the form of a white powder, precipitated immediately, and quantitatively. The filtration was followed by drying in vacuo. The crude product turned brownish at 203° and melted over the range 210-215° to a brown liquid. After one recrystallization from chloroform-ether, the salt darkened at 215° and melted at 227-232°.

The aqueous layer was treated in the usual way: made alkaline with a saturated solution of sodium carbonate, extracted with ether, the extract dried, and the solvent evaporated under reduced pressure. No residue remained.

(ii) In isoamyl alcohol

In an analogous reaction carried out in isoamyl alcohol instead of dioxane, the yield of crude product was 0.796 g. Recrystallization from 120 ml. of acetone afforded 0.560 g. of cotton-like white needles. The product contained nitrogen and melted at 149°. The hydrochloride was obtained in the same manner as in (i) and after one recrystallization, melted at 227-232°.

The methiodide was produced by dissolving a small amount of the basic compound in benzene, cooling to 0°, and adding dropwise ice-cold methyl iodide in excess. The solution was kept in a refrigeration unit overnight, and deposited small white crystals. The ionic iddine was found to be present by mixing a few drops of the suspension with nitric acid (2 N), diluting with distilled water and adding a few drops of 5% aqueous silver nitrate. The solution was then extracted with ether. The precipitate of silver iodide separated in the aqueous phase.

Neither condensation (i) nor (ii) could be reproduced in spite of numerous attempts.

Attempted condensation of cholest-5-en-3-one with paraformaldehyde and piperidine

The procedure described in the case of morpholine was adopted. The molar ratios of the above reactants were varied from 1:1:1 to 1:2:3, to 1:5; 7.5, and the refluxing time from half an hour to two hours. Absolute ethanol, n-butanol and isoamyl alcohol were used as solvents. The reaction mixture was treated in the usual way, and in all cases dark brown oils tending to form films were obtained.

4,4-Dimethylcholest-5-en-3-one

The ketone was prepared by direct methylation of cholest-5-en-3-one in dry tert.-butanol, with potassium tert.-butoxide and methyl iodide.

Commercial tert.-butanol was dried by refluxing with sodium, and then distilling. Potassium tert.-butoxide was prepared according to the method described in Organic Syntheses (159). A 200 ml. three-necked, round-bottomed flask equipped with a mechanical stirrer, a condenser fitted with a calcium chloride drying tube, the third neck being provided with a removable ground glass stopper, was used. The dissolution of 1.56 g. (0.035 mole) of potassium in 60 ml. of dry tert.-butanol required 45 minutes at room temperature with stirring. Then, 5.535 g. (0.013 mole) of cholest-5-en-3-one was added, and the colour of the solution immediately changed from slightly yellow to orange. The ground glass stopper was removed and replaced by a dropping funnel containing 4.85 ml. (0.078 mole) of methyl iodide. The latter was added dropwise to the solution maintained at room temperature by means of a cold water cooling bath. When about 1.5 ml. of methyl iodide had been added, the solution became turbid and the colour turned yellow. The addition of methyl iodide was completed in 20 minutes, and then 40 ml. of water was added. A white solid precipitated, was filtered with suction and dried. Recrystallization from 1-l. of 95% ethanol gave 3.65 g. (61 per cent of the theoretical) of shiny platelets, m.p. 173-174°. The reported yield was 63 per cent, and the melting point 176-177° (161).

Attempted condensation of 4,4-dimethylcholest-5-en-3-one with paraformaldehyde and morpholine hydrochloride

In a 25 ml. three-necked conical flask equipped with a mechanical stirrer, a condenser protected with a calcium chloride tube and a dry nitrogen inlet tube, were placed 0.62 g. (0.15 mmole) of the ketone, 0.185 g. (0.15 mmole) of morpholine hydrochloride, 0.04 g. (0.15 mmole) of paraformaldehyde, and 3 ml. of isoamyl alcohol. The mixture was stirred and heated to reflux in an oil bath, in a slow stream of dry nitrogen. Refluxing was continued for 4 hours and the colour of the solution became gradually brown. A white product precipitated upon cooling to room temperature. It was filtered with suction and recrystallized from 95% ethanol. White, shiny platelets were obtained which, after drying in vacuo, melted at 171-172°, undepressed by admixture of an authentic sample of 4,4-dimethylcholest-5-en-3-one. The recovery was 75 per cent.

Similar results were obtained when piperidine and dimethylamine hydrochlorides were used instead of morpholine hydrochloride in the above reaction.

Attempted condensation of 4,4-dimethylcholest-5-en-3-one with paraformaldehyde and morpholine

In a reaction similar to the above one, equimolecular amounts of the three reactants were refluxed in 3 ml. of isoamyl alcohol for four and a half hours. The recovery of the unreacted ketone was 85 per cent.

Cholest-4-en-3-one

(i) By isomerization of cholest-5-en-3-one

The procedure outlined by Fieser (145) was slightly modified. In a 500 ml. round-bottomed flask equipped with a condenser, 36 g. of cholest-5-en-3-one and 10 g. of anhydrous oxalic acid were dissolved and refluxed in 280 ml. of 95% ethanol for 15 minutes. The solution was allowed to cool to room temperature and was seeded with a few crystals of Δ^4-ketone. The first crop of the product (29.5 g.) separated as large, colourless needles, m.p. 80°. The second crop (4.3 g.) was obtained by concentration of the mother liquor and seeding; m.p. 79-80°. Total yield was 33.8 g., 94 per cent of the theoretical (60.7 per cent based on cholesterol used).

In the literature, a yield of 97 per cent and the melting point of 81-82° were reported (145).

(ii) By Oppenauer's dehydrogenation of cholesterol

The modification of Oppenauer's dehydrogenation (161) by Inhoffen (162) was adapted to the preparation of cholest-4-en-3-one. Aluminium isopropoxide was prepared according to the method described by Vogel (113). A dry 3-l. round-bottomed flask equipped with a reflux condenser fitted with a calcium chloride tube, was charged with 25 g. of cholesterol (purified by regeneration from 5α,6β-dibormocholestan 6β-ol) 27.6 g. of aluminium isopropoxide, 1250 ml. of dry toluene and 210 ml. of freshly redistilled cyclohexanone (m. p. 153-155°). The resulting solution was refluxed gently using an electric heating mantle. After five minutes, the solution developed a yellow colour, and after twenty minutes it turned slightly cloudy. Gentle boiling was continued for a total time of one

hour. The solution was then steam-distilled, the residue extracted several times with ether, the ether extract washed with 4% sulphuric acid, water, 10% wodium bicarbonate, twice with water, and dried over anhydrous magnesium sulphate. The solvent was evaporated on the steam bath under reduced pressure (water aspirator) till slight turbidity and 20 ml. of methanol was added. The evaporation was continued until slight cloudiness developed. The solution was seeded with crystals obtained by cooling a few drops of the solution in dry ice-acetone mixture. The flask was wrapped with a towel to ensure very slow cooling. The yield of the crude yellowish product was 18.5 g., m.p. 76-78°. After one recrystallization from 7:10 mixture of acetone-methanol, the material melted at 79-81° with 90 per cent recovery (yield from cholesterol 67.5 per cent of the theoretical).

Attempted condensations of cholest-4-en-3-one with paraformaldehyde and secondary amine hydrochlorides

Morpholine, piperidine, and dimethylamine hydrochlorides were used. The procedure outlined in the attempted condensations of cholest-5-en-3-one was followed. The molar ratios of the ketone paraformaldehyde and amine salts were varied from 1:1:1 to 1:2:3 to 1:5:7.5. Absolute ethanol, n-butanol and isoamyl alcohol were employed as solvents. Refluxing time was varied from half an hour to two hours in each case. A brown coloured visquous oil possessing film-forming tendencies was the only isolable product in all instances. The oil contained no nitrogen and could not be crystallized.

Attempted condensations of cholest-4-en-3-one with paraformaldehyde and secondary amines

In the previously described apparatus (cf. p.103), dry morpholine (0.13 g.; 0.15 mmole) and paraformaldehyde (0.04 g.; 0.15 mmole) were stirred for one hour at room temperature in a slow stream of dry nitrogen. A solution of 0.58 g. (0.15 mmole) of the ketone in 2 ml. of warm isoamyl alcohol was added and the mixture was stirred for 20 minutes at room temperature. Then it was brought to boiling in an oil bath and all solids dissolved after ten minutes. The solution was refluxed for one hour, and the colour became light yellow. An oil was obtained upon evaporation to dryness in vacuo. Crystallization took place within half an hour of standing at room temperature. After one recrystallization from 95% ethanol and drying, 0.52 g. of almost colourless needles was obtained, m.p. 78-80°, undepressed by admixture of an authentic sample of cholest-4-en-3-one. The recovery of the unreacted material was 80 per cent. A similar result was apparent when piperidine was substituted to morpholine.

When the refluxing time was two hours or longer, a brown amorphous material resulted, regardless of whether morpholine or piperidine was used. This material could not be crystallized. Varying the molar ratios of the reactants did not affect the course of the reaction.

Di-[x,x'-3-ketocholestanyl] methane

The synthesis of di-[x,x'-3-ketocholestanyl] methane involved the following steps:

1. Preparation of cholestanol by hydrogenation of the olefinic double bond of cholesterol.
2. Oxidation of cholestanol to cholestan-3-one.
3. Condensation of cholestan-3-one with paraformaldehyde in the presence of a secondary amine hydrochloride to give di-[x,x' 3-ketocholestanyl] methane.

1. Cholestanol

Cholestanol was prepared by catalytic reduction of cholesterol according to directions of Hershberg (164). Commercial cholesterol obtained from Brickman and Company was purified by regeneration from the dibromide (145).

The hydrogenation was carried out as follows:

Cholesterol (9.66 g.; 0.0025 mole), platinic oxide (0.2 g.) prepared according to the procedure described in Organic Syntheses (165), 125 ml. of c.p. ethyl acetate and four drops of 72% perchloric acid were placed in the reduction bottle of a PARR low pressure hydrogenation apparatus. The flask was evacuated, connected to the hydrogen tank of the apparatus, flushed out three times with hydrogen, and warmed up to 45° by means of an electric resistance heater mounted under the bottle. The heater was turned off, and the heat of the exothermic reaction of hydrogenation kept the solution at about the starting temperature. The initial pressure was 16 lbs./sq. in., and the flask was shaken mechanically for 20-30 minutes. The hydrogen was removed from the flask by suction (water aspirator) and replaced with nitrogen. The solution was then treated with two drops of 50% sodium hydroxide, and filtered with suction in order to remove the catalyst. The filtrate was kept in the refrigerator overnight and yielded a first crop of cholestanol which was collected by filtration. The mother liquor was evaporated to

dryness under reduced pressure, and the residue recrystallized from 120 ml. of boiling methanol. The total yield of cholestanol, m.p. 138-140.5°, was 8.3 g. (85 per cent of the theoretical). The melting point reported in the literature was 139-141° (164).

2. Cholestan-3-one

The preparation described in Organic Syntheses (166) was adopted. A solution of 9 g. of cholestanol in 9 ml. of benzene was added slowly with cooling (25-30°) to a solution of 11.7 g. of sodium dichromate in 9 ml. of glacial acetic acid, and 9 ml. of concentrated sulphuric acid in 54 ml. of water in a 500 ml. three-necked flask equipped with a mechanical stirrer. The mixture was stirred vigorously for seven hours at room temperature. Then, the benzene layer was separated, washed twice with water, once with 35 ml. of 5% potassium hydroxide, and twice with water again. The solution exhibited the tendency to emulsify and was centrifuged for 20 minutes. The benzene layer separated nicely and the solvent was removed under reduced pressure. The residual oil was dissolved in 55 ml. of boiling ethanol and left to crystallize at room temperature. The solution deposited colourless well-formed needles. The yield of thoroughly dried material was 6.5 g. (72 per cent of the theoretical), m.p. 129-130°. The melting point reported (166) was 129-130°.

Infrared absorption spectrum: 1710cm^{-1} (vs) (C=O)

3. Di-[x,x' 3-ketocholestanyl] methane

(i) A mixture of 0.58 g. (0.15 mmole) of cholestan-3-one, 0.05 g. (0.15 mmole) of paraformaldehyde, 0.2 g. (0.15 mmole) of morpholine hydrochloride, and 2.5 ml. of isoamyl alcohol was placed in a 25 ml. three-necked flask equipped with a mechanical stirrer, a condenser protected by a calcium chloride drying tube, and a nitrogen inlet tube. The flask was placed in an

oil bath and the mixture was heated to reflux with stirring in a slow stream of nitrogen. All solids dissolved when the temperature reached 115° (after five minutes of heating) and after 20-30 minutes of refluxing, solid material suddenly precipitated out of the solution. The mixture was allowed to cool to room temperature and was kept in a refrigerator overnight. The product was collected by filtration with suction, washed a few times with cold 95% ethanol and dried. White microcrystalline solid weighed 0.33 g. (35.4 per cent of the theoretical based on cholestan-3-one), and melted at 205-207° to a deep brown liquid. Recrystallization from benzene gave a similarly looking solid, m.p. 208-210°, which could not be raised by further recrystallizations.

Anal. Calcd. for $C_{55}H_{92}O_2$: C, 84.18%; H, 11.73%.

Found: C, 83.86%; H, 11.64%.

Infrared absorption spectrum: 1725cm^{-1} (s) and 1700cm^{-1} (m) (C=O)

(ii) A similar yield of the above product was obtained in an analogous reaction in which piperidine hydrochloride was substituted for morpholine hydrochloride.

(iii) When the reaction was carried out in absolute ethanol and refluxing was maintained up to two hours, the unreacted cholestan-3-one was recovered in 95 per cent yield.

(iv) In dry peroxide free dioxane, with refluxing time of two hours, products having melting points between 120° and 202° were obtained. Only a small amount of a product melting at 205-297° could be isolated and was not further investigated.

(v) The yield of the product was not affected when the molar ratio of the ketone to paraformaldehyde was changed from 1:1 to 2:1.

Reaction of 2,4-dinitrophenylhydrazine with di-[x,x' 3-ketocholestanyl] methane.

To a solution of 29 mg. of the ketone and 14 mg. of 2,4-dinitrophenylhydrazine in 35 ml. of ethanol, was added with a capillary tube one drop of concentrated hydrochloric acid. The reaction mixture was refluxed for 15 minutes on the steam bath. Upon cooling to room temperature, a small amount of an orange material separated. It was collected by filtration and dried; it melted at 220-222°. This product could not be recrystallized satisfactorily in spite of numerous attempts.

Di-[x,x'-di-3,6-ketocholestanyl] methane

The synthesis of the above compound involved the following steps:

1. Preparation of cholestane-3,6-dione by oxidation of cholesterol to cholest-4-en-6β-ol-3-one, and isomerization of the latter product.
2. Condensation of cholestane-3,6-dione with paraformaldehyde in the presence of a secondary amine hydrochloride to give di-[x,x'-di-3,6-ketocholestanyl] methane.

1. Cholestane-3,6-dione

a) Preparation of cholest-4-en-6β-ol-3-one

The procedure described by Fieser (167) was adopted. A solution of 28 g. of cholesterol (purified through the dibromide) in 140 ml. of benzene in a 1-l,beaker, was diluted with 140 ml. of glacial acetic acid and quickly

cooled to 20° (a supercooled solution is obtained in that manner). A solution of 17 g. of sodium dichromate dihydrate in 70 ml. of glacial acetic acid (also at 20°) was then added with stirring and an orange paste resulted. The mixture was kept at 15-20° for four hours and its viscosity decreased as the oxidation proceeded. It was allowed to stand at room temperature overnight, was diluted with an equal volume of water and extracted with three portions of ether 80 ml. each. The ethereal extract was evaporated to dryness under reduced pressure, and the green residue was dried for six hours in vacuo over sodium hydroxide pellets. The dry solid was digested with 140 ml. of warm petroleum ether (b.p. 60-90°), and the resulting green-yellowish solution was cooled in a refrigerator for one hour. The product which separated was filtered with suction and dried. The yield was 5.5 g., m.p. 171-174°. Recrystallization from n-hexane gave 4.4 g. (15.3 per cent of the theoretical) of white cotton-like needles, m.p. 182-184°. Fieser reported in two experiments 16-17 per cent yield, and the m.p. of 184-185° and 189-191°.

b) Isomerization of cholest-4-en-6-β-ol-3-one to cholestane-3,6-dione

A solution of 4.4 g. of cholest-4-en-6β-3-one in 70 ml. of 95% ethanol containing 1.5 ml. of concentrated hydrochloric acid was refluxed in a 200 ml. one-necked, round-bottomed flask equipped with a condenser. After 15 minutes of refluxing, some crystals of the dione had already separated. The total refluxing time was 1.75 hours. The mixture was first allowed to cool to room temperature and then was kept in a refrigerator overnight. The product was collected and dried; it weighed 4.0 g. (92 per cent of the theoretical), m.p. 169.5-171°.

The yield reported in the literature was 96 per cent and the m.p. 170-171°.

Infrared absorption spectrum (in Nujol): 1710cm^{-1} (vs) (C=O)

2. Di-[x,x'-di-3,6-ketocholestanyl] methane

(i) A mixture of 0.60 g. (0.15 mmole) of cholestane-3,6-dione, 0.05 g. (0.15 mmole) of paraformaldehyde, 0.2 g. (0.15 mmole) of morpholine hydrochloride, and 3 ml. of isoamyl alcohol was placed in a 25 ml. three-necked conical flask equipped with a mechanical stirrer, a condenser fitted with a calcium chloride drying tube, and a nitrogen inlet tube. The flask was placed in an oil bath and the mixture was heated to reflux, dry nitrogen being passed through the system. All solids dissolved when the temperature of the bath reached 105°. After 45 minutes of refluxing, the solution was dark yellow. Crystalline material separated upon cooling to room temperature. The mixture was treated with 0.5 ml. of water, and the organic layer was separated, dried over anhydrous magnesium sulphate and evaporated to dryness in vacuo. The solid residue was recrystallized from about 250 ml. of boiling acetone and afforded cotton-like needles. The yield was 0.254 g. (21.2 per cent of the theoretical based on cholestane-3,6-dione); m.p. 214-217°. Another two recrystallizations from the same solvent raised the melting point to 242-242.5°.

Anal. Calc'd for $C_{55}H_{88}O_4$; C, 82.51%; H, 11.83%

Found: C, 82.09%; H, 11.82%.

Infrared absorption spectrum (in Nujol): 1727cm^{-1} (s) and 1710cm^{-1} (vs) (C=O).

(ii) When absolute ethanol was used as solvent and the refluxing time was 5.5 hours, a mixture of amorphous and tarry materials was obtained. They were not investigated further.

(iii) In dioxane, the completeness of the reaction became apparent after 60-70 minutes of refluxing. The yield of the product was considerably lower than in isoamyl alcohol.

(iv) The yield of the product was not affected by varying the molar ratio of ketone and paraformaldehyde from 1:1 to 2:1.

Attempted reaction of 2,4-dinitrophenylhydrazine with di-[x,x'-di-3,6-ketocholestanyl] methane

The ketone (28 gm.) was placed in a thimble of a Soxhlet apparatus. The material was extracted for 24 hours by a solution of 14 mg. of 2,4-dinitrophenylhydrazine in 35 ml. of ethanol, to which one drop of concentrated hydrochloric acid was added with a capillary tube. The solvent was evaporated under reduced pressure. The residual semi-solid material remained oily after a thorough drying in vacuo over phosphorous pentoxide, and could not be purified.

Part II - Reduction of oximinocholanic acids

12-Oximinocholanic acid

12-Oximinocholanic acid was prepared from 3,12-dihydroxycholanic acid in three steps:

1. Oxidation of 3,12-dihydroxycholanic acid to 3,12-diketocholanic acid.

2. Clemmensen reduction of 3,12-diketocholanic acid to 12-ketocholanic acid.

3. Oximation of 12-ketocholanic acid to 12-oximinocholanic acid.

1. 3,12-Diketocholanic acid

3,12-Dihydroxycholanic acid was obtained from Brickman and Company, and was recrystallized from 70% ethanol; m.p. 172-173°; reported m.p. 172-173° (168). For oxidisation, the procedure outlined by Heilbron (150) for the oxidisation of acetylenic carbinols was adapted.

The oxidizing reagent was prepared according to the instructions of the latter author. To a solution of 13.35 g. of chromium trioxide in 20 ml. of water, 11. 5 ml. of concentrated sulphuric acid was added dropwise and with stirring, so as to avoid the formation of any precipitate. The cold solution was diluted to 50 ml., i.e. an 8 N solution with respect to oxygen.

In a 4-l. beaker, 30 g. of 3,12-dihydroxycholanic acid was dissolved in a minimum of 2.2-l. of reagent grade acetone, and the resulting solution was cooled to 20° in an ice-water bath. The oxidizing reagent solution was then added dropwise from a microburette until an orange-brownish colour persisted (about 45 ml. of the reagent was used). The acetone solution was decanted from the inorganic residue, and was diluted with water to a volume of about 6-l. The ketoacid precipitated immediately and was filtered with suction. The second crop was obtained by diluting the filtrate with another 2-l. of water. After thorough drying in vacuo over phosphorus pentoxide, the first and the second crops weighed respectively 23.40 g. and 5.35 g.; m.p. 184-185°. Total yield was 28.75 g. (96 per cent of the theoretical). Wieland (151) reported a yield of 18 g. of 3,12-diketocholanic acid (m.p. 185°) from 35 g. of 3,12-dihydroxycholanic acid-acetic acid complex.

2. 12-Ketocholanic acid

The procedure described by Wieland (169) was followed.

In a 1-l. one-necked, round-bottomed flask equipped with a condenser, 20 g. of 3,12-diketocholanic acid was dissolved in 240 ml. of 95% ethanol, and to the resulting solution was added 200 g. of c.p. zinc metal in bars and 100 ml. of concentrated hydrochloric acid. When the mixture had been refluxed on the steam bath for three hours, the evolution of hydrogen diminished greatly. Further portions of 40 ml. of concentrated hydrochloric acid were added each hour to the refluxing solution over a period of twelve hours (total of 480 ml. added). The solution was decanted from the unreacted zinc residue and the ketoester precipitated upon cooling to room temperature. After addition of 80 ml. of water, the product was extracted with three portions 150 ml. each of ether. The extract was neutralized with 10% sodium carbonate, washed once with water and dried over anhydrous magnesium sulphate. The solvent was evaporated on the steam bath under reduced pressure, the residue was triturated with 95% ethanol and the crystalline product collected and dried in vacuo over phosphorus pentoxide. The yield was 15.5 g. (74.8 per cent of the theoretical); m.p. 93-94°. Wieland (170) reported the melting point of 95° (no yield).

For the saponification of the ketoester, the procedure outlined by Wieland and Dane (171) was adapted.

In a 100 ml. round-bottomed flask, 15.5 g. of the ester in 60 ml. of 1 N methanolic potassium hydroxide was refluxed until a small sample of the solution remained clear upon dilution with water (one and a half hour refluxing time). Then the solution was greatly diluted with water and treated with 4% sulphuric acid till slightly acidic reaction to litmus. Free acid precipitated and was collected on the filter, dried, and recrystallized

twice from 95% ethanol. The dry product weighed 10.7 g. (75 per cent of the theoretical), and melted at 184-185°. Wieland reported the m.p. of 187° (no yield).

Infrared absorption spectrum: 1715cm^{-1} (sh) (COOH), 1705cm^{-1} (vs) (C=O).

3. 12-Oximinocholanic acid

A modification of the procedure of Schenck and Kirchhof (172) was used.

In a 500 ml. one-necked, round-bottomed flask equipped with a reflux condenser were placed 6.25 g. (0.116 mole) of 12-ketocholanic acid, 5.75 g. (0.082 mole) of hydroxylamine hydrochloride, 6.6 g. (0.080 mole) of fused sodium acetate and 265 ml. of 95% ethanol. The mixture was refluxed on the steam-bath for two hours, allowed to cool to room temperature and was slightly acidified with 30% acetic acid. The product separated and was collected by filtration with suction. After drying, it was redissolved in about 2-l. of boiling 95% ethanol, and hot water was injected into the alcoholic solution till slight turbidity occurred. The oxime precipitated in form of prisms within a few hours of standing at room temperature, and was filtered off. The second crop of pure product was obtained upon addition of more water to the filtrate. The combined crops were dried in vacuo over phosphorus pentoxide. The total yield was 6.3 g. (97% of the theoretical); the product sintered at 225°, melted at 235-236° to a dark brown liquid. According to Schenk (172), the oxime sintered at 185°, turned yellow at 225° and melted at about 245°.

Anal. Calc'd for: $C_{24}H_{39}O_3N$: N, 3.60%.

Found: N, 3.68%.

Infrared absorption spectrum: 3320cm^{-1} (s) (O-H), 1740cm^{-1} (m) (COOH)

1700cm-1 (vs) (C=O), 1647cm^{-1} (m) (C=N)

12β-Aminocholanic acid

Reduction of 12-oximinocholanic acid with sodium in alcohol

(i) In n-propanol

Reagent grade n-propanol (b.p. 96.5-97.5°) was dried before use by distilling it from sodium.

In a 100 ml. three-necked, round-bottomed flask equipped with a mechanical stirrer, a condenser fitted with a calcium chloride drying tube and a nitrogen inlet tube, 0.50 g. (0.0013 mole) of 12-oximinocholanic acid was dissolved in 58 ml. of dry n-propanol. The solution was stirred and heated to reflux in an oil bath in a slow stream of dry nitrogen. Sodium (2.5 g.; 0.10 mole) was then added in small pieces over a period of three and a half hours. Gradually, the solution became yellow. When the addition of sodium had been completed, the content of the flask was transferred to a 150 ml. beaker which was cooled in an ice-water bath. The solution was made slightly acidic with 4% sulphuric acid. Then the propanol was distilled off in vacuo in nitrogen atmosphere, and a certain amount of the product came out of solution. The precipitation was made complete by adjusting the pH to 5.6-5.8 with 1% sulphuric acid using a pH-meter. The yellow solid was filtered with suction and dried. The yield was 0.475 g. (98 per cent of the theoretical); m.p. 122-126° to an opaque visquous liquid which cleared at 138-140°. The product was dissolved in 20 ml. of hot acetone and the resulting yellowish solution was filtered with suction (water aspirator) through celite and carbon Nuchar -C190-N. The colourless filtrate was concentrated under reduced pressure to 10 ml. and heated up to boiling; hot water was then added dropwise until the solution became slightly turbid. Upon cooling to room temperature, the product precipitated in the form of needles which were collected on the filter and dried. They melted at

115-116° to an opaque mass which became fluid over the range 128-140°. Another recrystallization from acetone-water gave a product which melted at 115-116° to a liquid which became transparent at 120°.

Anal. Calc'd. for $C_{24}H_{41}O_2N$, $3H_2O$: C, 67.13%; H, 10.95%; N, 3.24
Found: C, 67.20%; H, 10.88%; N, 3.53

Infrared absorption spectrum: 3440cm^{-1} (m) (O-H), 3040cm^{-1} (sh) ($\overset{+}{N}H_3$), 1625cm^{-1} (m) ($\overset{+}{N}H_3$), 1550cm^{-1} (s) (COO^{-1}).

When the crude product was purified by recrystallization from the following solvents, materials having different melting points were obtained: from dimethylformanide-water, melting at 125-127°; from ethanol-water, sintering at 123°, melting at 127-129°; from methanol-water, sintering at 124°, melting at 131°. The infrared absorption spectra of all these products were identical with the spectrum of the material purified by recrystallization from acetone water.

The hydrochloride of 12β-aminocholanic acid was prepared by treating 40 mg. of the amino acid with 0.5 ml. of concentrated hydrochloric acid, evaporating to dryness under reduced pressure, and repeating this process five times. The white residue, when heated, began to turn yellow at 220°, and melted at 245-247°. Recrystallization from methanol-water gave white powder-like product which melted at 257-258°.

Infrared absorption spectrum: 1705cm^{-1} (vs) (C=O); 1610cm^{-1} (m) ($\overset{+}{N}H_3$).

(ii) <u>In n-butanol</u>

When the reduction of 12-oximinocholanic acid was carried out in n-butanol, a similar yield of 12β-aminocholanic acid was obtained as in n-propanol.

(iii) In isoamyl alcohol

The procedure outlined in (i) was followed and a product obtained in good yield was recrystallized several times from ethanol-water and from acetone-water. After a thorough drying in vacuo at 80° over phosphorus pentoxide, it melted at 174-176° to give an opaque mass which became fluid and transparent at 185-187°. The nitrogen content was half of the one required by 12-aminocholanic acid. This material was not further investigated.

Attempted reduction of 12-oximinocholanic acid with sodium amalgam in absolute ethanol

The sodium amalgam was prepared according to the procedure described by Vogel (173). In a 100 ml. three-necked, round-bottomed flask equipped with a mechanical stirrer, a condenser fitted with a calcium chloride tube and a nitrogen inlet tube, 0.20 g. (0.51 mmole) of 12-oximinocholanic acid was dissolved in 20 ml. of dry ethanol, and the solution was heated to reflux in an oil bath. Then 26.6 g. of 3% sodium amalgam was added, and the mixture was stirred and refluxed for six hours in a slow stream of nitrogen. At this time the major part of the amalgam seemed to have reacted, and the supernatant solution was decanted, allowed to cool, diluted with water to 50 ml., and neutralized with 4% sulphuric acid. A white product which precipitated was collected by filtration with suction and dried; it weighed 0.180 g., melted at 178-179° to a milky liquid which cleared completely at 198-201°. One recrystallization from ethanol gave platelets, m.p. 180-182°. These were identified as 12-ketocholanic acid by a mixed melting point determination (183-184°) with an authentic sample of the ketoacid. An oxime derivative of the reaction product was prepared. A mixed melting point determination with an original sample of 12-oximinocholanic acid showed no

depression (238-240°).

3,12-Dioximinocholanic acid

This acid was prepared from 3,12-dihydroxycholanic acid in two steps:

1. Oxidation of 3,12-dihydroxycholanic acid to 3,12-diketocholanic acid.
2. Oximation of 3,12-diketocholanic acid to 3,12-dioximinocholanic acid.

1. 3,12-Diketocholanic acid

The preparation described above (cf. p.114) as one of the steps in the synthesis of 12-oximinocholanic acid was adopted.

2. 3,12-Dioximinocholanic acid

A modification of the procedure outlined by Schenck and Kirchhof (174) was used.

In a 1-l. one-necked, round-bottomed flask equipped with a condenser, a solution of 12.5 g. (0.033 mole) of 3,12-diketocholanic acid, 23 g. (0.33 mole) of hydroxylamine hydrochloride, and 26.4 (0.32 mole) of fused sodium acetate in 500 ml. of 95% ethanol was refluxed for two and a half hours. After the reaction mixture had cooled to room temperature, the resulting crystalline slurry was poured into 200 ml. of water with stirring, and the crystalline product was removed by filtration and dried. The yield was quantitative. The product sintered at 170°, melted at 184°. The melting point could not be raised by further recrystallization from ethanol.

Schenck (174) reported sintering at 170°; m.p. 188-189°; no yield was given.

3α,12β-diaminocholanic acid

Reduction of 3,12-dioximinocholanic acid with sodium in alcohol

(i) In n-propanol

In a 100 ml. three-necked, round-bottomed flask equipped with a mechanical stirrer, a condenser guarded by a calcium chloride drying tube and a nitrogen inlet tube, 0.40 g. (0.001 mole) of 3,12-dioximinocholanic acid was dissolved in 60 ml. of dry n-propanol. The solution was stirred and heated to reflux in an oil bath, dry nitrogen being passed through the system. Sodium (4 g.; 0.17 mole) was then added in small pieces over a period of four hours. Gradually, the solution became brownish. When the addition of sodium was completed, the reaction mixture was cooled in an ice-water bath and brought to a pH of about 9 with 4% sulphuric acid. The propanol was evaporated under reduced pressure, and the pH of the aqueous solution was further adjusted to 8.6-8.8 with 1% sulphuric acid, using a pH-meter. A yellowish product precipitated and was collected by filtration with suction and dried. The yield was 0.245 g. (65 per cent of the theoretical). Three recrystallizations from methanol-water gave white product which melted at 244-245°.

Anal. Calc'd for: $C_{24}H_{42}O_2N_2 \cdot H_2O$: C, 70.58%; H, 10.78%; N, 6.86%

Found: C, 70.35%; H, 10.63%; N, 6.59%.

Infrared absorption spectrum: 3380-cm^{-1} (s) (O-H, NH_2), 1660cm^{-1} (sh) (NH_2), 1623cm^{-1} (s) ($\overset{+}{N}H_3$), 1575cm^{-1} (vs) (COO^-).

(ii) In n-butanol

In a similar experiment in which n-propanol was used instead of n-butanol, the solution remained colourless during the first three hours of refluxing. The yield of the crude product was 73 per cent of the theoretical.

3β,12β,24-trihydroxycholane

Purification of tetrahydrofuran*

The peroxides were destroyed by shaking vigorously one quart of the solvent for a few minutes with equal amounts of ferrous sulphate heptahydrate and sodium bisulfate. Then, a small amount of solid sodium hydroxide was added in order to destroy iron complexes. The solution was filtered and dried over sodium hydroxide pellets for several days with occasional shaking. The solvent was distilled, the last 50 ml. being discarded, and the distillate was dried a few times over sodium wire until the sodium did not become yellow on standing. Finally, the tetrahydrofuran was distilled from lithium aluminium hydride.

Reduction of 3,12-dioximinocholanic acid with lithium aluminium hydride in tetrahydrofuran

(i) Lithium aluminium hydride (0.365 g.; 0.0096 mole) was dissolved in 20 ml. of dry tetrahydrofuran in a 100 ml. three-necked, round-bottomed flask equipped with a reflux condenser fitted with a calcium chloride drying tube. A second neck of the flask was provided with a dropping funnel protected by a calcium chloride tube, and containing a solution of 0.30 g. (0.76 mmole) of 3,12-dioximinocholanic acid in 30 ml. of dry tetrahydrofuran. The third neck of the flask was equipped with a nitrogen inlet tube. The flask was surrounded by an electric heating mantle and equipped with a magnetic stirrer. The entire apparatus was swept with dry nitrogen and the hydride suspension was stirred and refluxed for half an hour. The dioxime solution was then added dropwise over a period of forty minutes and a white complex was formed as soon as the solution came in contact with the hydride

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* The gift of several quarts of tetrahydrofuran from DuPont Co. Of Canada is gratefully acknowledged.

suspension. The resulting mixture was stirred and refluxed for a further period of two hours. After the stirred slurry cooled to room temperature, it was hydrolysed by dropwise addition of 50 ml. of a saturated sodium potassium tartrate (Rochelle salt) solution. Tetrahydrofuran was then evaporated on the steam bath under reduced pressure (water aspirator) and a semi-solid material separated in the aqueous phase. It was extracted with three 40 ml. portions of n-butanol. The alcoholic extract was washed once with 1% sulphuric acid, twice with water, and dried over anhydrous magnesium sulphate. The solvent, after filtration, was evaporated in vacuo, leaving an amorphous residue which weighed after drying 0.225 g. (83 per cent of the theoretical). This product contained no nitrogen, and was recrystallized in the following way: it was dissolved in 2 ml. of 95% boiling ethanol, and hot water was added dropwise till slight cloudiness occurred; the solution was then seeded. The crystals used for seeding were obtained by cooling a few drops of the solution in a dry ice-acetone mixture for 2-3 minutes, and keeping the resulting solid in a refrigerator overnight. Upon crystallization of the main crop, long white needles were obtained, and they were recrystallized from acetone-water, yielding a product melting at 166-169°.

Anal. Calc'd for: $C_{24}H_{42}O_3$: C, 76.19%; H, 11.11%

Found: C, 75.86%; H, 11.43%

Infrared absorption spectrum: 3340cm^{-1} (vs) (H-O); 1075, 1055, 1042cm^{-1} (s), 1011cm^{-1} (vs) (C=O).

The benzoate derivative of 3ξ,12ξ,24-trihydroxycholane was prepared by dissolving 20 mg. of the triol in 1 ml. of pyridine and adding two drops of benzoyl chloride. The solution was heated on the steam bath for a few

minutes, then was poured on crashed ice. An oil settled on the bottom, the supernatant liquid was decanted and the oil was rubbed with a glass rod until it solidified. The solid was dried in vacuo for twenty-four hours over phosphorus pentoxide. The infrared absorptional spectrum showed the following bands: 3400cm^{-1} (s) (O-H); 1780cm^{-1} (m) and 1715cm^{-1} (s) (C=O); 3060cm^{-1}, 1600cm^{-1}, 705cm^{-1} (aromatic ring vibrations).

(ii) In an experiment similar to (i), the molar ratio of 3,12-dioximinocholanic acid to lithium aluminium hydride was 1:2. The product contained no nitrogen and melted over a wide range of temperature.

Infrared absorption spectrum: 3400cm^{-1} (s) (O-H); 1705cm^{-1} (s) (C=O).

7,12-Dioximinocholanic acid

The synthesis of 7,12-dioximinocholanic acid was carried out in two steps:

1. Clemmensen reduction of 3,7,12-triketocholanic acid (dehydrocholic acid) to 7,12-diketocholanic acid.
2. Oximation of 7,12-diketocholanic acid to 7,12-dioximinocholanic acid.

1. 7,12-Diketocholanic acid

Dehydrocholic acid was obtained from Brickman and Company, and was used without further purification.

For Clemmensen reduction, the procedure of Wieland and Boersché (170) was adopted. In order to ensure complete solution of dehydrocholic acid, an additional amount of 62.5% of ethanol was required.

In a 1-l. one-necked, round-bottomed flask equipped with a condenser, 20 g. of dehydrocholic acid was dissolved in 325 ml. of 95% ethanol, and to the resulting solution, 200 g. of c.p. zinc metal in bars and 40 ml. of

concentrated hydrochloric acid were added. The mixture was heated to reflux on the steam bath for one hour, and then further portions of concentrated hydrochloric acid, 20 ml. each, were added every hour over a period of 20 hours. The total refluxing time was twenty-two hours. The supernatant solution was decanted from the unreacted zinc residue, and on cooling to room temperature, the first crop of the diketoester separated. The material was filtered off with suction, and the filtrate deposited the second crop upon dilution with 50 ml. of water. Both crops were combined and recrystallized from about 50 ml. of 95% ethanol. The product was collected by filtration with suction and dried in a vacuum oven at 80° for 8 hours. The yield was 15 g. (75 per cent of the theoretical), m.p. 157-159°. Wieland reported 80 per cent yield and the melting point of 162°.

Ethyl 7,12-diketocholanate (15 g.) was saponified by refluxing it for one hour in 60 ml. of 1 N methanolic potassium hydroxide. The solution was diluted with a large amount of water, warmed up to about 40°, and neutralized with warm 1% sulphuric acid. The free acid precipitated and was collected by suction filtration. One recrystallization from 95% ethanol gave 11 g. (78 per cent of the theoretical) of the product melting at 175-176° (lit. m.p. 177°).

2. 7,12-Dioximinocholenic acid

The procedure described by Schenck and Kirchhof (175) was followed.

In a 1-l. one-necked, round-bottomed flask equipped with a reflux condenser, a solution of 10 g. (0.026 mole) of 7,12 di-ketocholenic acid, 20 g. (0.28 mole) of hydroxylamine hydrochloride in 70 ml. of water, 370 ml. of 95% ethanol and 105 ml. of 10% sodium hydroxide was refluxed on the steam bath for one hour. After fifteen minutes of refluxing, the crystals of the reaction product began to separate. The product was filtered with suction

and dried. The yield was quantitative, m.p. 238-240°. One recrystallization from ethanol-water raised the melting point to 243-244° (dec.). The melting point given in the literature was 273° (dec.).

Anal. Calc'd for $C_{24}H_{38}N_2O_4$: C, 68.89%; H, 9.10%; N, 6.69%.

Found: C, 68.97%; H, 8.76%; N, 6.62%

Infrared absorption spectrum: 1655cm^{-1} (m) and 1637cm^{-1} (m) (C=N).

7β,12β-Diaminocholanic acid

Reduction of 7,12-dioximinocholanic acid with sodium in alcohol

(i) In n-propanol

In a 100 ml. three-necked, round-bottomed flask equipped with a mechanical stirrer, a condenser fitted with a calcium chloride drying tube and a nitrogen inlet tube, 0.20 g. (0.5 mmole) of 7,12-dioximinocholanic acid was dissolved in 40 ml. of dry n-propanol. The solution was stirred and heated toreflux in an oil bath in a slow stream of dry nitrogen. Sodium (2 g.; 0.085 mole) was added in small pieces to the stirred and refluxing solution over a period of three hours. Gradually, the reaction mixture became brown. It was cooled in an ice-water bath and brought to pH of about 9, first with 4% and then with 2% sulphuric acid. Propanol was evaporated under reduced pressure, and 1% sulphuric acid was added carefully using a pH-meter till the product precipitated (pH 8.3-8.6). The solid was filtered off and dried. The crude product was yellowish and weighed 0.155 g. (83 per cent of the theoretical). It was dissolved in about 8 ml. of 95% ethanol, and the solution was filtered through celite and carbon Nuchar-C190-N. The slightly coloured filtrate was concentrated to 4 ml. by warming on the steam bath and simultaneously blowing a stream of nitrogen into the flask; the vapours

of solvent were removed by suction (water aspirator). Upon standing at room temperature, the solution deposited needles which, after drying, melted at 125-135°. Further two recrystallizations from acetone-water afforded long needles melting at 128-130° to a mass which became fluid at 132°.

Anal. Calc'd for: $C_{24}H_{42}O_2N_2$, 1.5 H_2O: C, 69.06%; H, 10.31%; N, 6.71%
Found: C, 68.52%; H, 10.32%; N, 6.57%.

Infrared absorption spectrum: 3400cm^{-1} (m) (O-H), 1635cm^{-1}, 1630cm^{-1}, 1560cm^{-1} (sh), (NH_2,$\overset{+}{N}H_3$); 1550cm^{-1} (m) (COO^-).

(ii) In n-butanol

In an experiment similar to (i), the colour of the reaction mixture was lighter and the yield of the crude product was 85 per cent of the theoretical.

7ξ-Amino-,12-oximino-24-hydroxycholane

Reduction of 7,12-dioximinocholanic acid with lithium aluminium hydride in tetrahydrofuran

Lithium aluminium hydride (0.73 g.; 0.02 mole) was dissolved in 50 ml. of dry tetrahydrofuran in a 300 ml. three-necked, round-bottomed flask equipped with a reflux condenser fitted with a calcium chloride drying tube. A sedond neck of the flask was provided with a dropping funnel guarded by a calcium chloride drying tube, and containing a solution of .60 g. (0.0015 mole) of 7,12-dioximinocholanic acid in 80 ml. of dry tetrahydrofuran. The third neck of the flask was provided with a nitrogen inlet tube. The flask was equipped with an electric heating mantle and a magnetic stirrer. The entire apparatus was swept with dry nitrogen, and the hydride suspension was

stirred and refluxed for half an hour. The dioxime solution was then added dropwise over a period of fourty minutes. The white complex began to form after ten minutes, and when the addition was completed, a large amount of the slurry was already present in the reaction mixture. Stirring and refluxing were continued for a further period of two and a half hours. After the suspension had cooled to room temperature, it was hydrolysed by dropwise addition of 100 ml. of a saturated sodium potassium tartrate (Rochelle salt) solution. Tetrahydrofuran was then evaporated under reduced pressure, and a white precipitate separated from the aqueous solution. The product was extracted with four 50 ml. portions of n-butanol. The extract was washed once with 1% sulphuric acid, twice with water, and was dried over anhydrous magnesium sulphate. The solution was filtered and butanol was evaporated on the steam bath under reduced pressure. The crystalline residue was dried, weighed 0.450 g. (77.6 per cent of the theoretical), melted at 203-209° and became transparent at 215°. Recrystallization from 95% ethanol gave shiny plates, browning at 220°, melting at 228-230°. Another recrystallization from the same solvent raised the melting point to 234-236° (turning yellow at 222°).

Anal. Calc'd for $C_{24}H_{38}O_2N_2$: C, 71.79%; H, 9.74%; N, 7.17%

Found: C, 71.15%; H, 10.17%; N, 7.15%

Infrared absorption spectrum: 3300cm^{-1} (vs) (O-H); 1660cm^{-1} (m) (C=N, NH_2).

7ξ-Amino-12-keto-24-hydroxycholane

7ξ-Amino,-12-oximino,-24-hydroxycholane (15 mg.) was dissolved in 3 ml. of 95% ethanol in a 25 ml. round-bottomed flask equipped with a reflux condenser and one drop of 4% hydrochloric acid was added to the solution. The reaction mixture was refluxed for 20 minutes, then was concentrated to a volume of 1 ml. by warming on the steam bath and simultaneously blowing a stream of nitrogen into the flask; the vapours of solvent were removed by suction (water aspirator). Short needles separated on standing at room temperature, they were filtered with suction and dried; m.p. 180-183°. Recrystallization from ethanol-water gave needles melting at 183-184°.

Anal. Calc'd for: $C_{24}H_{37}ON$, 1.5 H_2O: C, 71.39%; H, 9.95%

Found: C, 70.83%; H, 9.98%.

Infrared absorption spectrum: 3300cm^{-1} (vs) (O-H); 1700cm^{-1} (m) (C=O); 1655cm^{-1} (m) (NH_2).

Analyses

The melting points were determined in a Thiele-Dennis melting point tube containing Dow Corning silicone fluid No. D. C. 550, and are uncorrected.

Carbon, hydrogen microanalyses of di-[x,x' 3-ketocholestanyl] methane and of di-[x,x'-di-3,6-ketocholestanyl] methane were carried out by the Schwarzkopf microanalytical laboratory in Woodside, N.Y., U.S.A.; the remaining analyses were performed by W. Menser laboratory in Zurich, Switzerland.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. An investigation of the Mannich reaction of saturated and unsaturated ketosteroids containing active hydrogen atoms in rings A or/and B was carried out under a variety of experimental conditions.

2. When cholest-5-en-3-one was allowed to react with morpholine and paraformaldehyde using isoamyl alcohol or dioxane as solvents, a nitrogen-containing product was isolated once in each instance. These condensations could not be reproduced in spite of numerous attemps.

3. The ketones reacted preferentially with formaldehyde rather than with free amines or their hydrochlorides which were used as the third reactant.

4. An explanation was offered for the higher reactivity of cholest-5-en-3-one as compared to that of cholest-4-en-3-one. The former reacted in the presence of either free amine or its hydrochloride; the latter only in the presence of an amine hydrochloride. The reaction products were brown visquous oils devoid of nitrogen.

5. When saturated ketones, cholestan-3-one and cholestane-3,6-dione, were brought into reaction with formaldehyde and amine hydrochlorides, the condensation products were identified as methylene-bis-ketones. Their structures were postulated to be di-[2,2'-3-ketocholestanyl] methane and di-[4,4'-di-3,6-ketocholestanyl] methane, respectively, on the basis of the reaction mechanism of brominations of cholestan-3-one and cholestane-3,6-dione, and infrared spectroscopic results.

6. A reaction mechanism was proposed for the reaction of saturated keto-steroids with formaldehyde. Since there was no evidence for the effective participation of an amine hydrochloride, two alternate schemes were presented.

7. The infrared absorption spectrum of cholestan-3-one was recorded for the first time in potassium bromide. By comparison with the spectrum determined in solution there was a 5-10cm^{-1} shift of maxima towards lower frequencies.

8. The preparation of 3,12-diketocholanic acid (dehydrodesoxycholic acid) from 3,12-dihydroxycholanic acid (desoxycholic acid) was significantly improved as to the yield (from 70 to over 90 per cent) and to the ease of isolation of the product.

9. The reduction of various oximinocholanic acids using sodium in alcohol and lithium aluminium hydride was carried out under modified conditions.

10. 12β-Aminocholanic acid and its hydrochloride, 3α,12β-diamino- and 7β,12β-diamino-cholanic acids were synthesized from the corresponding eximinocholanic acids using sodium in n-propanol or n-butanol. The infrared absorption spectra of the above compounds were determined and constituted additional evidence that these steroidal amino acids possess a dipole structure. The equatorial conformation was allotted to amino substituents on the basis of the mode of preparation.

11. The reaction of 3,12-dioximinocholanic acid with lithium aluminium hydride in tetrahydrofuran was investigated and found to proceed in an unusual way. The carboxyl group was reduced to a primary alcohol,

whereas the oxime groups were transformed to secondary alcohols. The resulting 3β,12β,24-trihydroxycholane was characterized as its benzoyl derivative and by the infrared absorption spectrum.

12. A reaction mechanism was proposed for the reaction of 3,12-dioximinocholanic acid with lithium aluminium hydride.

13. The reduction of 7,12-dioximinocholanic acid with lithium aluminium hydride was carried out in tetrahydrofuran. It resulted in the reduction of the carboxyl group to a primary alcohol and in the reduction of one oxime group to a primary amine. The amine substituent was postulated to be located in position 7 rather than 12 because of lesser steric hindrance. The product, 7β-amino-12-oximino-24-hydroxycholane, was also identified by the infrared absorption spectrum.

14. An acidic hydrolysis of the latter compound afforded a product which, in accordance with the postulation of its precursor, was assigned the structure 7β-amino-12-keto-24-hydroxycholane. It was further characterized by the infrared spectrum.

15. The infrared absorption spectra of the following ketocholanic acids were recorded for the first time [in potassium bromide], and the correlation between their structures and absorption maxima was discussed:

    a) 12-Ketocholanic acid

    b) 3,12-Diketocholanic acid

    c) 7,12-Diketocholanic acid

    d) 3,7,12-Triketocholanic acid

16. The infrared absorption spectra of the following oximinocholanic acids were determined for the first time [in potassium bromide], and the correlation between their structures and absorption maxima were discussed:

    a) 12-Oximinocholanic acid

    b) 3,12-Dioximinocholanic acid

    c) 7,12-Dioximinocholanic acid

    d) 3,7,12-Trioximinocholanic acid

17. The following new compounds were prepared:

    a) Di-[x,x'-3-ketocholestanyl] methane, m.p. 208-210°

    b) Di-[x,x'-di-3,6-ketocholestanyl] methane, m.p. 242-242.5°

    c) 12β-Aminocholanic acid, m.p. 115-116°

    d) 3α,12β-Diaminocholanic acid, m.p. 244-245°

    e) 7β,12β-Diaminocholanic acid, m.p. 128-130°

    f) 3ξ,12ξ,24-Trihydroxycholane, m.p. 166-169°

    g) 3ξ-Amino-12-oximino-24-hydroxycholane, m.p. 234-236°

    h) 3ξ-Amino-12-keto-24-hydroxycholane, m.p. 183-184°.

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