ON THE SYNTHESIS OF THE 12-OXYGEN ANALOGUES OF CORTICOSTERONE

Sainte-Marie

ON THE SYNTHESIS OF THE 12-OXYGEN ANALOGUES OF CORTICOSTERONE

A Thesis

by

Dorothée Sainte-Marie

Submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

McGill University

April 1944

ACKNOWLEDGEMENTS

The candidate wishes to thank Dr. R. D. H. Heard for the care and time spent in directing this research, the National Research Council and the Banting Research Foundation for their financial support, Dr. D. L. Thomson for the recommendations which were instrumental in obtaining this support, Dr. B. K. Wasson for frequent advice and, with Charles E. Frosst and Company, for supplies of pregnane-3(α),12(β)-diol-20-one and etiodesoxycholic acid necessary for the work, Miss Lykke Groth for her careful technical assistance throughout the year, and Mrs. Dorothy Jewitt for microanalyses.

TABLE OF CONTENTS

GENERAL INTRODUCTION

The accepted emergency function of the adrenal cortex indicates the therapeutic application of the adrenal cortical hormones in conditions of stress. While the clinical aspects have received considerable attention, the results are confusing, owing mainly to the use of whole adrenal cortical extract which contains a mixture of cortical hormones several of which possess antagonistic actions. It may be assumed that, under physiological conditions, each cortical hormone is secreted independently, in amount governed by the physiological needs of the moment. Moreover, there is great variation in the relative content of the cortically active steroids in different preparations of cortical extracts obtained by the same method. Consequently, the true answer may be expected only from careful investigations carried out with each crystalline hormone. To date, the therapeutic value of desoxycorticosterone acetate (XXIX)* has been investigated and has been found unsatisfactory. The other hormones of the adrenal cortex, especially those possessing gluconeogenic activity, have not received adequate attention, owing to the tedious and costly methods required for their isolation from tissue extracts. Hence, the synthesis of the compounds

---

*The formulae of compounds mentioned in the text are listed in numerical sequence in the appendix.

from the bile acids is desirable. Reichstein[1], Gallagher[2], and Kendall[3] have developed procedures for transposing the 12-oxygen atom in compounds of the desoxycholic acid series to the 11-position, which leads to corticosterone (XXX) and its derivatives. As these methods involve a large number of steps and the products are obtained in poor yield, it was decided to investigate the gluconeogenic activity of that series of compounds in which the ring C oxygen atom is substituted in the 12- rather than the 11-position. As the 12-oxygen analogues of corticosterone are obtainable directly from the bile acids, the advantage of the possible use of the latter is obvious. Since the beginning of the work, the preparation of this series of compounds has been reported by Fuchs and Reichstein[4] by way of etio-desoxycholic acid (XXIV), thionyl chloride and diazomethane, a route radically different from that under examination by us.

PART ONE

Synthesis of the $C_{17}\alpha$-ketol Side-chain by Oxidation of the C_{17} Methyl Ketone with Lead Tetraacetate.

I. Introduction

The object of this study was to produce a sufficient amount of the 12-oxygen analogues of corticosterone (XXX) to investigate the gluconeogenic activity of this series of compounds. As they are prepared directly by oxidative degradation of the bile acid side-chain, the problem of the synthesis of cortically active compounds would be greatly simplified, if they were found to be physiologically active.

Dimroth and Schweizer[5] first observed that compounds with hydrogen atoms activated by an adjacent carbonyl group could be oxidized with lead tetraacetate in varying yields with the formation of the corresponding acetates.

In 1939, Ehrhart, Ruschig and Aumüller[6] obtained desoxycorticosterone acetate (XXIX) by the action of lead tetraacetate on progesterone (XXXI) and also 21-acetoxy-pregnenolone (XXXII) from the corresponding C_{17} methyl ketone (XXXIII). Two years later, these investigators together with Bockmühl secured patents[7] for the action of lead tetraacetate and of other lead tetraacylates, such as lead tetrabutyrate and lead tetrabenzoate, on C_{17} methyl ketones possessing a double bond

between positions 4, 5, and 6 of the steroid nucleus, and either a hydrogen atom, a hydroxyl group, an alkyl group or an acyl group in the 3-position, and which may or may not possess an esterified or a non-esterified hydroxyl group in the 11- or the 12-position. Among the compounds studied are progesterone (XXXI), 12-acetoxy-progesterone (XXXIV) and pregnenolone (XXXIII). In the several examples given, they claim yields varying between 84 and 100 per cent. Later, Reichstein and Montigel[8] disproved these results. By means of the same reaction, but using a trace of acetic anhydride as catalyst, they obtained a yield of less than 3 per cent of desoxycorticosterone acetate (XXIX) and a 19 per cent yield of 21-acetoxy-pregnenolone acetate (XXXV). By treating allopregnanolone acetate (XXXVI) with lead tetraacetate in the same way, these investigators isolated the corresponding $C_{17}\alpha$-ketol acetate (XXXVII) in 53 per cent yield.

II. Discussion of Results

The immediate object was the preparation of $\Delta^{4:5}$-pregnene-21-ol-3,12,20-trione-monoacetate (XLVI), which it was proposed to achieve by the series of reactions outlined on page iv of the appendix. In the first instance, the action of lead tetraacetate on pregnane-3(α)-ol-12,20-dione (XLI) was studied. This compound was prepared by the conversion of pregnane-3(α), 12(β)-diol-20-one (XXXVIII) into the 3-monosuccinate (XXXIX) with succinic anhydride, by an adaptation of the method of

Schwenk, Riegel, Moffett, and Stahl[9] for succinoylation of desoxycholic acid, followed by oxidation of the monosuccinate with chromic anhydride to the corresponding 12-keto compound (XL) and saponification of the latter to the free pregnane-3-ol-12,20-dione (XLI). Treatment of this product with lead tetraacetate produced a small amount of pregnane-3-ol-12,20-dione-monoacetate (XLII), which was isolated from the oily mixture by chromatographic analysis, but most of the recovered material consisted of unchanged starting material (XLI). Some reducing activity (alkaline silver diamine) was found in some of the oily fractions which could not be crystallized, which suggests the presence of the 21-acetoxyl grouping. The action of lead tetraacetate on pregnane-3,12-diol-20-one (XXXVIII) directly was then studied. It was found that oxidation of the 21-methyl group and acetylation of the 3-hydroxyl group proceed simultaneously to give a mixture of pregnane-3,12-diol-20-one-3-monoacetate (XLVII), pregnane-3,12,21-triol-20-one-3,21-diacetate (XLVIII), and pregnane-3,12,21-triol-20-one-21-monoacetate (XLIII), as well as some unchanged starting material (XXXVIII). These compounds could not be isolated after chromatographic analysis. After oxidation with chromic anhydride, the mixture proved separable chromatographically and the following compounds were obtained in pure form: pregnane-3,12,20-trione (XLIX), which arises on oxidation of unchanged starting material (XXXVIII); pregnane-3-ol-12,20-dione-monoacetate (XLII); pregnane-3,21-diol-12,20-dione-diacetate (L); and,

pregnane-21-ol-3,12,20-trione-monoacetate (XLIV), which may be converted into the desired end-product, $\Delta^{4:5}$-pregnene-21-ol-3,12,20-trione-monoacetate (XLVI), on bromination of the activated C_4-methylene group and subsequent removal of hydrogen bromide.

As a considerable amount of pregnane-3,21-diol-12, 20-dione-diacetate (L) was obtained, its possible conversion into pregnane-21-ol-3,12,20-trione-monoacetate (XLIV) was studied. It was proposed to achieve this by saponification to the free dihydroxy compound (LI), protection of the 21-hydroxyl group and oxidation of the 3-hydroxyl group with chromic anhydride, followed by hydrolysis to the free 21-hydroxy compound, if desired. To study the various possibilities, desoxycorticosterone (LII) and 21-hydroxy-pregnenolone (XXXIII) have been used as models. The conversion of desoxycorticosterone (LII) into the acid succinate (LIII) was studied and the mildest possible conditions for complete succinoylation determined. The same conditions were then applied to 21-hydroxy-pregnenolone (XXXIII) in the hope of obtaining the 21-monosuccinate (LIV). At the same time, the disuccinoyl derivative of 21-hydroxy-pregnenolone was prepared (LV). In this case, however, more vigorous conditions seem necessary for complete succinoylation, as a small fraction of the product was proved identical by mixture melting point with the monosuccinoyl derivative (LIV) which was found to give the correct carbon and hydrogen analyses for the structure assigned to it.

At this point the problem was set aside to investigate the oxidative degradation of the bile acid side-chain. If the partial succinoylation of 21-hydroxy-pregnenolone were proved possible, the same technique could be applied to pregnane-3, 21-diol-12,20-dione (LI) to protect the 21-hydroxyl group while oxidizing the 3-hydroxyl group. It may be of interest to mention that Dr. Hans Selye* has carried out biological tests with desoxycorticosterone succinate (LIII) and has found it to be a more powerful anesthetic than desoxycorticosterone acetate (XXIX).

The results obtained in these lead tetraacetate oxidations indicate that it is preferable to oxidize pregnane-3, 12-diol-20-one (XXXVIII) directly without forming the 12-keto derivative (XLI). It may be that a greater concentration of lead tetraacetate is needed to form the α-ketol derivative of pregnane-3-ol-12,20-dione (XLI), as an appreciable quantity of starting material was recovered unchanged. It seems more probable, however, that the time of reaction (thirty-nine hours) should be lengthened, for only a fraction of the lead tetraacetate was used up in that time to form pregnane-3-ol-12,20-dione-monoacetate (XLII) and the same concentration of lead tetraacetate (1.25 mole) with a longer period of heating (sixty hours) produced a 10 per cent yield of pregnane-21-ol-3,12,20-trione-monoacetate (XLIV) from pregnane-3,12-diol-20-one

---

*Dr. Hans Selye, unpublished results.

(XXXVIII) after subsequent oxidation with chromic anhydride. The purpose of forming pregnane-3-ol-12,20-dione (XLI) before treating with lead tetraacetate was to obtain a more crystalline, and therefore more easily isolated, product. It is better, however, to oxidize with chromic anhydride after treatment with lead tetraacetate, without isolating the intermediate hydroxy derivatives. The effect of a larger concentration of lead tetraacetate (1.66 mole) on pregnane-3,12-diol-20-one (XXXVIII) was to increase the amount of acetylation, as, after treatment with chromic anhydride, only a 4 per cent yield of the desired product (XLIV) was obtained, while pregnane-3,21-diol-12,20-dione-diacetate (L) was isolated in 9.5 per cent yield. The yield of conversion of the latter (L) to the desired compound (XLIV) would either be low or would, at any rate, involve several steps. It would seem highly desirable to prevent acetylation of the 3-hydroxyl group by causing lead tetraacetate to react with pregnane-3,12,20-trione (XLIX) rather than with the diol (XXXVIII), but a study by Heard and Wasson[10] on cholestanone (LVI) has shown that both 3- and 20-keto steroids react with lead tetraacetate to give the corresponding α-ketol. Hence, lead tetraacetate oxidation of pregnane-3,12,20-trione would lead to the formation of a ring A cyclic α-ketol, as well as of the desired side-chain α-ketol and must therefore be ruled out. The behaviour of a 12-keto steroid under the same conditions was also tested by these

investigators. They were unable to form a ring C cyclic α-ketol, presumably due to the strong steric hindrance about C_{11}.

Thus, it is possible to synthesize pregnane-21-ol-3, 12,20-trione-monoacetate (XLIV) by oxidation with lead tetra-acetate, followed by reaction with chromic anhydride, from pregnane-3,12-diol-20-one (XXXVIII). By bromination of the active C_4 methylene group and subsequent removal of hydrogen bromide $\Delta^{4:5}$-pregnene-21-ol-3,12,20-trione-monoacetate (XLVI) may be prepared and its gluconeogenic activity tested fully. The method should be further studied to find the optimum reaction conditions. If a satisfactory yield could be obtained, the method would be preferable to the longer procedure of Fuchs and Reichstein[4], which has been repeated with some alterations in Part Three of this thesis to compare overall yields.

III. Experimental Work

1. Partial Succinoylation of Pregnane-3,12-diol-20-one (XXXVIII to XXXIX):

Two and one half g. of pregnane-3,12-diol-20-one were added to 8.75 g. (2.7 moles) of succinic anhydride in pyridine (30 ml.), heated two hours on the boiling water-bath and left sixteen hours at room temperature under anhydrous conditions. The mixture was poured into chilled 10 per cent sulphuric acid (150 ml.), extracted with ether, washed with dilute sulphuric acid, and then with 10 per cent sodium carbonate (3 x 50 ml.). On acidification of the alkaline washings with 50 per cent

hydrochloric acid, the product was collected with ether (3 x 50 ml.) and the solvent distilled. Yield of crystalline product: 2.8 g. (87 per cent). Two recrystallizations from methanol and one from ether: petroleum ether gave faintly yellow, transparent polygonal plates, m.p. 172°-174°. The compound is quite soluble in methanol. Evaporation of the neutral ether yielded 61.9 mg. of starting material (XXXVIII). Milder conditions (sixteen hours at room temperature) resulted in the formation of only 20 per cent of the expected monosuccinate.

$C_{25}H_{38}O_6$	requires	C-69.12%	H-8.83%
	found	C-69.71% 69.82%	H-9.16% 9.18%

2. Oxidation of Pregnane-3,12-diol-20-one-3-monosuccinate (XXXIX to XL):

To 2.5 g. of pregnane-3,12-diol-20-one-3-monosuccinate (XXXIX) in glacial acetic acid were added 2.5 g. (4 moles) of chromic anhydride dissolved in 80 per cent acetic acid. During the addition the mixture was kept at room temperature or slightly below by occasional immersion in an ice-water bath. After sixteen hours at room temperature, the mixture was diluted to ten volumes (about 250 ml.) with water, extracted with ether (3 x 80 ml.), the solvent evaporated and the crystalline residue recrystallized twice from methanol to give stout white rectangular plates, m.p. 175°-176°. The compound is not very soluble in ether or cold methanol; it is

extremely so in hot methanol. It was saponified directly without determining the yield.

$C_{25} H_{36} O_6$	requires	C-69.40%	H-8.38%
	found	C-69.95%	H-8.77%
		69.77%	8.85%

Solid material insoluble in water and only slightly so in ether separated at the interface during the above extraction; by mixture melting point it was identified as starting material (XXXIX).

3. Saponification of Pregnane-3-ol-12, 20-dione-monosuccinate (XL to XLI):

Pregnane-3-ol-12,20-dione-monosuccinate (XL) was saponified sixteen hours at room temperature in 15 ml. methanolic potassium hydroxide (10 per cent). The lustrous minute crystals produced by the reaction were filtered off and were recrystallized once from ether:petroleum ether and twice from ether to give large white rosettes, m.p. 154°-155°. The compound is not very soluble in ether. The methanolic filtrate was diluted to ten volumes with water, extracted with ether (3 x 50 ml.) and the solvent distilled to give a yellow oil (510.1 mg.), which was oxidized as such with lead tetraacetate, without determining the yield of crystalline product.

$C_{21} H_{32} O_3$	requires	C-75.90%	H- 9.72%
	found	C-76.42%	H-10.06%
		76.59%	10.17%

4. Action of Lead Tetraacetate on Pregnane-3-ol-12,20-dione (XLI):

Pregnane-3-ol-12,20-dione (XLI) (510 mg.) in lead tetraacetate-stable glacial acetic acid (32.3 ml.) was heated at 60° for thirty-nine hours with lead tetraacetate (1.25 mole) under anhydrous conditions. The reaction mixture was then diluted with water (10 volumes), and the precipitated oily products collected with ether (3 x 100 ml.). The combined ethereal extracts were washed neutral with 5 per cent sodium carbonate solution (3 x 50 ml.) and water (3 x 50 ml.), and were taken to dryness (388.8 mg. of a yellow oil). The product could not be induced to crystallize, and was, therefore, adsorbed on a column of alumina (column number S-1) and fractions eluted therefrom with various solvent mixtures as outlined on the following page. Obtained were:

a) Pregnane-3-ol-12,20-dione-monoacetate (XLII) (fractions 5-10, 57.3 mg., 16.5 per cent yield), m.p. 161°-162°, eluted with petroleum ether:benzene (2.5/1), crystallized from ether:petroleum ether, and identified by mixture melting point with the authentic product obtained by acetylation of pregnane-3-ol-12,20-dione (XLI)(see paragraph 5 of this section).

b) Starting material (XLI)(fractions 13-35, 224.7 mg., 44 per cent yield), m.p. 154°-155°, eluted with petroleum ether:benzene (1/1), absolute benzene, benzene:ether (40/1, 20/1), crystallized from ether:petroleum ether, and identified by mixture melting point with the authentic product. Per cent starting material recovered: 44.

Wt. Adsorbed Mixture: 388.8 mg.

COLUMN No. S-1 FRACTION eluted: 91 per cent

12 GMS. OF ALUMINA; 13.8 CM. BY 1.0 CM.
G. Merck, acid washed.

FRACTION NO.	ELUANT NATURE	ELUANT VOL. ML.	ELUATE WT. MG.	ELUATE NATURE after crystallization	ELUATE M.P. after crystallization °C	ELUATE MAIN COMPONENT
1-2	petroleum ether: benzene (9:1)	50	1.4	brown oil		
3-4	petroleum ether: benzene (4:1)	"	0.9	brown oil		
5-10	petroleum ether: benzene (2.5:1)	"	57.3	white crystals (rosettes)	161-162	pregnane-3-ol-12,20-dione monoacetate
11-12	petroleum ether: benzene (1.5:1)	"	8.5	yellow oil		
13-24	petroleum ether: benzene (1:1)	"	124.2	white rosettes	153-155	pregnane-3-ol-12,20-dione
25-29	absolute benzene	"	46.6	white rosettes	152-155	pregnane-3-ol-12,20-dione
30-31	benzene:ether (40:1)	"	14.7	white rosettes	148-156	pregnane-3-ol-12,20-dione
32-35	benzene:ether (20:1)	"	39.2	white rosettes	146-151	pregnane-3-ol-12,20-dione
36-39	benzene:ether (5:1)	"	36.6	yellow oil		
40-41	benzene:ether (3:1)	"	11.1	yellow oil		
42-45	benzene:ether (1:1)	"	11.4	yellow oil		
46-47	absolute ether	"	2.9	yellow oil		
			T = 354.8	mg.		

c) Oily fractions which could not be crystallized but which exhibited strong reducing power, thereby suggesting the presence of the 21-acetoxyl grouping. To test for reducing activity, a few crystals of a compound were dissolved in four to six drops of methanol, and one or two drops of alkaline silver diamine were added. The formation of a black precipitate within two minutes was taken as evidence of reducing activity. A control test on a known reducing substance should always be carried out at the same time. The alkaline silver diamine solution must be reasonably fresh.

5. Acetylation of Pregnane-3-ol-12,20-dione (XLI to XLII):

Pregnane-3-ol-12,20-dione (XLI) (19.5 mg.) was dissolved in pyridine (5 drops) and an equal volume of acetic anhydride added. After sixteen hours at room temperature under anhydrous conditions, the mixture was diluted to 10 volumes with 10 per cent hydrochloric acid, extracted with ether (3 x 10 ml.), the combined ethereal extracts washed with cold 10 per cent sodium carbonate (3 x 10 ml.), and then with water (3 x 10 ml.), and evaporated. The crude crystalline residue (25.2 mg.) was recrystallized twice from ether: petroleum ether, m.p. 161°-162°, and then sublimed in high vacuo at 180°. The white powdery sublimate had a melting point of 162°-163°. The yield of crystalline product was equal to theoretical.

$C_{23}H_{34}O_4$	requires	C-73.74%	H-9.15%
	found	C-73.69% 73.70%	H-9.50% 9.24%

6. Action of Lead Tetraacetate on Pregnane-3,12-diol-20-one (XXXVIII):

A. Using 1.25 mole lead tetraacetate -

Pregnane-3,12-diol-20-one (XXXVIII) (518 mg.) in glacial acetic acid (32.6 ml.) was heated at 60° for sixty hours with lead tetraacetate (1.25 mole). The mixture was then diluted with water (10 volumes), and the oily precipitate collected with ether (3 x 30 ml.). The combined ethereal extracts were washed neutral with 5 per cent sodium carbonate solution and water, and were taken to dryness to yield an oil (605 mg.) which could not be crystallized. The oily mixture was adsorbed on a column of alumina (column number S-2) and fractions eluted therefrom with various solvents, as outlined on the following page. As none of these fractions could be induced to crystallize, they were recombined into two groups and treated as follows:

Group a. Fractions 1-15 (153.5 mg.) were combined and dissolved in glacial acetic acid. To this solution was added chromic anhydride (154 mg.) in 80 per cent acetic acid. During the addition the mixture was kept at room temperature or slightly below by occasional immersion in an ice-water bath. After sixteen hours at room temperature, the mixture was diluted to ten volumes (about 250 ml.) with water and extracted with ether (3 x 80 ml.). The combined ethereal extracts were washed with cold 5 per cent sodium carbonate (2 x 80 ml.) and then with water (3 x 80 ml.). Evaporation of the solvent yielded a yellow oily residue (85.7 mg.). Only a trace of organic acids

Wt. Adsorbed Mixture: 604.8 mg.

COLUMN No. S-2

FRACTION eluted: 76 per cent

{ 18 GMS. OF ALUMINA; 20.7 CM. BY 1 CM.
{ G. Merck, acid washed.

FRACTION NO.	ELUANT		ELUATE			
	NATURE	VOL.	WT.	NATURE	M.P.	MAIN COMPONENT
		ML.	MG.		ϕ°C	
1-4	petroleum ether: benzene (4:1)	50	93.5	white oil		strongly reducing
5-6	petroleum ether: benzene (2.5:1)	"	6.6	white oil		
7-12	petroleum ether: benzene (1:1)	"	26.6	white oil		
13-15	absolute benzene	"	26.8	white oil		
16-20	benzene:ether (40:1)	"	70.5	white oil		strongly reducing
21-22	benzene:ether (20:1)	"	7.4	white oil		
23-24	benzene:ether (9:1)	"	10.4	white oil		
25-33	benzene:ether (3:1)	"	188.3	white oil		
34-35	benzene:ether (2:1)	"	9.1	white oil		
36-41	benzene:ether (1:1)	"	10.4	white oil		strongly reducing
42-43	absolute ether	"	1.7	white oil		
44-45	absolute chloroform	"	1.6	yellow oil		
46-47	absolute ethyl acetate	"	3.0	yellow oil		
48-49	ethyl acetate: acetone (1:1)	"	1.3	yellow oil		
			T = 457.2	mg.		

was observed on acidification (50 per cent hydrochloric acid) of the sodium carbonate washings. The original aqueous layer was extracted with chloroform (3 x 80 ml.) and washed (3 x 80 ml.). The solvent was distilled and a yellow oily residue (39.5 mg.) remained. The two residues (125.2 mg.) were combined and adsorbed on a column of alumina (column number S-3) and fractions eluted therefrom with various solvents, as outlined on the following page. Obtained were:

(i) Pregnane-3-ol-12,20-dione-monoacetate (XLII) (fractions 1-3, 65.7 mg., 11 per cent yield), eluted with petroleum ether:benzene (9:1), m.p. 159°-161° and identified by mixture melting point with the authentic product.

(ii) Several oily fractions which could not be crystallized.

Group b. Fractions 16-49 (303.7 mg.) were combined and dissolved in glacial acetic acid. To this solution was added chromic anhydride (304 mg.) in 80 per cent acetic acid. The mixture was then submitted to the same treatment as Group A. The yellow oily residue (156.6 mg.) obtained by ether extraction of the diluted reaction mixture was combined with that obtained by chloroform extraction (22.8 mg.) and adsorbed on a column of alumina (column number S-4) and fractions eluted therefrom with various solvents, as outlined on page 20. Obtained were:

(i) Pregnane-3,12,20-trione (XLIX) (fractions 3-4, 34.1 mg., 7 per cent yield), m.p. 202°-203°, eluted with petroleum ether:benzene (4/1) and identified by mixture melting

Wt. Adsorbed Mixture: 125.2 mg.

COLUMN No. S-3

FRACTION eluted: 90 per cent

{ 4 GMS. OF ALUMINA; 9.2 CM. BY 0.5 CM.
{ G. Merck, acid washed.

FRACTION NO.	ELUANT		ELUATE			
	NATURE	VOL.	WT.	NATURE	M.P. after crystallization °C	MAIN COMPONENT
		ML.	MG.			
1-3	petroleum ether: benzene (9:1)	25	65.7	semi-cryst'ne	159-161	pregnane-3-ol-12,20-dione-monoacetate
4-5	petroleum ether: benzene (4:1)	"	8.0	white oil		non-reducing
6-7	petroleum ether: benzene (2.5:1)	"	7.2	"		
8-11	petroleum ether: benzene (1:1)	"	17.7	"		
12-13	absolute benzene	"	3.4	"		
14-15	benzene:ether (20:1)	"	5.5	"		
16-17	benzene:ether (3:1)	"	3.3	"		
18-19	benzene:ether (1:1)	"	1.0	"		
20-21	absolute ether	"	1.2	"		
22-23	absolute chloroform	"	0.3	yellow oil		
			T = 113.3	mg.		

point with the authentic product obtained by oxidation of pregnane-3,12-diol-20-one (XXXVIII) by Mr. John Phinney.

(ii) Pregnane-21-ol-3,12,20-trione-monoacetate (XLIV) (fractions 5-16, 60.7 mg., 10 per cent yield), eluted with petroleum ether:benzene (4/1, 2.5/1, 1/1), absolute benzene, and benzene:ether (40/1), in the form of minute colourless needles, m.p. 191°-192° (Reichstein: 189°-191°)[4] after recrystallization from ether:petroleum ether. The compound reduces alkaline silver diamine, thus indicating the presence of a C_{21}-hydroxyl group (free or esterified) adjacent to the C_{20}-carbonyl group.

$C_{23}H_{32}O_5$	requires	C-71.09%	H-8.31%
	found	C-70.34% 70.42%	H-8.67% 8.69%

(iii) Several oily fractions which could not be crystallized.

B. Using 1.66 mole lead tetraacetate -

Pregnane-3,12-diol-20-one (XXXVIII) (2 g.) in lead tetraacetate-stable glacial acetic acid (226 ml.) was heated at 70° for 65 hours with lead tetraacetate (1.66 mole). The mixture was then diluted with water (10 volumes), and the oily products extracted with ether (4 x 500 ml.). The combined ethereal extracts were washed neutral with 5 per cent sodium carbonate solution (3 x 500 ml.) and with water (3 x 500 ml.), dried over sodium sulphate and taken to dryness. The yellow oily residue (2.28 g.) was dissolved in glacial

Wt. Adsorbed Mixture: 179.4 mg.

COLUMN No. S-4 FRACTION eluted: 83 per cent

5.5 GMS. OF ALUMINA; 12.65 CM. BY 0.5 CM.
G. Merck, acid washed.

TION NO.	ELUANT		ELUATE			
	NATURE	VOL. ML.	WT. MG.	NATURE after crystallization	M.P. after crystallization °C	MAIN COMPONENT
1-2	petroleum ether: benzene (9:1)	25	29.3	white oil		
3-4	petroleum ether: benzene (4:1)	"	34.1	white, minute crystals	190-194	pregnane-3,12,20-trione
5	petroleum ether: benzene (4:1)	"	3.5	white needles	191-192	pregnane-21-ol-3,12,20-trione-monoacetate
6-7	petroleum ether: benzene (2.5:1)	"	8.0	white needles	191-192	"
8-11	petroleum ether: benzene (1:1)	"	30.0	white needles	191-192	"
12-14	absolute benzene	"	15.1	white needles	191-192	"
15-16	benzene:ether (40:1)	"	4.1	white needles	191-192	"
17-18	benzene:ether (3:1)	"	16.6	white oil		
19-22	benzene:ether (1:1)	"	5.2	white oil		
23-24	absolute ether	"	1.1	white oil		
25-26	absolute chloroform	"	1.2	yellow oil		
			T = 148.2	mg.		

acetic acid and oxidized with chromic anhydride (2.3 g.) in 80 per cent acetic acid. During the addition the mixture was kept at room temperature or slightly below by occasional immersion in an ice-water bath. After sixteen hours at room temperature, the mixture was diluted to ten volumes (about 400 ml.) with water and extracted with ether (4 x 100 ml.). The combined ethereal extracts were washed with cold 5 per cent sodium carbonate (2 x 100 ml.) and then with water (3 x 100 ml.). The solvent was distilled to give a yellow oily residue (1.2066 g.). The original aqueous layer was then extracted as in A, first with chloroform to give a yellow oily residue weighing 194.7 mg. and then with benzene to give a yellow oily residue weighing 9.8 mg. The residues (1.4111 g.) were combined and adsorbed on a column of alumina (column number S-5) and fractions eluted therefrom with various solvents, as outlined on pages 23 and 24. Obtained were:

(i) Pregnane-3-ol-12,20-dione-monoacetate (XLII) (fraction 1, 196.3 mg., 9 per cent yield), m.p. 161°-162°, eluted with petroleum ether and identified by mixture melting point with the authentic product (see paragraph 5 of this section).

(ii) Oily material (fraction 2, 107.8 mg.), eluted with absolute petroleum ether, which could not be crystallized; probably a mixture of pregnane-3-ol-12,20-dione-monoacetate (XLII) and pregnane-3,21-diol-12,20-dione-diacetate (LI).

(iii) Pregnane-3,21-diol-12,20-dione-diacetate (L)

(fractions 3-15, 246.5 mg., 9.5 per cent yield), eluted with absolute petroleum ether, petroleum ether:benzene (40/1, 20/1, 10/1, 4/1), m.p. 146°-147° after crystallization from ether:petroleum ether. The compound crystallizes in the form of needles and reduces alkaline silver diamine, which indicates the presence of a 21-acetoxyl grouping adjacent to the 20-carbonyl group.

$C_{25}H_{36}O_6$	requires	C-69.41%	H-8.38%
	found	C-69.66% 69.79%	H-8.87% 8.74%

(iv) Pregnane-21-ol-3,12,20-trione-monoacetate (XLIV) (fractions 17-27, 84.0 mg., 4 per cent yield), eluted with petroleum ether:benzene (4/1, 2.5/1, 1/1), m.p. 190°-192°, and identified by mixture melting point with the product previously obtained (column number S-4).

(v) Fraction 16 (168.0 mg.), eluted with petroleum ether-benzene (4/1). This fraction, which has been only partially crystallized, is evidently a mixture of pregnane-3, 21-diol-12,20-dione-diacetate (LI) and pregnane-21-ol-3,12, 20-trione-monoacetate (XLIV).

(vi) Several oily fractions (28-61) which could not be crystallized.

Wt. Adsorbed Mixture: 1.4111 g.

COLUMN No. S-5

FRACTION eluted: 76 per cent

{ 43 GMS. OF ALUMINA; 15.4 CM. BY 1.7 CM.
{ G. Merck, acid washed.

FRACTION NO.	ELUANT		ELUATE			
	NATURE	VOL. ML.	WT. MG.	NATURE after crystallization	M.P. after crystallization °C.	MAIN COMPONENT
1	absolute petroleum ether	150	196.3	white rosettes	161-163	pregnane-3-ol-12,20-dione-monoacetate
2	absolute petroleum ether	"	107.8	white oil		
3-5	absolute petroleum ether	"	49.1	white needles	146-147	pregnane-3,21-diol-12,20-dione-diacetate
6-7	petroleum ether: benzene (40:1)	"	8.5	white needles	146-147	"
8-9	petroleum ether: benzene (20:1)	"	11.2	white needles	146-147	"
10-11	petroleum ether: benzene (9:1)	"	8.3	white needles	146-147	"
12-15	petroleum ether: benzene (4:1)	"	169.4	white needles	146-147	"
16	petroleum ether: benzene (4:1)	"	168.0	white oil		
17-19	petroleum ether: benzene (4:1)	"	46.1	white needles	191-193	pregnane-21-01-3,12,20-trione-monoacetate
20-21	petroleum ether: benzene (2.5:1)	"	11.2	white needles	191-193	"
22-27	petroleum ether: benzene (1:1)	"	26.7	white needles	191-193	"
28-29	absolute benzene	"	9.0	white oil		
30-34	benzene:ether (40:1)	"	41.5	white oil		
35-37	benzene:ether (20:1)	"	20.6	white oil		

Continued on following page

Wt. Adsorbed Mixture: 1.4111 g.

COLUMN No. S-5 (continued) FRACTION eluted: 76 per cent

{ 43 GMS. OF ALUMINA; 15.4 CM. BY 1.7 CM.
{ G. Merck, acid washed.

FRACTION NO.	ELUANT		ELUATE			
	NATURE	VOL.	WT.	NATURE after crystallization	M.P. after crystallization °C	MAIN COMPONENT
		ML.	MG.			
38-39	benzene:ether (8:1)	150	12.5	white oil		
40-41	benzene:ether (4:1)	"	9.0	white oil		
42-44	benzene:ether (1:1)	"	17.9	white oil		
45-46	absolute ether	"	4.9	white oil		
47-48	ether:chloroform (100:1)	"	4.3	white oil		
49-50	ether:chloroform (40:1)	"	4.6	white oil		
51-52	ether:chloroform (20:1)	"	1.7	white oil		
53-54	ether:chloroform (7:1)	"	1.7	white oil		
55-56	ether:chloroform (1:1)	"	1.1	white oil		
57-58	absolute chloroform	"	1.1	white oil		
59-60	acetic acid:methanol:chloroform (1:1:2)	"	112.3	white oil		
61	alumina extracted with chloroform and chloroform with water to remove alumina	"	34.9			
			T= 1.0697 g.			

7. Saponification of Desoxycorticosterone Acetate* (XXIX to LII):

A solution of desoxycorticosterone acetate (XXIX) (536 mg.) in methanol (54 ml.) was saponified with potassium bicarbonate (536 mg. in 18 ml. water) for sixteen hours at room temperature. The methanol was then distilled in vacuo using as little heat as possible and gradually replacing the evaporated methanol with water. The aqueous mixture was extracted with ether:chloroform (5/1) (4 x 30 ml.), the combined extracts were washed with water (3 x 30 ml.), taken to dryness on the boiling water-bath, transferred to a 10 ml. flask and evaporated to dryness in a bell-jar under vacuum and in an atmosphere of nitrogen, using heat only occasionally. The light yellow oily product was recrystallized from dry acetone: absolute ether[11] to give shiny white rosettes, m.p. 142°-143° (Reichstein, 141°-142°)[11]. The yield of crude product was practically theoretical.

8. Succinoylation of Desoxycorticosterone (LII to LIII):

Desoxycorticosterone (LII) (100 mg.) was added to succinic anhydride (350 mg.) (3.33 moles) in pyridine (3 ml.). The mixture was kept at room temperature. Samples (0.75 ml.) of the mixture were taken after three, seven, and twenty-four hours, respectively, and were treated as follows:

---

*The desoxycorticosterone acetate has been generously supplied by the Ciba Pharmaceutical Products Inc.

Each sample was acidified with 5 per cent hydrochloric acid (15 ml.) and extracted with ether:chloroform (5/1) (4 x 5 ml.). The combined extracts were washed with cold 5 per cent sodium bicarbonate (3 x 5 ml.) and with water (3 x 5 ml.), were taken to dryness, and were weighed (neutral fraction). The sodium bicarbonate extracts were acidified with 50 per cent hydrochloric acid, extracted with ether: chloroform (5/1) (4 x 10 ml.), washed with water (3 x 15 ml.), and the combined extracts were taken to dryness and were weighed (acid fraction, containing monosuccinate).

Time (hours)	Wt. Neutrals (LII)	Wt. Acid Succinate (LIII)
3	12.3 mg.	13.3 mg.(41% theoretical)
7	3.9 mg.	23.9 mg.(73% theoretical)
24	2.5 mg.	27.0 mg.(83% theoretical)

From the above data it would seem that about ten hours is the minimum time required for succinoylation. The product (LIII) crystallized from chloroform:ether as yellow rosettes (m.p. 193°-201°), which could not be completely decolorized even after charcoal treatment.

$C_{25}H_{34}O_6$	requires	C-69.73%	H-7.97%
	found	C-67.41% 67.64%	H-7.67% 7.59%
$C_{25}H_{34}O_6.H_2O$	requires	C-66.96%	H-8.10%

The succinoylation was repeated on 550 mg. desoxycorticosterone

to furnish enough material to Dr. Hans Selye for biological assay as described in the discussion of results in Part One of this thesis.

9. Saponification of 21-Acetoxy-pregnenolone* (XXXII to XXXIII):

A solution of 21-acetoxy-pregnenolone (XXXII) (900 mg.) in methanol (90 ml.) was saponified with potassium bicarbonate (900 mg. in 30 ml. water) for sixteen hours at room temperature. The methanol was then distilled in vacuo using as little heat as possible and gradually replacing the evaporated methanol with water. The aqueous mixture was extracted with ether:chloroform (5/1) (4 x 300 ml.). The combined extracts were washed with water (3 x 300 ml.) and taken to dryness. The product (XXX) (688.0 mg., 86 per cent yield after one recrystallization from benzene) exhibited a broad melting point range from 163° to 182° (Reichstein[11] records a lower and equally broad melt from 139° to 159°), and further recrystallizations from benzene and from chloroform:ether failed to sharpen the melting point. The purity of the compound was ascertained by combustion analysis and by conversion of a sample to the diacetate, which now melted sharply at 163°-164° (Reichstein, 164°)[12] (see paragraph 10 of this section).

$C_{21}H_{32}O_3$	requires	C-75.90%	H-9.72%
	found	C-75.91%	H-9.82%
		76.14%	9.84%

*The 21-acetoxy-pregnenolone has been generously supplied by the Ciba Pharmaceutical Products Inc.

10. Complete Acetylation of 21-Hydroxy-pregnenolone (XXXIII to XXXV):

Fifty mg. of 21-hydroxy-pregnenolone (XXXIII) were dissolved in pyridine (1 ml.) and an equal volume of acetic anhydride (large excess). After standing seventeen hours at room temperature under anhydrous conditions, the mixture was diluted to 10 volumes with 10 per cent hydrochloric acid, extracted with ether (3 x 10 ml.), washed with cold 5 per cent sodium carbonate (3 x 10 ml.) and with water (3 x 10 ml.). The ether was distilled and a melting point of the white crystalline product was taken: m.p. 163°-164° (Reichstein, 164°)[12]. The yield was not determined.

11. Complete Succinoylation of 21-Hydroxy-pregnenolone (XXXIII to LV):

Fifty mg. of 21-hydroxy-pregnenolone (XXXIII) were added to succinic anhydride (175 mg.) (11.7 moles) in pyridine (1.5 ml.). After sixteen hours at room temperature, the mixture was heated two hours at 80°. It was then acidified with 5 per cent hydrochloric acid,,extracted with ether:chloroform (5/1) (5 x 1/3 volume of aqueous layer) and the combined extracts were washed with cold 5 per cent sodium bicarbonate (3 x 1/3 volume of ether:chloroform layer) and with water and were taken to dryness (neutral fraction, weight: 1.6 mg.). The sodium bicarbonate extracts were acidified with 50 per cent hydrochloric acid, extracted with ether:chloroform (5/1), washed with water and taken to dryness. The crystalline product (77 mg., 96 per cent theoretical) was recrystallized

from acetone:ether, m.p. 163°-178°. By fractional crystallization a few mg. of a substance melting at 196°-202° was isolated which showed no melting point depression on mixture with the product of the partial succinoylation of 21-hydroxy-pregnenolone (XXXIII) described below. As the results of carbon and hydrogen analyses point to this product as 21-succinoyl-pregnenolone (LIV), it is possible that the conditions used in this experiment were not sufficiently vigorous to effect complete succinoylation of 21-hydroxy-pregnenolone ((XXXIII). It is more probable that the disuccinoyl derivative (LV) of 21-hydroxy-pregnenolone (XXXIII) is difficult to purify and the experiment should be repeated on a larger scale in order to isolate it from the mixture (see paragraph 10 of this section for results of combustion analysis).

12. Partial Succinoylation of 21-Hydroxy-pregnenolone (XXXIII to LIV):

Two hundred mg. of 21-hydroxy-pregnenolone (XXXIII) were added to succinic anhydride (700 mg.) (11.7 moles) in pyridine (7 ml.). After ten hours at room temperature, the mixture was acidified with 5 per cent hydrochloric acid and extracted with ether:chloroform (5/1). As the white crystalline product is only slightly soluble in chloroform, ether, and benzene, the extraction was incomplete and the yield could not be determined. The combined extracts were taken to dryness and the residue recrystallized several times from acetone, m.p. 203°-207°.

$C_{25}H_{36}O_6$ (monosuccinate)	requires	C-69.41%	H-8.38%
	found	C-68.77%	H-8.49%
		68.85%	8.57%
$C_{25}H_{36}O_6 . 1/3 H_2O$	requires	C-68.50%	H-8.45%
$C_{29}H_{40}O_9$ (disuccinate)	requires	C-65.39%	H-7.57%

PART TWO

Synthesis of Etiodesoxycholic Acid by Oxidation of:

a) Ternorcholanyldiphenylethylene;

b) Ternorcholanyldiphenylcarbinol.

I. Introduction

The recognized method of degradation of the side-chain of the bile acids is that of Wieland[13], which he applied to cholanic acid, and which is an adaptation of the Barbier-Locquin[14] degradation of mono- and di-basic saturated aliphatic acids*. The object of this section of the investigation was to study the direct oxidation of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) and 3,12-diacetoxy-ternorcholanyldiphenylcarbinol (XVI) to etiodesoxycholic acid (XXIV) with chromic anhydride, in order to circumvent the tedious and costly six-step procedure of Hoehn and Mason[15], outlined on page two of the appendix (compounds XVII to XXIV). Heard and Wasson[10] have obtained etiodesoxycholic acid (XXIV) in 33 per cent yield from 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) by running these six steps in sequence without separation of any of the intermediates. However, this expensive

---

*The step-wise Barbier-Wieland degradation of desoxycholic acid (I) is outlined on pages i and ii of the appendix.

method is not suited to large scale production, as it requires the handling of large quantities of ozone and of periodic acid. For this reason, even a 5 to 10 per cent yield of etiodesoxycholic acid obtained by direct chromic anhydride oxidation of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) or of the corresponding carbinol (XVI) is more advantageous than a higher yield obtained by ozonolysis and periodic acid oxidation. Hoehn and Mason[15] have obtained etiodesoxycholic acid (XXIV) in 15 per cent yield by direct oxidation of the diacetoxy-ternorcholanyldiphenylethylene (XVII) with chromic anhydride at room temperature. Schwenk[16] found selenium dioxide oxidation of the diacetoxy-ternorcholanyldiphenylethylene to be much more convenient than ozonolysis; the yield is about the same. Reichstein and von Arx[17] have repeated the work of Hoehn and Mason.

Kendall[18] has obtained 90 per cent yields of nordesoxycholic (VII) and bisnordesoxycholic (XIII) acids by performing a two-phase oxidation in acetic acid and chloroform at room temperature and adding sulphuric acid to prevent the reaction from coming to a stop before all the starting material is used up. The degradation of a bisnor- acid gives lower yields than the degradation of the corresponding bile acid or even of the corresponding nor- acid owing to the greater steric hindrance when the terminal carbon atom of the side-chain more closely approaches the steroid nucleus, thus more vigorous conditions must be used in the degradation of a bisnor- acid.

Sawlewicz and Reichstein[19] have obtained 3-acetoxy-etiolithocholic acid (LVII) in 12 per cent yield by chromic anhydride oxidation of the corresponding acetoxy-ternordiphenylethylene on the boiling water-bath. This reaction was repeated by Hoehn and Mason[20]. Steiger and Reichstein[21] have reported a 20 per cent yield of $\Delta^{5:6}$-etiolithocholenic acid (LVIII) by chromic anhydride oxidation at 42° of the corresponding acetoxy-ternordiphenylethylene, provided the double bond is protected by formation of the dibromide. Morsman, Steiger, and Reichstein[22] degraded cholic acid (LIX) to the corresponding C_{17} methyl ketone (LX), without proceeding further to the etio acid (LXI).

Some work has been done on the less common bile acids: Ishihara[23] studied the oxidative degradation of chenodesoxycholic acid (LXII) to etiochenodesoxycholic acid (LXIII) and the corresponding C_{17} methyl ketone (LXIV). Kimura and Sugiyama[24] performed a similar series of reactions on hyodesoxycholic acid (LXV). As a result of this reaction, they isolated pregnane-3 ,6-diol-20-one (LXVI).

As in the dehydration of 3,12-diacetoxy-ternorcholanyldiphenylcarbinol (XVI) to 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) the yield is only 50 per cent, it would be highly desirable to oxidize the ternorcholanyldiphenylcarbinol directly to etiodesoxycholic acid (XXIV), providing satisfactory yields were obtained. Wieland, Schlichtling, and Jacobi[13] had earlier obtained a 19 per cent yield of etiocholanic acid (LXVIII) based on bisnorcholanic acid (LXVII)

by chromic anhydride oxidation of the corresponding ternorcholanyldiphenylcarbinol on the boiling water-bath. Dalmer, von Werder, Honigmann, and Heyns[25] have reported a 19.5 per cent yield of 3-acetoxy-etioallocholanic acid (LXIX) by chromic acid oxidation at 100° of the corresponding acetoxy-ternorcholanyldiphenylcarbinol. Ehrenstein and Stevens[26] obtained etiocholic acid (LXI) in 16.5 per cent yield on chromic anhydride oxidation of the corresponding acetoxy-ternorcholanyldiphenylcarbinol on the boiling water-bath.

Several investigators have studied the direct oxidation of C_{24}-C_{27} steroid compounds to the corresponding C_{17} methyl ketone, etio acid and C_{17} ketone:

Wallis and Fernholz[27] have obtained a 2.4 per cent yield of dehydroandrosterone (LXXI) by chromic anhydride oxidation of cholesteryl acetate dibromide (LXX) at 65°. More recently, Wallis and Brink[28] have prepared 3,12-diacetoxy-etiocholanone-17 (XXVIII) by oxidation of 3,12-diacetoxy-norcholanyldiphenylmethane (LXXII) with chromic anhydride and sulphuric acid at 50° and in 3.5 per cent yield (based on material used up) from 3,12-diacetoxy-bisnorcholanyldiphenyl-ethylene (XVII) passing through the dibromide.

In the past year, Reich and Reichstein[29] have studied the direct oxidation of diacetoxy-desoxycholic acid methyl ester (IIa) into 3,12-diacetoxy -etiocholanone-17 (XXVIII), pregnane-3,12-diol-20-one-diacetate (XIX), and diacetoxy-etiodesoxycholic acid (XXIII), using the method of Ruzicka[30] (chromic anhydride in 80 per cent acetic acid at 70°). Each

of the three products was obtained in 0.15% yield. Fieser[31] has carried out a Ruzicka oxidation on 3,12-dibenzoxy-desoxycholic acid methyl ester (IIb) and says that the semicarbazone thus obtained appears to be that of 3,12-dihydroxy-etiocholanone-17 (XXVIIIa).

Kendall[3] has obtained the 3:9 epoxide of 11-keto-etiocholanic acid (LXXIV) in 63 per cent yield from the 3:9 epoxide of 11-keto-ternorcholanyldiphenylethylene (LXXIII) by a five-step oxidation, using the method of Hoehn and Mason[15]. If a satisfactory method of opening the 3:9 epoxide ring were found, this procedure would be worthwhile adopting as the formation of the 3:9 epoxide ring furnishes a means of transferring the 12-oxygen atom of the desoxycholic acid series to the 11-position as in the corticosterone series.

II. Discussion of Results

The purpose of this study was to investigate methods of oxidizing 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) and the corresponding carbonol (XVI) directly to etiodesoxycholic acid (XXIV), instead of by the indirect method of Hoehn and Mason[15], outlined on page ii of the appendix. These workers obtained a 2 per cent yield of etiodesoxycholic acid (XXIX) from desoxycholic acid (I). As their method involves some twenty-two steps, it would be of considerable advantage to shorten it at this point. However, owing to the increasing steric hindrance as the terminal carbon atom approaches the steroid nucleus, more vigorous conditions must

be used to oxidize a bisnor- acid than those required to oxidize the corresponding bile acid or the corresponding nor- acid. Thus, it is difficult to obtain satisfactory yields of an etio- acid from the direct oxidation of a bisnor- acid.

The preparation of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) from desoxycholic acid (I) was carried out by Charles E. Frosst and Company. Oxidation of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) with chromic anhydride in carbon tetrachloride-acetic acid by an adaptation of the Kendall[18] method of oxidation of nor- and bisnor-diacetoxy-ethylenes (V and XI) and subsequent saponification yielded a large amount of saponified starting material (XVIIa) (yield, 29 per cent), a small amount of pregnane-3,12-diol-20-one (XXXVIII) (yield, 6 per cent), and pure etiodesoxycholic acid (XXIV) in 4 per cent yield. Chromic anhydride oxidation of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) according to the method of Hoehn and Mason[15] yielded a minute quantity (2 per cent) of pregnane-3-12-diol-20-one (XXXVIII), and pure etiodesoxycholic acid in 2.5 per cent yield. Thus, the former conditions give the better yield. This may be due partly to the fact that the use of an organic phase prevents contact to some extent of the desired product with the chromic anhydride, thus putting an obstacle to further oxidation. The higher yield may also be due to the formation of etiodesoxycholic acid anhydride in the presence of the sulphuric acid in the oxidizing medium, thus preventing further oxidation.

The 2.5 per cent yield of etiodesoxycholic acid obtained by the method of Hoehn and Mason[15] is lower than that claimed by these investigators. It must be remembered that different workers have varying criteria of purity for determining yields. Actually, the yield of crude solid acidic material obtained in the above repetition of the work of Hoehn and Mason was 20 per cent.

A two-phase oxidation of 3,12-diacetoxy-ternorcholanyldiphenylcarbinol (XVI) produced etiodesoxycholic acid in 5 per cent yield. As the yield of conversion of the carbinol to the corresponding ethylene (XVII) is only 50 per cent, the amount of etiodesoxycholic acid (XXIV) produced is equivalent to a 7 per cent yield from the ethylene.

More strenuous conditions than were used by Kendall[18] for the degradation of nor- and bisnor- cholanyldiphenylethylene were obtained by lengthening the time of reaction from one and a half hours to twenty-four hours. It may be possible to increase the yield by using a slightly higher temperature.

In these oxidations not a trace of 3,12-dihydroxy-etiocholanone-17 (XXVIIIa) has been found. It may have been present in quantities too small to be purified and identified.

It might be possible to obtain higher yields of etiodesoxycholic acid (XXIV) by working with the dimethylcholanylcarbinol and the corresponding ethylene as there is less steric hindrance in these compounds than in the more space-filling diphenyl compounds and the absence of phenyl groups

will admit the use of a higher temperature. Accordingly, several oxidations were carried out on material supplied to us as 3,12-diacetoxy-ternorcholanyldimethylcarbinol. The results are at present very confusing and cannot yet be described, owing to the fact that evidence is accumulating which suggests the original starting material is incorrectly formulated.

III. Experimental Work

1. Chromic Acid Oxidation (I) of 3,12-Diacetoxy-ternorcholanyldiphenylethylene (XVII), by an adaptation of the Kendall[18] method of oxidation of the nor- and bisnorcholanyldiphenylethylenes:

To a solution of 5 g. of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) in 36 ml. dry chloroform kept at 10°-12° (by immersion in an ice-bath) were added with mechanical stirring 53 ml. glacial acetic acid, and then 8.8 ml. of 10 normal chromic anhydride (10 equivalents) over a period of at least one minute. After fifteen minutes, 8.8 ml. of 10 normal sulphuric acid in 80 per cent acetic acid were added over a period of ten minutes. The ice-bath was then removed and the mixture was stirred for twenty-four hours, the temperature remaining at 20°.

Extraction

The oxidation mixture was then diluted with 176 ml. of water and the chloroform layer was separated. The aqueous layer was shaken three times more with chloroform (3 x 200 ml.) and the combined chloroform extracts were washed twice with water (2 x 300 ml.) and then four times with normal sodium

hydroxide (4 x 300 ml.). The neutral and alkaline fractions were treated as outlined in the diagram on page 40.

Separation of Chromic Acid Oxidation Mixture I into Acidic, Ketonic and Non-Ketonic Fractions

chloroform

-water washing

——N NaOH; acidified with 50% HCl; extracted with ether and washed with water. The ether was evaporated off.

-water washing

Acetylated Neutral Fraction I

Acetylated Acid Fraction I

Mixture was saponified in 30 ml. methanol and 15 ml. aqueous 6N KOH by refluxing 3 hours at 80° on the boiling water bath, cooled, diluted to 300 ml. with water, extracted with ether.

—Mixture was saponified with 30 ml. 2N aqueous KOH, 3 hours at 80° on the boiling water-bath, cooled, and acidified with 50% HCl. Filtered.

Neutral Fraction I

Acid Fraction I (0.1758 g.)

water layer, acidified with 50% HCl

—ether fraction, washed with water and taken to dryness

—recrystallized 3 times from acetone.

Milky white emulsoid

Neutral Fraction I (2.0464 g.)

White powder (39.9 mg.), m.p. 272°-281°. (Hoehn and Mason[15], 283°-286° for etiodesoxycholic acid). Identified as etiodesoxycholic acid (XXIV) by formation of the methyl ester (XXV) and of dehydroetiodesoxycholic acid methyl ester (XXVI).

-mixture extracted with ether, washed with water and taken to dryness.

—Girard[32] Separation

Non-Ketonic Fraction I (1.6024 g.)

Ketonic Fraction I (0.2959 g.)

Total yield of pure etiodesoxycholic acid: 4 per cent theoretical.

Acids I (neutral fraction)

(1.5011 g., m.p. 244°-274°)

——recrystallized 3 times from acetone.

White powder (74.1 mg.), m.p. 275°-284°. This acid was proved identical with etiodesoxycholic acid by mixture melting point.

The acid separated from the neutral fraction is believed due to hydrolysis during the saponification of an anhydride formed in the original oxidation mixture by the union of two molecules of etiodesoxycholic acid with loss of a molecule of water.

Girard[32] Separation of Ketonic and Non-Ketonic Fractions. Girard's reagent P (6.6264 g.) was added to the neutral fraction I (2.0464 g.), dissolved in 66 ml. absolute ethanol and 16.5 ml. standard acetic acid were added. The mixture was refluxed one hour. It was then cooled and poured into enough ice-water to obtain a 10 per cent solution of ethanol and sufficient standard normal sodium hydroxide to neutralize the acetic acid. The pH was adjusted to 6.5-7.0 (it should not blue brom thymol). The mixture was then extracted with ether (3 x 300 ml.), washed with water (3 x 300 ml.), the combined ethereal extracts were taken to dryness to give 1.6024 g. non-ketonic substances. The aqueous layer was acidified in the cold with 50 per cent hydrochloric acid to give a final concentration of one normal or 0.5 normal. The acidified mixture was let stand two hours at room temperature and was then extracted with ether, washed with water and the combined ethereal extracts were taken to dryness to give 0.2959 g. ketonic substances.

The above is the procedure followed in all Girard separations mentioned in this thesis, using amounts of the reagents proportional to the weight of the neutral fraction.

Chromatographic Separation of Ketonic Fraction I. The ketonic fraction was dissolved in 3.5 ml. benzene, and

petroleum ether was added to the point of cloudiness (5 ml.). The mixture was adsorbed on a column of alumina (column S-8) and fractions were eluted therefrom with various solvent mixtures, as outlined on the following page. Obtained were:

1. Pregnane-3,12-diol-20-one (XXXVIII) (fractions 12-21, 113.9 mg.), eluted with petroleum ether: benzene (4/1, 1/1), m.p. 172°-174°, after crystallization from ether:petroleum ether, and identified by mixture melting point with the authentic product obtained from Charles E. Frosst and Company. The compound crystallizes as minute, colourless needles. Yield: 4.5 per cent theoretical. Yield (based on starting material used up): 6 per cent theoretical.

2. Several oily or amorphous fractions which could not be purified.

Chromatographic Separation of Non-Ketonic Fraction I.

The non-ketonic fraction was dissolved in 4 ml. benzene and petroleum ether was added to the point of cloudiness (8.5 ml.). The mixture was adsorbed on a column of alumina (column S-9) and fractions eluted therefrom with various solvent mixtures, as outlined on page 44. Obtained were:

1. A substance (compound "165") (fraction 1-2, 292.9 mg.), probably of a non-steroid nature, eluted with absolute petroleum ether, m.p. 165°-166° after crystallization from ether:petroleum ether and from methanol. The compound crystallizes as faintly yellow needles. On carbon and hydrogen analysis, the compound behaved explosively.

KETONIC FRACTION I. Wt. Adsorbed Mixture: 295.9 mg.

COLUMN No. S-8 FRACTION eluted: 94.5 per cent

(9 GMS. OF ALUMINA; 9 CM. BY 1 CM.
(G. Merck, acid washed.

FRACTION NO.	ELUANT		ELUATE			
	NATURE	VOL. ML.	WT. MG.	NATURE	M.P. °C.	MAIN COMPONENT
1	original benzene: petroleum ether mixture	-	132.7	oil		
2	absolute petroleum ether	40	4.3	"		
3	petroleum ether: benzene (20:1)	"	0.5	"		
4	petroleum ether: benzene (10:1)	"	3.3	amorphous solid	about 64	
5	petroleum ether: benzene (4:1)	"	0.1	amorphous solid	about 64	
6	petroleum ether: benzene (1:1)	"	0.2	amorphous solid	about 64	
7	absolute benzene	"	1.1	amorphous solid	about 64	
8	benzene:ether (40:1)	"	1.3	amorphous solid	about 64	
9-10	benzene:ether (20:1)	"	13.7	amorphous solid	104	
11	benzene:ether (8:1)	"	4.6	amorphous solid	104	
12-20	benzene:ether (4:1)	"	111.0	minute, colourless needles	172-174	pregnane-3,12-diol-20-one
21	benzene:ether (1:1)	"	2.9	"	172-174	"
22-23	absolute ether	"	4.9	wax		
24-25	absolute chloroform	"	0.1	"		
		T	280.7	mg.		

NON-KETONIC FRACTION I. Wt. Adsorbed Mixture: 1.6024 g.

COLUMN No. S-9 FRACTION eluted: 100 per cent.

(48 GMS. OF ALUMINA; 17 CM. BY 1.8 CM.
(G. Merck, acid washed.

FRACTION NO.	ELUANT		ELUATE			
	NATURE	VOL.	WT.	NATURE	M.P.	MAIN COMPONENT
		ML.	MG.		°C	
1	original benzene: petroleum ether mixture	-	249.2	yellow needles	165-166	substance "165"
2	absolute petroleum ether	150	43.7	yellow needles	165-166	"
3	petroleum ether: benzene (20:1)	"	2.6	yellow oil		
4	petroleum ether: benzene (8:1)	"	4.3	yellow oil		
5	petroleum ether: benzene (4:1)	"	4.9	yellow oil		
6-7	petroleum ether: benzene (1:1)	"	14.5	yellow oil		
8-37	absolute benzene		1,029.5	white rosettes	205-207	3,12-di-hydroxy-ternor-cholanyldiphenylethylene
38-46	benzene:ether (20:1)	"	189.4	white rosettes	204-207	"
47-48	benzene:ether (8:1)		11.8	white rosettes	203-207	"
49-51	benzene:ether (4:1)	"	22.1	amorphous solid	100-107	
52	benzene:ether (1:1)	"	18.3	amorphous solid	about 102	
53-55	benzene:ether (1:1)	"	54.2	minute rosettes	299-301	21:22 oxide?
56	absolute ether	"	2.9	amorphous solid	117-137	
57-58	absolute chloroform	"	4.3	amorphous solid	117-137	
			T 1,651.7	mg.		

	Found	C-85.57%	H-5.81%
Benzopinacol:	requires	C-85.22%	H-6.05%, m.p. 186°,
	formula:	$(C_6H_5)_2$-C(OH)-C(OH)-$(C_6H_5)_2$.	

Compound "165" may be impure benzopinacol or is almost certainly a short-chain break-down product of the starting material (XVII).

2. Saponified starting material (3,12-dihydroxy-ternorcholanyldiphenylethylene (XVIIa) (fractions 8-48, 1.2307 g., 29 per cent yield), eluted with absolute benzene, benzene: ether (20/1, 8/1), m.p. 205°-207°, after crystallization from ether:petroleum ether, and identified by mixture melting point with the authentic product (see paragraph 2 of this section). The compound crystallizes as small white rosettes from methanol and as colourless rectangular plates from ether:petroleum ether.

$C_{34}H_{44}O_2$	requires	C-84.30%	H-9.17%
	found	C-81.53%	H-8.81%
		81.41%	8.97%
$C_{34}H_{44}O_2.H_2O$	requires	C-81.28%	H-9.16%

2. Saponification of 3,12-Diacetoxy-ternorcholanyldiphenylethylene:(XVII to XVIIa):

One gram of starting material, dissolved in 80 ml. ethanol, was heated two hours at 80° on the boiling water-bath with 40 ml. 6 normal aqueous potassium hydroxide. The mixture was diluted to ten volumes with water, cooled and extracted with ether (6 x 250 ml.), the combined ethereal extracts

were washed with water (3 x 500 ml.) and the solvent was distilled. The white oily residue could be crystallized from methanol only by seeding with some crystals which could not be extracted from the original water layer. Recrystallization from ether:petroleum ether and, again, from methanol yielded white rosettes, m.p. 218°-225°. A 50:50 mixture of this product and of that isolated from column S-9 (described in the previous paragraph, m.p. 205°-207°) melted at 205°-215°. The two products with different melting points are probably identical. Scholz[33] has obtained samples of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) which differed in melting point by twenty degrees, but were proved identical and gave the correct carbon and hydrogen analyses. The same probably applies to the saponified compound (XVIIa).

$C_{34}H_{44}O_2$	requires	C-84.30%	H-9.17%
	found	C-81.42%	H-8.73%
$C_{34}H_{44}O_2.H_2O$	requires	C-81.28%	H-9.16%

3. Methylation of Etiodesoxycholic Acid I (XXIV to XXV):

The acid (70 mg.) was dissolved in 10 ml. anhydrous methanol and 0.45 ml. of acetyl chloride was added dropwise, cooling the mixture when necessary. After sixteen hours at room temperature, under anhydrous conditions, the mixture was diluted to ten volumes with water, extracted with ether (3 x 35 ml.), and the combined ethereal extracts were washed with 10 per cent sodium carbonate (3 x 35 ml.) and with water

(3 x 35 ml.). The solvent was distilled and the residue was crystallized from ether:petroleum ether to give one large, white rosette (42.3 mg., 58 per cent yield), m.p. 146°-147° (Hoehn and Mason, 145°-146°)[34]. It was noted that the methylation was complete, no precipitate being formed on acidification of the alkaline washings with 50 per cent hydrochloric acid.

4. Chromic Acid Oxidation II of 3,12-Diacetoxy-ternorcholanyldiphenylethylene (XVII) (by an adaptation of the method of Hoehn and Mason)[15]:

To a solution of 3 g. of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) in 106 ml. glacial acetic acid, cooled to 15°, was added a solution of 18 g. chromic anhydride in 16 ml. of 60 per cent acetic acid. After twenty-four hours at room temperature, the mixture was diluted to ten volumes with water, extracted with ether (3 x 300 ml.), and separated into acidic, ketonic, and non-ketonic fractions as outlined on the following page:

Separation of Chromic Acid Oxidation Mixture II into Acidic, Ketonic and Non-Ketonic Fractions

ether

washed with water

N NaOH; acidified with 50% HCl; extracted with ether and washed with water. The ether was distilled.

Acetylated Neutral Fraction II (0.9646 g.)

Mixture was saponified in 30 ml. methanol and 15 ml. aqueous 6N KOH by refluxing 3 hours at 80° on the boiling H_2O bath, cooled, diluted to 300 ml. with water, extracted with ether.

Acetylated Acid Fraction II (0.4918 g.)

Mixture was saponified with 46 ml. 2N KOH, 3 hours at 80° on the boiling water-bath, cooled, and acidified with 50% HCl. Filtered.

Neutral Fraction II (0.7147 g.)

Acid Fraction II (0.2646 g.)

recrystallized 3 times from acetone.

water layer acidified with 50% HCl.

No precipitate.

ether fraction washed with water and taken to dryness

White powder (41.9 mg.), m.p. 276°-281°. Identified as etiodesoxycholic acid (XXIV) by mixture melting point with the etiodesoxycholic acid obtained in the Kendall oxidation of 3,12-diacetoxy-ternorcholanyldiphenylethylene.

Neutral Fraction II

Girard[32] separation

Ketonic Fraction II (0.2821 g.)

Non-ketonic Fraction II (0.3109 g.)

crystallized from methanol.

Faintly yellow needles, m.p. 164°-166°. Identified by mixture melting point with previous samples as substance "165" (see column S-9 in paragraph 1 of this section).

Chromatographic Separation of Ketonic Fraction II. The ketonic fraction was dissolved in 3.5 ml. benzene and petroleum ether was added to the point of cloudiness (1 ml.). The mixture was adsorbed on a column of alumina (column S-10) and fractions eluted therefrom with various solvent mixtures, as outlined on the following page. Obtained were:

1. Pregnane-3,12-diol-20-one (XXXVIII) (fractions 4-5, 3.8 mg., 2 per cent yield), eluted with petroleum ether:benzene (20/1, 8/1), m.p. 165°-167°, identified by mixture melting point with the authentic product.

2. The remainder of the material could not be crystallized, the greater fraction (0.2387 g.) was not adsorbed at all on the column, being very likely short-chain degradation products of the oxidation.

Chromatographic Separation of the Mother Liquors of Non-Ketonic Fraction II. The mother liquors of the non-ketonic fraction were dissolved in benzene and petroleum ether was added to the point of cloudiness. The mixture was adsorbed on a column of alumina (column S-11) and fractions eluted therefrom with various solvent mixtures, as outlined on page 51. Obtained were:

1. Compound "165" (208.7 mg.), eluted with absolute petroleum ether, m.p. 165°-167° after crystallization from methanol and proved identical with previous samples of the same substance by mixture melting point (see column S-9 in paragraph 1 of this section).

KETONIC FRACTION II. Wt. Adsorbed Mixture: 282.1 mg.

COLUMN No. S-10 FRACTION eluted: 95.5 per cent

(8.5 GMS. OF ALUMINA; 9 CM. BY 1 CM.
(G. Merck, acid washed.

FRACTION NO.	ELUANT		ELUATE			
	NATURE	VOL.	WT.	NATURE	M.P.	MAIN COMPONENT
		ML.	MG.		°C.	
1	original benzene: petroleum ether mixture	-	238.7			
2-3	absolute petroleum ether	45	10.4	yellow oil		
4	petroleum ether: benzene (20:1)	"	3.3	white rosettes	165-167	pregnane-3,12-diol-20-on
5	petroleum ether: benzene (8:1)	"	0.5	white rosettes	165-167	"
6	petroleum ether: benzene (4:1)	"	0.1	yellow oil		
7	petroleum ether: benzene (2:1)	"	0.1	yellow oil		
8	absolute benzene	"	0.5	yellow oil		
9	benzene:ether (20:1)	"	0.6	yellow oil		
10	benzene:ether (8:1)	"	0.7	yellow oil		
11	benzene:ether (4:1)	"	0.9	yellow oil		
12-13	benzene:ether (1:1)	"	12.1	yellow oil		
14	absolute ether	"	1.5	yellow oil		
15	absolute chloroform	"	0.2	yellow oil		
			T 269.4	mg.		

NON-KETONIC FRACTION II. Wt. Adsorbed Mixture: 286.0 mg.

COLUMN No. S-11 FRACTION eluted: 94.3 per cent

(9.3 GMS. OF ALUMINA; 10 CM. BY 1 CM.
(G. Merck, acid washed.

FRACTION NO.	ELUANT		ELUATE			
	NATURE	VOL.	WT.	NATURE	M.P.	MAIN COMPONENT
		ML.	MG.		0°C	
1	original benzene: petroleum ether mixture	-	186.3	yellow needles	165-167	substance "165"
2-3	absolute petroleum ether	50	22.4	yellow needles	163-167	"
4	petroleum ether: benzene (20:1)	"	2.8	yellow oil		
5	petroleum ether: benzene (8:1)	"	2.8	yellow oil		
6-7	petroleum ether: benzene (4:1)	"	11.2	yellow crystals	127-137	
8-9	petroleum ether: benzene (1:1)	"	19.3	yellow crystals	97-108	
10	absolute benzene	"	2.4	yellow oil		
11	benzene:ether (20:1)	"	0.9	yellow oil		
12	benzene:ether (8:1)	"	2.3	yellow oil		
13	benzene:ether (4:1)	"	2.5	yellow oil		
14-15	benzene:ether (1:1)	"	11.4	yellow oil		
16	absolute ether	"	3.6	yellow oil		
17-18	absolute chloroform	"	1.9	yellow oil		
		T	269.8	mg.		

2. A small quantity (fractions 6-7, 11.2 mg.) of an unidentified product eluted with petroleum ether:benzene (4/1), m.p. 127°-137°, after crystallization from methanol.

3. A small quantity (fractions 8-9, 19.3 mg.) of an unidentified product eluted with petroleum ether:benzene (1/1), m.p. 97°-108°, after crystallization from methanol and possibly identical with the product obtained in fractions 6-7 (m.p. 127°-137°).

5. Methylation of Etiodesoxycholic Acid II (XXIV to XXV):

The acid (41.9 mg.) was dissolved in 7 ml. anhydrous methanol and 0.32 ml. acetyl chloride was added dropwise, cooling the mixture when necessary. After sixteen hours at room temperature under anhydrous conditions, the mixture was diluted to ten volumes with water, extracted with ether (3 x 20 ml.), the ethereal extracts were washed with 10 per cent sodium carbonate (3 x 20 ml.) and then with water (3 x 20 ml.). Weight of crude oily residue: 45.5 mg. It was noted that the methylation was complete, as no precipitate was formed on acidification of the alkaline washings with 50 per cent hydrochloric acid.

6. Methylation of Mother Liquors of Etiodesoxycholic Acids I and II:

The combined oily residues (1.5943 g.) were dissolved in 70 ml. methanol and 3.15 ml. acetyl chloride were added. After sixteen hours at room temperature under anhydrous conditions, the mixture was diluted to ten volumes with water,

extracted with ether (3 x 250 ml.), the combined ethereal extracts were washed with 10 per cent sodium carbonate (3 x 250 ml.) and then with water (3 x 250 ml.). The ether was evaporated off. The crude oily methyl esters (0.7158 g.) could not be crystallized. Acidification of the alkaline washings as well as of the original aqueous layer with 50 per cent hydrochloric acid revealed the presence of 0.8273 g. of unmethylated or partially methylated acids which were filtered. It seems likely that these compounds were dibasic acids formed by the opening of ring D of the steroid nucleus and which could not be completely methylated under the conditions used, one of the α-carbon atoms being tertiary.

7. Oxidation of Etiodesoxycholic Acid Methyl Ester (XXV to XXVI)[15]:

To a solution of the methyl esters of acids I and II (88.8 mg.) in 25 ml. acetone, a mixture of 2 normal potassium dichromate (4.4 ml.) and 5 normal sulphuric acid (6.1 ml.) was added. After two and a quarter hours at room temperature, the mixture was diluted to ten volumes with water, extracted with ether (3 x 100 ml.), the combined ethereal extracts were washed with 10 per cent sodium carbonate (3 x 100 ml.) and then with water (3 x 100 ml.). On evaporation of the solvent, the product crystallized out as large white rosettes (80.3 mg.). The compound was recrystallized twice from methanol, m.p. 171°-173° (Hoehn and Mason, 169°-170°, 171°-172°)[15,34]. Yield: 91.5 per cent theoretical. Acidification of the alkaline washings revealed no trace of acids.

8. Oxidation of Mother Liquors of Etiodesocycholic Acid Methyl Esters I and II:

To a solution of the combined mother liquors (0.7158 g.) in 200 ml. acetone a mixture of 2 normal potassium dichromate (35.2 ml.) and 5 normal sulphuric acid (14.1 ml.) was added. After two and a quarter hours at room temperature, the mixture was diluted to ten volumes with water, extracted with ether (3 x 700 ml.), the combined ethereal extracts were washed with 10 per cent sodium carbonate (3 x 700 ml.) and then with water (3 x 700 ml.). On evaporation of the solvent, the product (0.4234 g.) crystallized out on standing and was proved identical with the product of the previous oxidation by mixture melting point (see paragraph 7 of this section). Acidification of the alkaline washings revealed the presence of a trace of either dibasic or unmethylated acids.

9. Hydrolysis of Dehydroetiodesoxycholic Acid Methyl Ester (XXVI to XXVII):

Dehydroetiodesoxycholic acid methyl ester (XXVI) (323.0 mg.) was heated for three hours at 80° on the boiling water-bath in a mixture of 20 ml. methanol and 10 ml. 6 normal potassium hydroxide. The mixture was cooled, diluted to 200 ml. with water, and acidified with 50 per cent hydrochloric acid. The yellow powder, which precipitated out, was filtered and dried, m.p. 163°-175° (Hoehn and Mason, 177°-178.5°)[15] The impurity of the product is apparently due to the conditions of hydrolysis which were too vigorous.

10. Chromic Acid Oxidation of 3,12-Diacetoxy-ternorcholanyldiphenylcarbinol (XVI), by an adaptation of the Kendall[18] method of oxidation of the nor- and bisnorcholanyldiphenylethylenes:

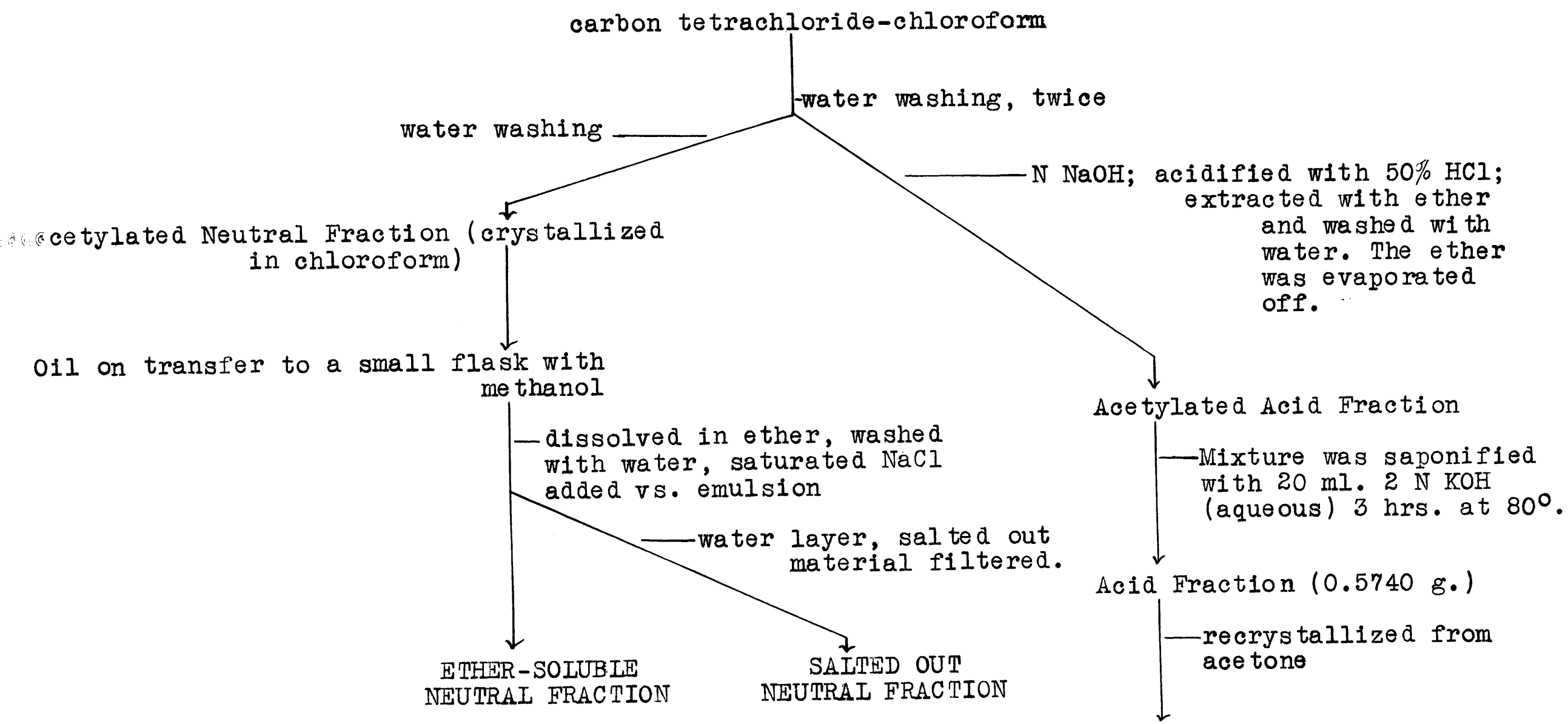
To a solution of 12.2 g. of 3,12-diacetoxy-ternorcholanyldiphenylcarbinol (XVI) in 104 ml. dry carbon tetrachloride kept at 10°-12° were added with mechanical stirring 156 ml. glacial acetic acid, and then 17.32 g. chromic anhydride in 26 ml. water (10 equivalents) over a period of at least one minute. After fifteen minutes, 26 ml. of 10 normal sulphuric acid in 80 per cent acetic acid were added over a period of ten minutes. The ice-bath was then removed and the mixture was stirred for twenty-four hours at room temperature.

Extraction. The oxidation mixture was then diluted with 518 ml. water and the carbon tetrachloride layer was separated. The aqueous layer was shaken thrice with chloroform (3 x 400 ml.) and the combined chloroform-carbon tetrachloride extracts were washed twice with water (2 x 400 ml.) and then four times with normal sodium hydroxide (4 x 400 ml.). The neutral and alkaline fractions were treated as outlined on pages 57 and 58.

As in the Kendall oxidation of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII), the greater part of the etiodesoxycholic acid (XXIV) formed in the reaction is first obtained as an anhydride which is hydrolyzed when the neutral fraction is saponified and can be recovered on acidification of the aqueous layer after extraction of the neutral components with ether.

The total weight of etiodesoxycholic acid (XXIV) obtained was 296.1 mg. As 2.4883 g. 3,12-dihydroxy-ternorcholanyldiphenylethylene (XVII) were produced in this oxidation, this means that 5 g. of starting material were merely dehydrated (the yield on dehydration being about 50 per cent). Therefore, the net yield of etiodesoxycholic acid (XXIV) was 5 per cent.

Separation of Chromic Acid Oxidation Mixture into Acidic, Ketonic and Non-Ketonic Fractions



(Continued on following page)

Separation of Chromic Acid Oxidation Mixture into Acidic, Ketonic and Non-Ketonic Fractions ... cont'd.

ETHER-SOLUBLE NEUTRAL FRACTION

aponified in 320 ml. methanol and 155 ml. N KOH. Product precipitated out almost immediately on addition of KOH as shiny white crystals, cooled, filtered.

Crystalline Neutrals (2.4883 g.), m.p. 207°-209°. Proved identical by mixture melting point with 3,12-dihydroxy-ternorcholanyl-diphenylethylene (XVIIa). Yield: 20 per cent theoretical.

Mother Liquors

Methanol was evaporated off, mixture was diluted with water and extracted with ether

water layer, acidified with 50% HCl, extracted with ether, taken to dryness

ether layer, taken to dryness

oily residue (4.1569 g.)

Yellow oil (2.0000 g.)

recrystallized 4 times from acetone

White powder (231.8 mg.), m.p. 278°-282°, identified as etiodesoxycholic acid (XXIV) by mixture melting point with the authentic product.

SALTED OUT NEUTRAL FRACTION

Saponified 3 hrs. at 80° in 40 ml. MeOH with 20 ml. 6N KOH, cooled, diluted to 400 ml., extracted with ether

ether layer, washed with water and taken to dryness.

Salted out Neutrals (111.7 mg.), yellow oil which could not be crystallized.

water layer, acidified with 50% HCl, extracted with ether, washed with water and taken to dryness

Salted out Acids (neutral fraction) (427.4 mg.), yellow oil which could not be crystallized from acetone.

PART THREE

Synthesis of the $C_{17\alpha}$-ketol Side-Chain from Etiodesoxycholic Acid

I. Introduction

When the study on the synthesis of the 12-oxygen analogues of corticosterone (XXX) was begun, there was available no published work concerned with the synthesis of the desired compounds. Since then, it has become known that Fuchs and Reichstein[4] have synthesized the 12-acetoxy- (LXXXII), the 12-hydroxy- (LXXXV), and the 12-keto- (LXXXVI) analogues of corticosterone from etiodesoxycholic acid (XXIV), as outlined on page vii of the appendix. Assuming that the yield of 3,12-diacetoxy-etiodesoxycholic acid (XXIII) from pregnane-3,12-diol-20-one-diacetate (XIX) is 50 per cent as obtained by Hoehn and Mason[15], these investigators have obtained pregnane-21-ol-3,12,20-trione-monoacetate in 19.5 per cent yield from pregnane-3,12-diol-20-one-diacetate (XIX). The corresponding yields of the 12-hydroxy- (LXXXV) and the 12-acetoxy- (LXXXII) analogues were 11 per cent and 22 per cent, respectively. The gluconeogenic activity of the 12-oxygen analogues of corticosterone has not been sufficiently investigated by these workers to permit any generalizations.

II. Discussion of Results

The oxidation of etiodesoxycholic acid (XXIV) to dehydroetiodesoxycholic acid (XXVII) was first studied. Oxidation of the starting material (XXIV) with chromic anhydride (3 oxygen equivalents) in glacial acetic acid and a trace of water for two hours gave a 54-64 per cent yield of dehydroetiodesoxycholic acid (XXVII). Oxidation with potassium dichromate (2.2 oxygen equivalents) in glacial acetic acid and water for twenty-eight hours gave only a 20 per cent yield of the desired product (XXVII). As described in Part Two of this thesis, conversion of etiodesoxycholic acid (XXIV) to its methyl ester (XXV), oxidation with potassium dichromate (2.2 oxygen equivalents) to the dehydro- methyl ester (XXVI) and saponification of the ester to dehydroetiodesoxycholic acid (XXVII) gave an impure product in low yield.

Dehydroetiodesoxycholic acid (XXVII) was then converted to the acid chloride (LXXXVII) with thionyl chloride. The crude acid chloride was transformed without being weighed into the corresponding 21-diazo- compound (LXXXVIII), which in turn was converted without weighing into the desired pregnane-21-ol-3,12,20-trione-monoacetate (XLIV) by heating at 100° in purified glacial acetic acid.

The crude, brown oily mixture obtained is at present being purified. A few mg. of the desired pregnane-21-ol-3, 12,20-trione-monoacetate (XLIV) have already been obtained and identified by mixture melting point with the product

obtained in the lead tetraacetate oxidation of pregnane-3,12-diol-20-one (XXXVIII). As the mixture is very impure, a considerable amount of work remains to be done before yields can be determined.

III. Experimental Work

1. Saponification of Diacetoxy-etiodesoxycholic Acid (XXIII to XXIV):

An unweighed quantity of the oily starting material, dissolved in 80 ml. methanol, was saponified three hours at 80° on the boiling water-bath with 40 ml. 6 normal aqueous potassium hydroxide. The mixture was then diluted to 800 ml. with water, extracted with ether (3 x 300 ml.), the combined ethereal extracts were washed with water and taken to dryness; a few mg. of a yellow oil were obtained. The aqueous layer from the above extraction was acidified carefully with 50 per cent hydrochloric acid and the voluminous white precipitate so obtained was filtered and washed with water. Recrystallization from acetone yielded 956.1 mg. of etiodesoxycholic acid (XXIV), m.p. 291°-294°. Repeated recrystallizations of the mother liquors with acetone yielded 1.5531 g. product (XXIV), m.p. 285°-290°.

2. Oxidation (I) of Etiodesoxycholic Acid (XXIV to XXVII):

To 450 mg. starting material (XXIV) in 25 ml. glacial acetic acid were added 238.5 mg. (three oxygen equivalents) of chromic anhydride in two drops of water and 9 ml. glacial acetic acid. The mixture was let stand two hours at room

temperature and then diluted to ten volumes with water. The aqueous mixture was extracted with ether (3 x 100 ml.), the combined ethereal extracts were washed with normal sodium hydroxide (3 x 100 ml.) and with water (3 x 100 ml.) and taken to dryness. There was a trace of neutral products. The alkaline extracts were acidified carefully with 50 per cent hydrochloric acid. At first only a diffuse cloudiness was observed, but on prolonged standing (sixteen hours) the material separated out as white, star-like crystals which were filtered and washed. The product, m.p. 180°-181° (Hoehn and Mason[15], 177°-178.5°), weighed 210 mg. Extraction of the mother liquors with ether, evaporation of the ether after washing with water, and recrystallization from ether-petroleum ether yielded 187.8 mg. of product, m.p. 177°-179°. Total yield: 64 per cent theoretical.

3. Oxidation (II) of Etiodesoxycholic Acid (XXIV to XXVII):

To 1.6000 g. starting material (XXIV) in 50 ml. glacial acetic acid were added 952.4 mg. (three oxygen equivalents) of chromic anhydride in four drops of water and 20 ml. glacial acetic acid. The mixture was let stand two and a quarter hours at room temperature and was treated exactly as the corresponding material in the preceding paragraph. Weight crystalline product obtained: 601.6 mg., m.p. 179°-180°. Weight product recovered from the mother liquors: 267.8 mg., m.p. 173°-176°. Total yield: 54.5 per cent theoretical.

4. Oxidation (III) of Etiodesoxycholic Acid (XXIV to XXVII):

To 214 mg. starting material (XXIV) in 15 ml. glacial acetic acid was added 1.4 ml. of 2 normal potassium dichromate (2.2 oxygen equivalents). The mixture was let stand twenty-eight hours at room temperature. It was then diluted to ten volumes with water, extracted with ether (3 x 50 ml.), the combined ethereal extracts were washed with normal sodium hydroxide (3 x 50 ml.) and with water (3 x 50 ml.), and were taken to dryness. Weight of neutral residue: 9.0 mg. The alkaline washings were acidified carefully with 50 per cent hydrochloric acid, whereupon a small amount of flocculent precipitate separated out. As this did not crystallize or increase on standing, the mixture was extracted with ether, the combined ethereal extracts were washed with water and were taken to dryness. The white oily residue was recrystallized from ether, after removing a small amount of ether insoluble impurity, to give 43.6 mg. of a white powder, m.p. 178°-181°. Yield: 20 per cent theoretical.

5. Action of Thionyl Chloride on Dehydroetiodesoxycholic Acid (XXVII to LXXXVII):

The starting material (XXVII) (1.1965 g.) was dissolved in 8.83 ml. purified thionyl chloride. The mixture was let stand thirty minutes at 0° and twenty hours at room temperature, under anhydrous conditions. The mixture turned dark red. The solvent was then distilled off under vacuum, using as little heat as possible. The red crystalline resi-

due was dessicated two hours under vacuum (calcium chloride). The product was neither weighed nor purified.

6. Action of Diazomethane on Dehydro-etiodesoxycholyl Chloride (LXXXVII to LXXXVIII):

The starting material (LXXXVII), dissolved in 10 ml. dry benzene, was added to the diazomethane[35] obtained from 5.4 g. nitroso-methyl urea in 83.5 ml. dry ether, the temperature during the addition being maintained at -15°. The mixture was let stand two hours at 0° and twenty-two hours at room temperature. The solvents were then distilled off under vacuum. The dark red residue was neither weighed nor purified.

7. Action of Glacial Acetic Acid on 21-Diazo-pregnane-3,12,20-trione (LXXXVIII to XLIV):

The starting material (LXXXVIII) was dissolved in 8.33 ml. purified glacial acetic acid and the mixture was heated at 100° for fifty-five minutes. The dark brown solid mixture so obtained is at present being purified. A few mg. of the desired pregnane-21-ol-3,12,20-trione-monoacetate (XLIV) have already been obtained and identified by mixture melting point with the product obtained in the lead tetraacetate oxidation of pregnane-3,12-diol-20-one (XXXVIII). As the mixture is very impure, a considerable amount of work remains to be done before yields can be determined.

SUMMARY

1. Pregnane-3-ol-12,20-dione (XLI) has been prepared by partial succinoylation of pregnane-3,12-diol-20-one (XXXVIII) to the corresponding 3-monosuccinate (XXXIX), oxidation to pregnane-3-ol-12,20-dione-monosuccinate (XL), and saponification of the latter.

2. Treatment of pregnane-3-ol-12,20-dione (XLI) with lead tetraacetate yields mainly pregnane-3-ol-12,20-dione-monoacetate (XLII), and only a trace of compounds possessing reducing activity (i.e., α-β-ketols).

3. Treatment of pregnane-3,12-diol-20-one (XXXVIII) with lead tetraacetate effects simultaneous oxidation of the C_{21} methyl group and acetylation. Oxidation of the mixture so obtained with chromic anhydride yields pregnane-3,12,20-trione (XLIX), pregnane-3-ol-12,20-dione-monoacetate (XLII), pregnane-3,21-diol-12,20-dione-diacetate (L), and pregnane-21-ol-3,12,20-trione-monoacetate (XLIV).

4. Desoxycorticosterone (LII) is converted into the succinate (LIII) by treatment with succinic anhydride in pyridine at room temperature.

5. A two-phase oxidation in chloroform-acetic acid of 3,12-diacetoxy-ternorcholanyldiphenylethylene (XVII) with chromic anhydride gives a slightly higher yield of etiodesoxycholic

acid (XXIV) than the more usual chromic anhydride oxidation in acetic acid.

6. A higher yield of etiodesoxycholic acid (XXIV) is obtained by performing a two-phase oxidation with chromic anhydride in carbon tetrachloride-acetic acid directly on 3,12-diacetoxy-ternorcholanyldiphenylcarbinol (XVI) without preliminary dehydration to the corresponding ethylene (XVII).

7. It is preferable to oxidize etiodesoxycholic acid (XXIV) directly to dehydroetiodesoxycholic acid (XXVII) with chromic anhydride rather than to oxidize the methyl ester (XXV) and saponify the dehydroetiodesoxycholic acid methyl ester (XXVI) so obtained. The product obtained by direct oxidation of the acid is isolated in purer form and in higher yield.

8. Treatment of dehydroetiodesoxycholic acid (XXVII) with thionyl chloride, diazomethane, and acetic acid yields a mixture of reducing substances (alkaline silver diamine) which is in the process of separation. A few mg. only of the desired pregnane-21-ol-3,12,20-trione-monoacetate (XLIV) have as yet been isolated from the mixture.

REFERENCES

1. P. Hegner and T. Reichstein, Helv. Chim. Acta, 26, 721, 1943.
2. T. F. Gallagher, O.S.R.D. conference communication, confidential.
3. E. C. Kendall, O.S.R.D. conference communication, confidential.
4. H. G. Fuchs and T. Reichstein, Helv. Chim. Acta, 26, 511, 1943.
5. O. Dimroth and R. Schweizer, B. 56, 1,375, 1923.
6. G. Ehrhart, H. Ruschig, W. Aumüller, Z. Angew. Ch., 52, 363, 1939.
7. M. Bockmühl, G. Ehrhart, H. Ruschig, W. Aumüller, U. S. Patents 2,230,772 and 2,230,773, Feb. 4, 1941; Chem. Zentr. II, 170, 1939.
8. T. Reichstein and T. Montigel, Helv. Chim. Acta, 22, 1,212, 1939.
9. E. Schwenk, B. Riegel, R. B. Moffett, E. Stahl, J. Am. Chem. Soc., 65, 549, 1943.
10. R. D. H. Heard, National Research Council Progress Report, confidential.
11. M. Steiger and T. Reichstein, Helv. Chim. Acta, 20, 1,164, 1937.
12. T. Reichstein and J. v. Euw, Helv. Chim. Acta, 21, 1,182, 1938.
13. H. Wieland, O. Schlichting, R. Jacobi, Zeit. Physiol. Chem., 161, 80, 1926.

14. P. Barbier, R. Locquin, Compt. rend., 156, 1,443, 1913.

15. W. M. Hoehn and H. L. Mason, J. Am. Chem. Soc., 60, 1,493, 1938.

16. E. Schwenk, O.S.R.D. conference communication, confidential.

17. T. Reichstein and E. von Arx, Helv. Chim. Acta, 23, 747, 1940.

18. E. C. Kendall and W. F. McGuckin, O.S.R.D. conference communication, confidential.

19. J. Sawlewicz and T. Reichstein, Helv. Chim. Acta, 20, 949, 1937.

20. W. M. Hoehn and H. L. Mason, J. Am. Chem. Soc., 62, 569, 1940.

21. M. Steiger and T. Reichstein, Helv. Chim. Acta, 20, 1,040, 1937.

22. H. Morsman, M. Steiger, T. Reichstein, Helv. Chim. Acta, 20, 1, 1937.

23. Ishihara, J. Biochem. 27, 265, 1938; Chem. Zentr. I, 2,315, 1940.

24. Kimura and Sugiyama, J. Biochem. 29, 409, 1939; Chem. Zentr. II, 2,792, 1939.

25. O. Dalmer, F. v. Werder, H. Honigmann, K. Heyns, Ber., 68, 1,814, 1935.

26. M. Ehrenstein and T. O. Stevens, J. Org. Chem., 5, 660, 1940.

27. E. S. Wallis and E. Fernholz, J. Am. Chem. Soc., 57, 1,504, 1935.

28. E. S. Wallis and N. G. Brink, O.S.R.D. communication, confidential.

29. H. Reich and T. Reichstein, Helv. Chim. Acta, 26, 2,102, 1943.

30. L. Ruzicka, Helv. Chim. Acta, 17, 1,389, 1,395, 1934; 18, 61, 430, 668, 1,483, 1935; 20, 1,283, 1,291, 1937.

31. L. F. Fieser, O.S.R.D. communication, confidential.

32. Girard and Sandulesco, Helv. Chim. Acta, 19, 1,095, 1936.

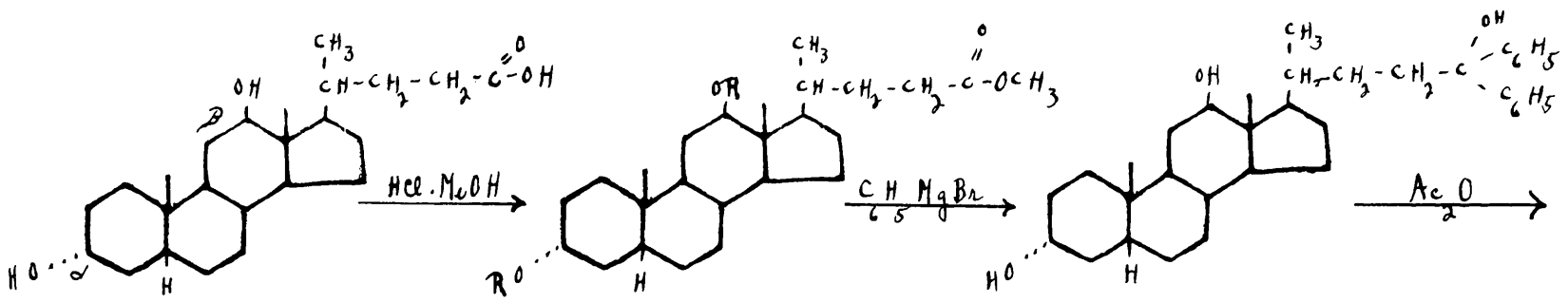
33. C. R. Scholz, private communication from W. Bergmann, confidential.

34. W. M. Hoehn and H. L. Mason, J. Am. Chem. Soc., 60, 2,824, 1938.

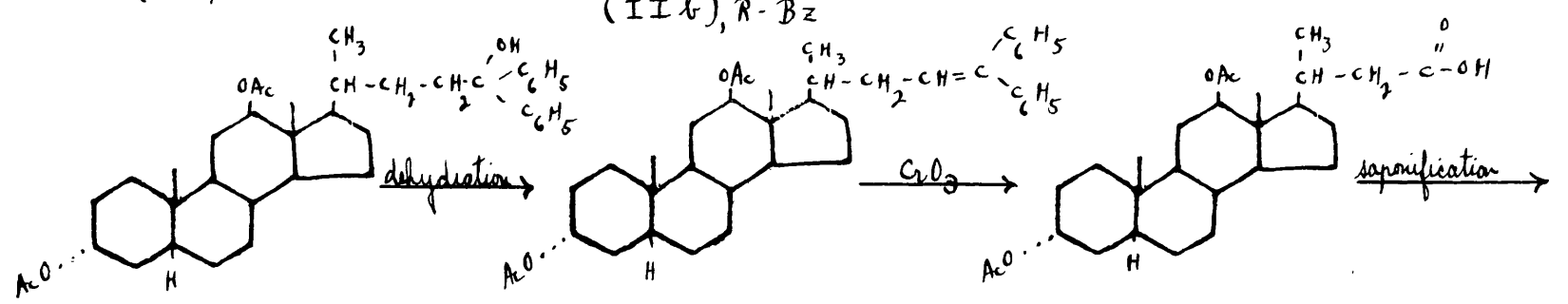
35. Org. Syn., Coll. Vol. II (Blatt), page 165.

APPENDIX

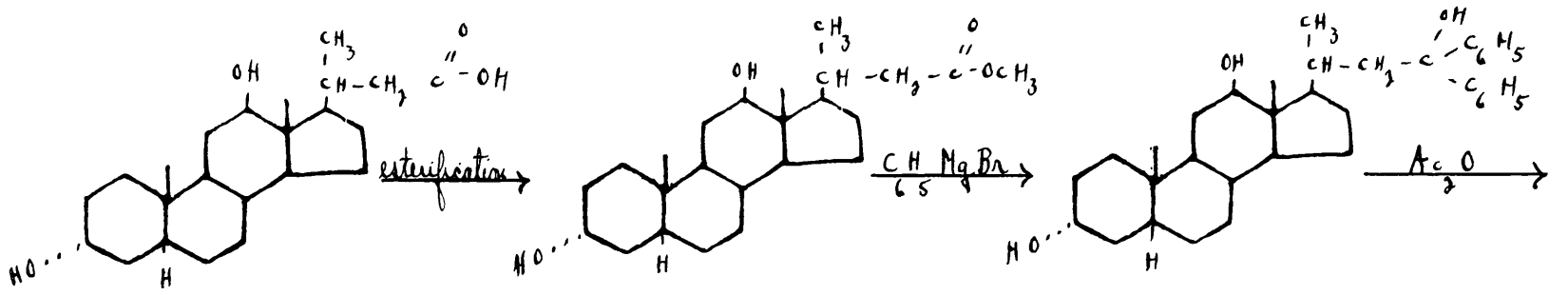
Barbier-Wieland Degradation of Desoxycholic Acid (compounds I to XXIV):



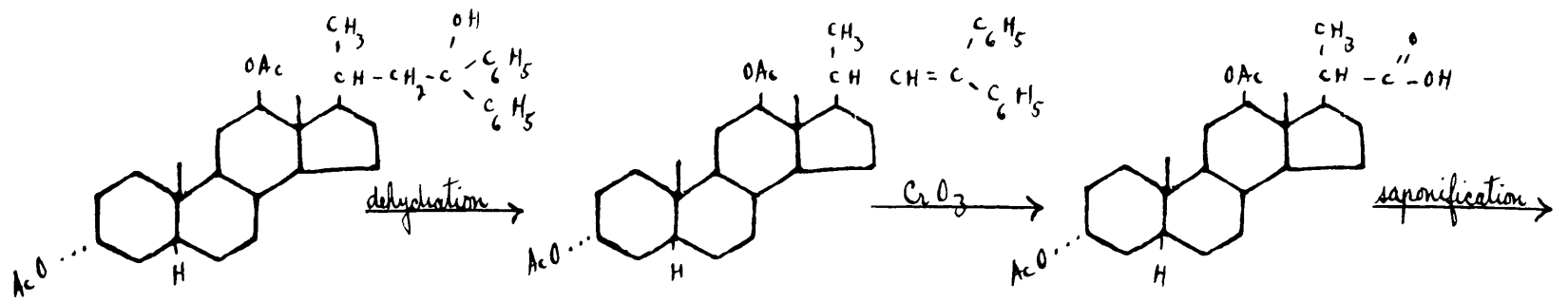
(I) (II), R = H; (IIa), R = Ac; (IIb), R = Bz (III)



(IV) (V) (VI)

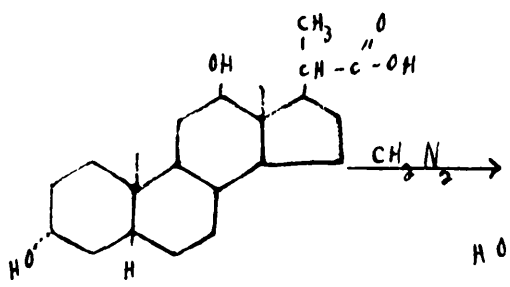


(VII) (VIII) (IX)

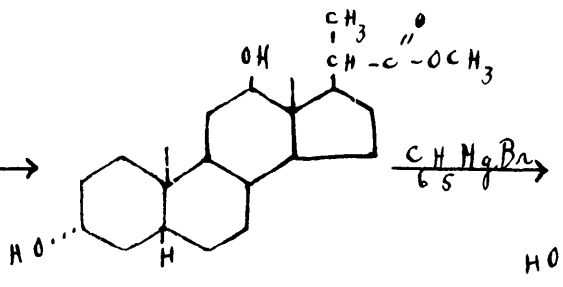


(X) (XI) (XII)

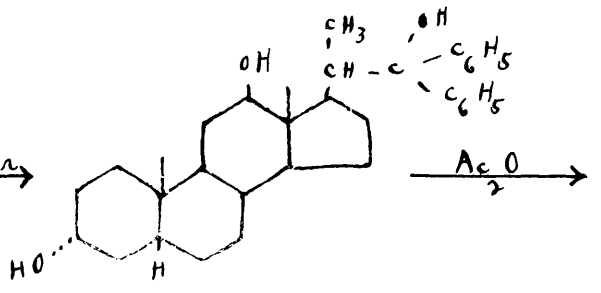
Barbier-Wieland Degradation of Desoxycholic Acid (continued):



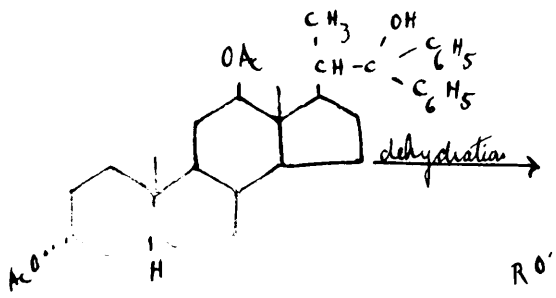
(XIII)



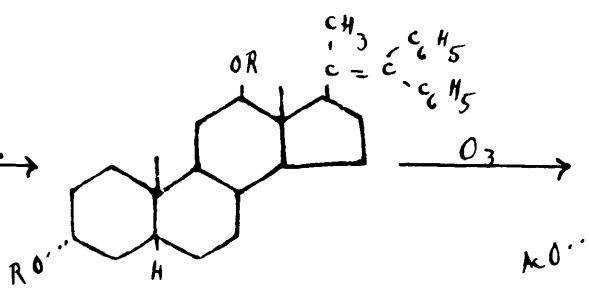
(XIV)



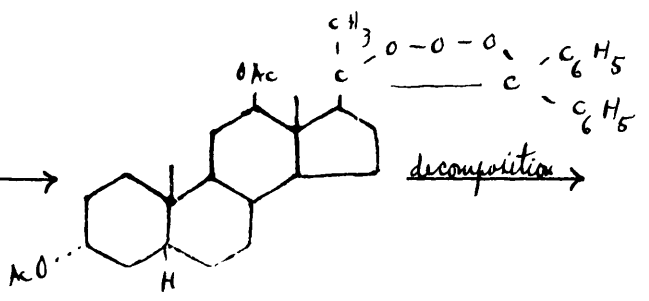
(XV)



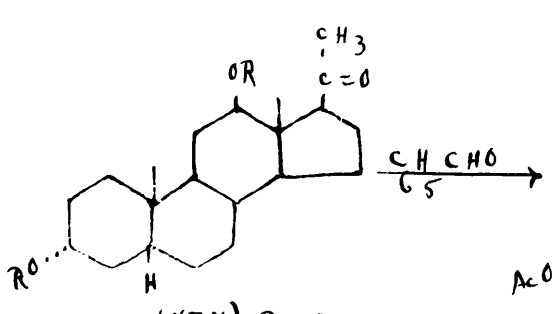
(XVI)



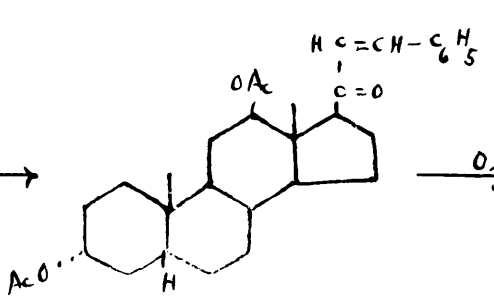
(XVII a), R = H
(XVII), R = Ac



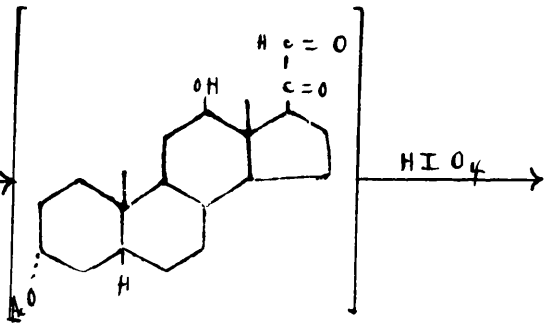
(XVIII)



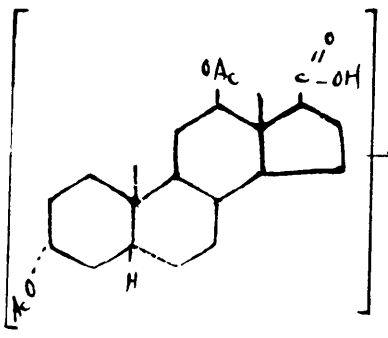
(XIX), R = Ac
(XX), R = H



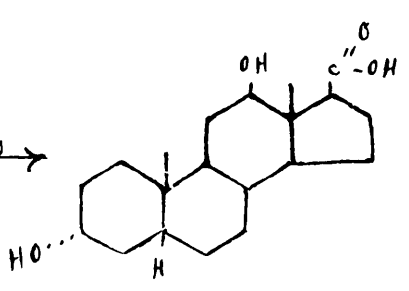
(XXI)



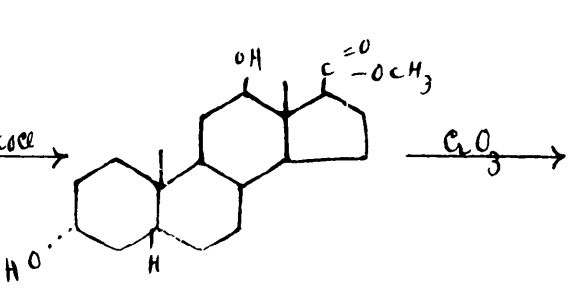
(XXII)



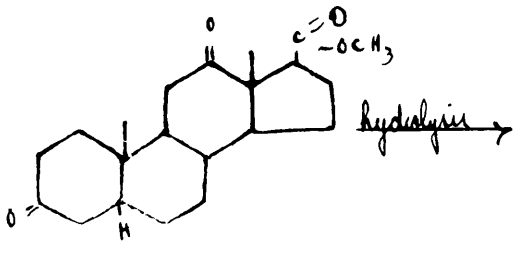
(XXIII)



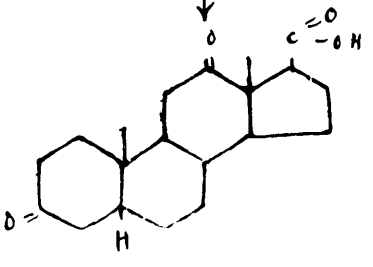
(XXIV)



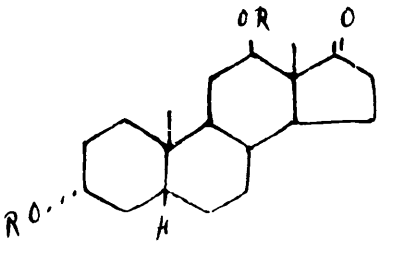
(XXV)



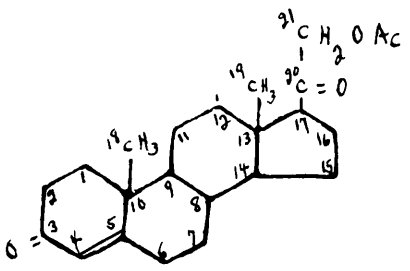
(XXVI)



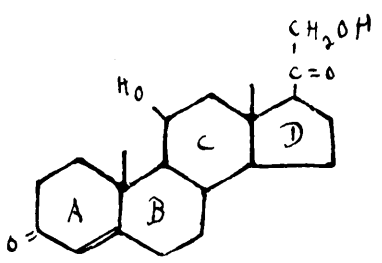
(XXVII)



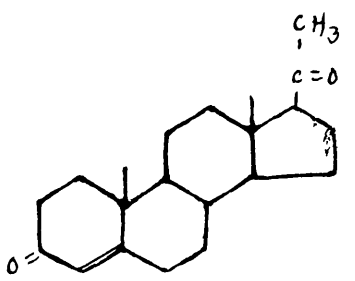
(XXVIII), R = Ac
(XXVIII a), R = H



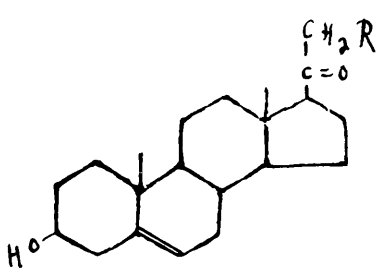
(XXIX)



(XXX)

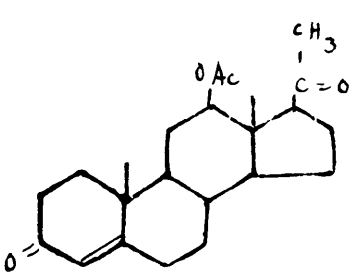


(XXXI)

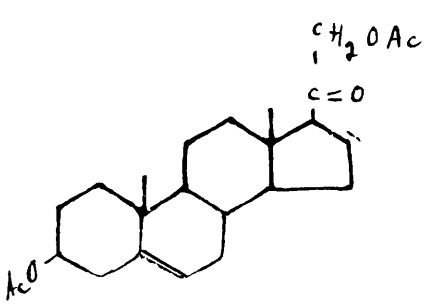


(XXXII), R= OAc

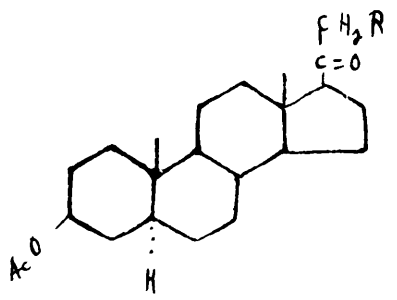
(XXXIII), R= H



(XXXIV)

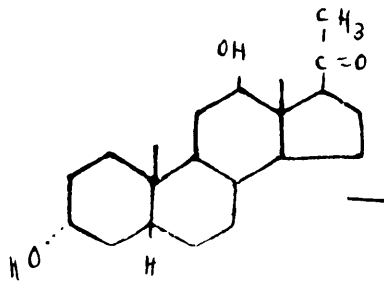


(XXXV)

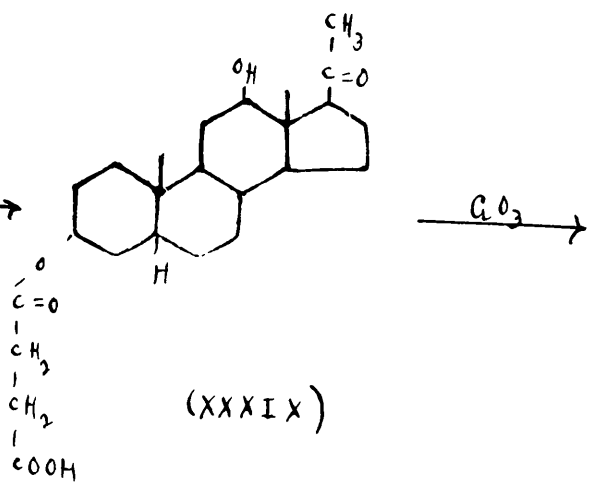


(XXXVI), R= H

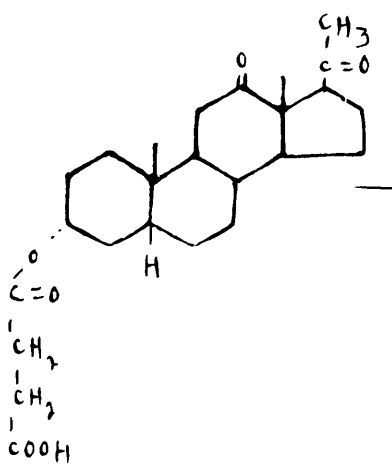
(XXXVII), R= OAc



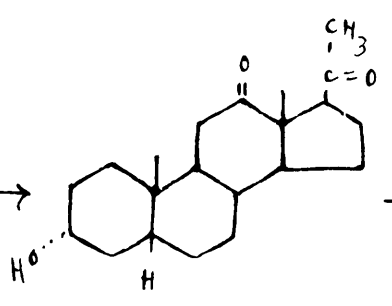
(XXXVIII)



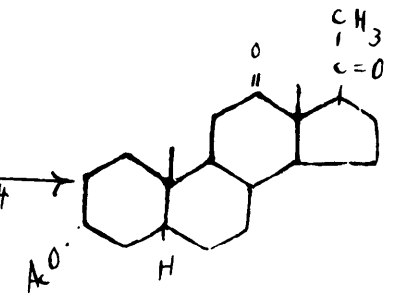
(XXXIX)



(XL)

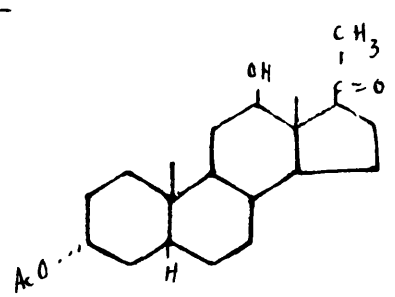


(XLI)

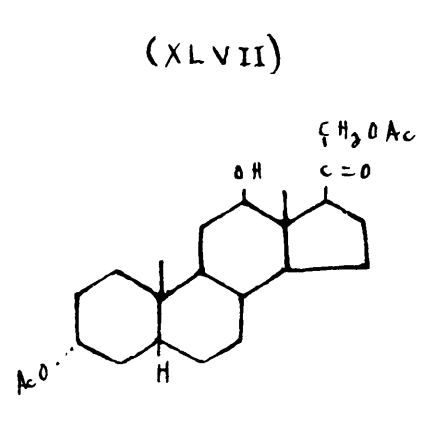


(XLII)

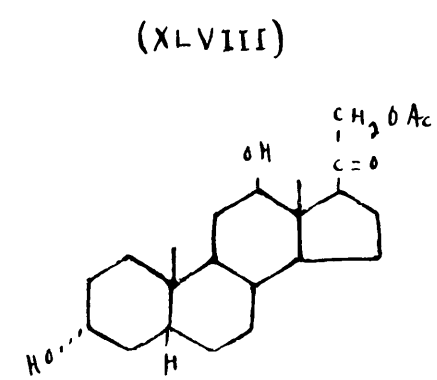
(XXXVIII)



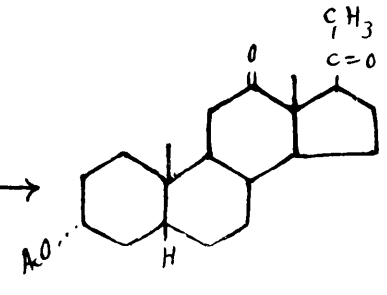
(XLVII)



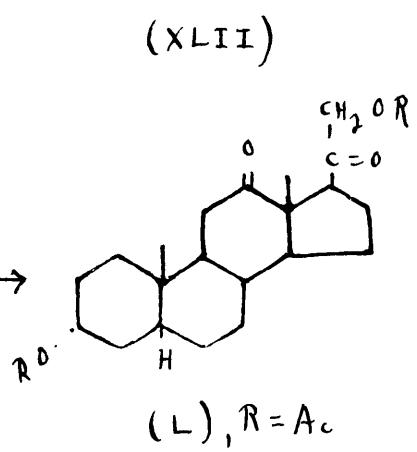
(XLVIII)



(XLIII)

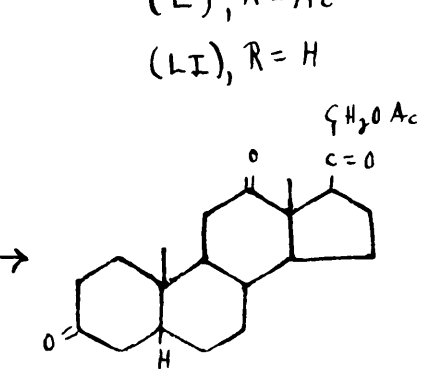


(XLII)

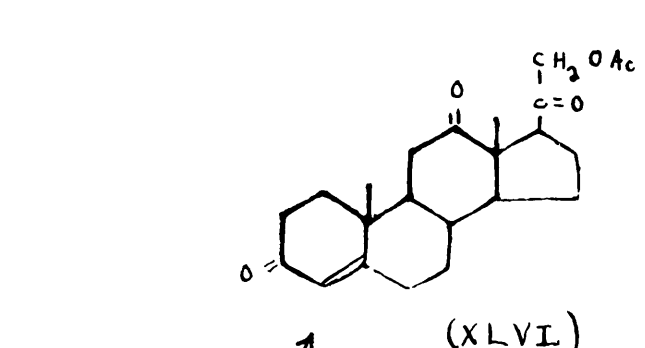


(L), R = Ac

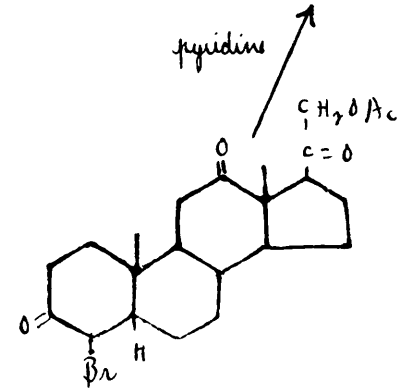
(LI), R = H



(XLIV)



(XLVI)



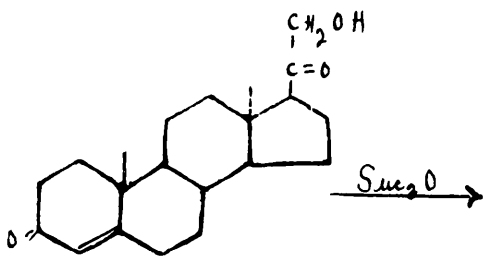
(XLV)

pyridine

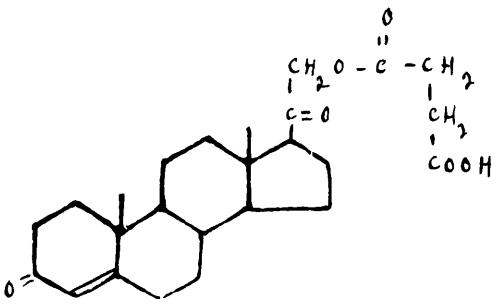
CrO_3

$Pb(OAc)_4$

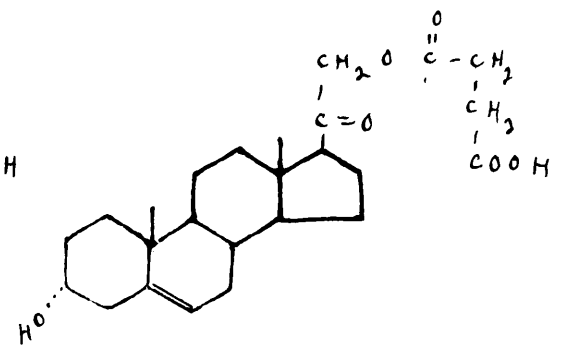
Br_2



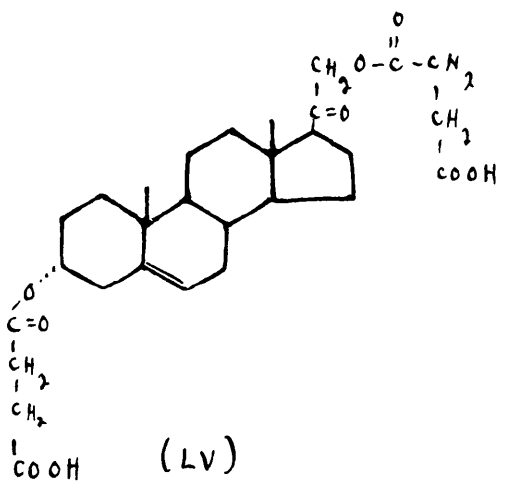
(LII)



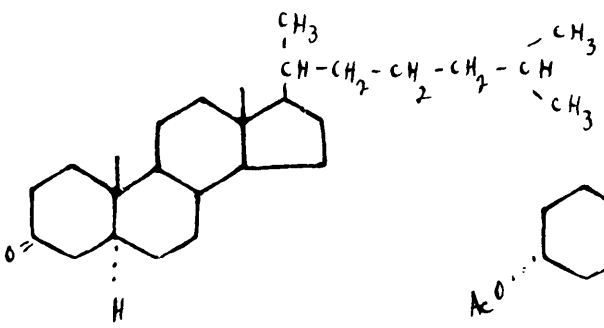
(LIII)



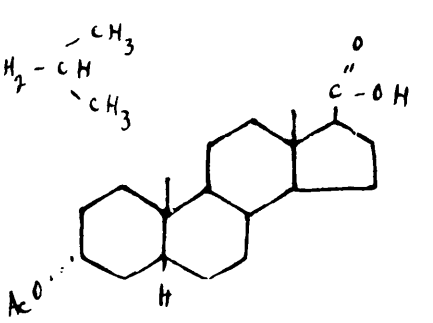
(LIV)



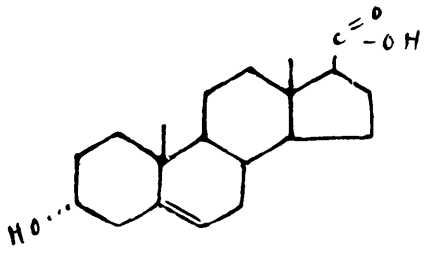
(LV)



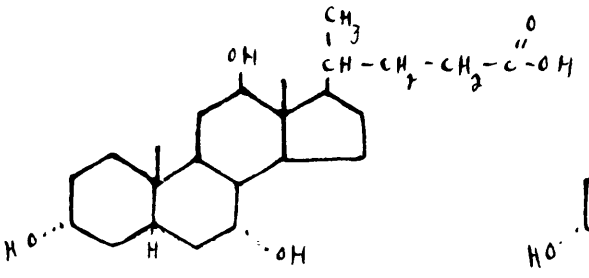
(LVI)



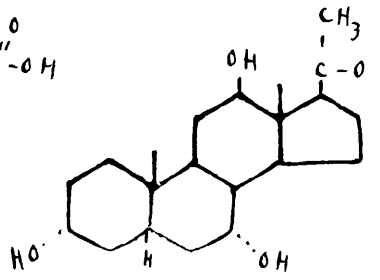
(LVII)



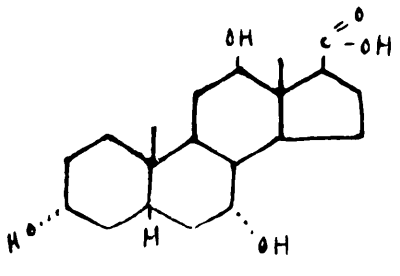
(LVIII)



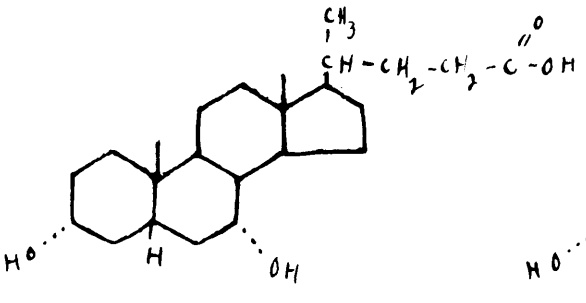
(LIX)



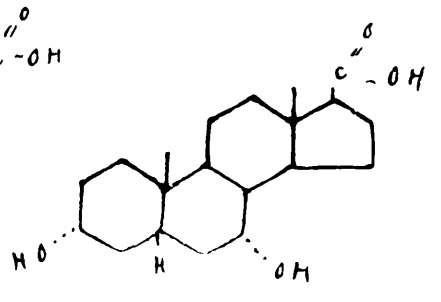
(LX)



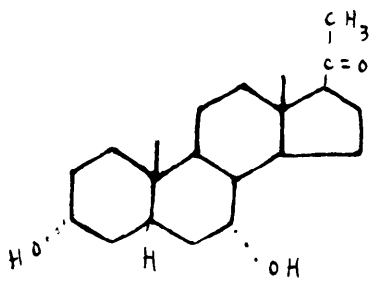
(LXI)



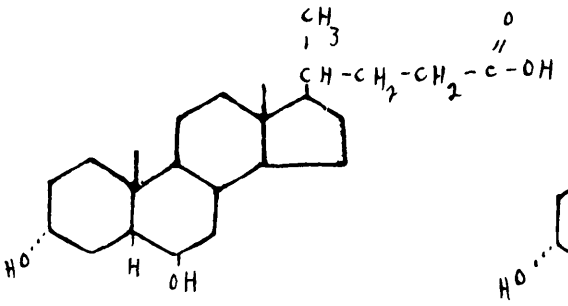
(LXII)



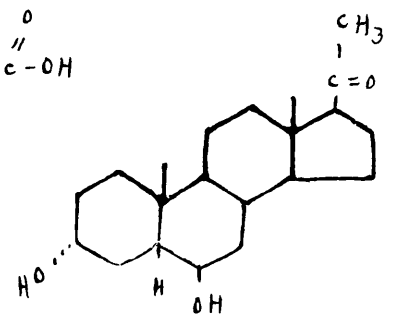
(LXIII)



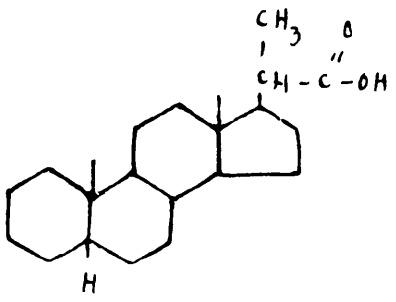
(LXIV)



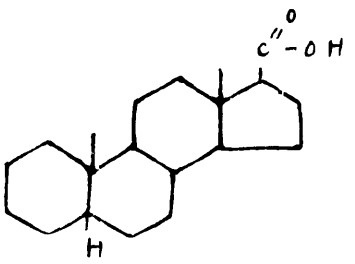
(LXV)



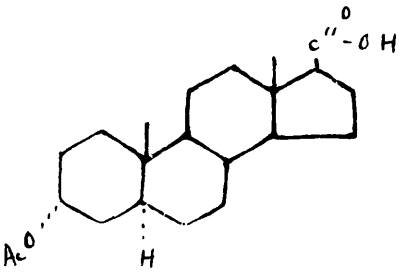
(LXVI)



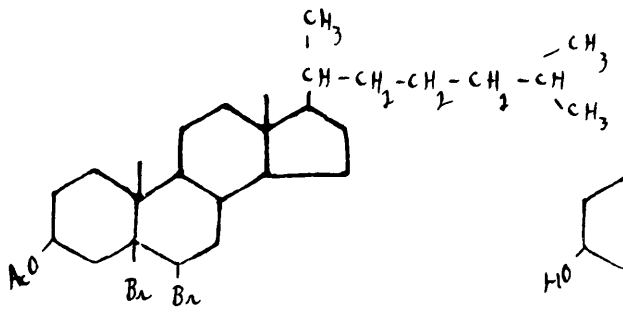
(LXVII)



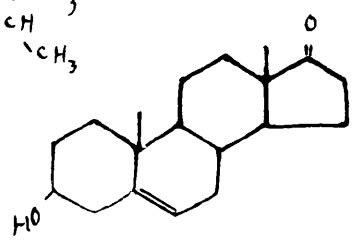
(LXVIII)



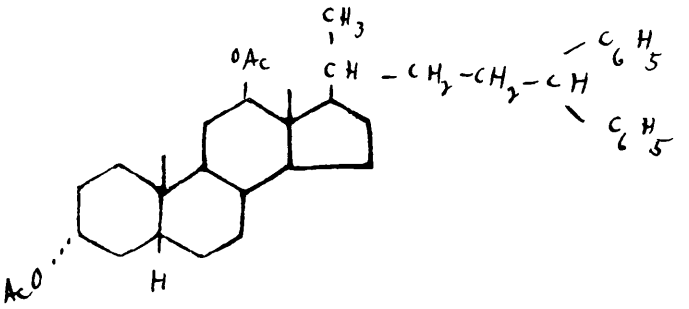
(LXIX)



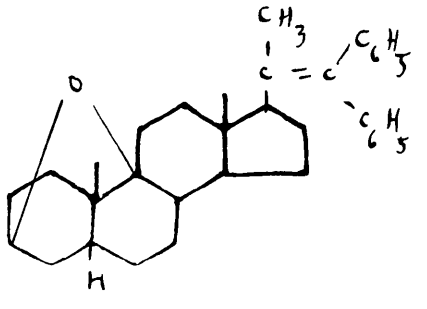
(LXX)



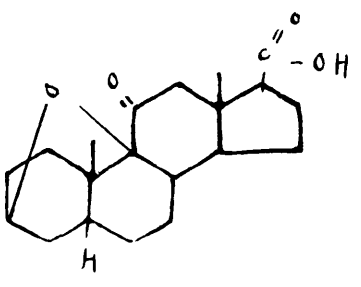
(LXXI)



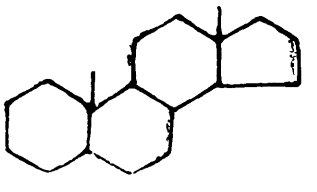
(LXXII)

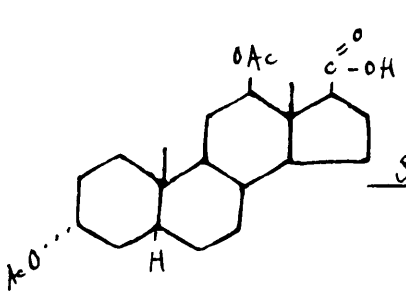


(LXXIII)



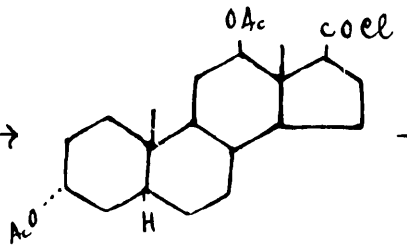
(LXXIV)





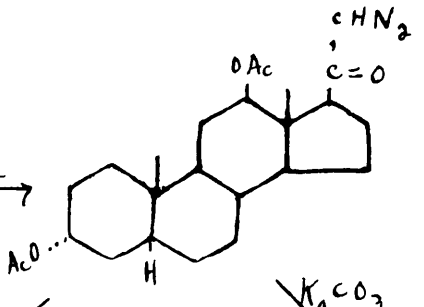
(XXIII)

$SOCl_2$



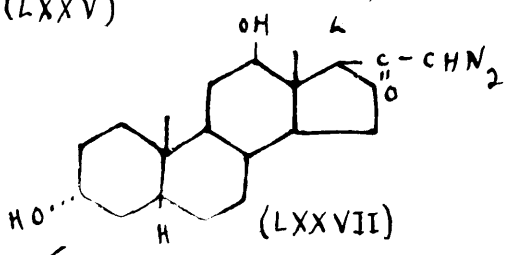
(LXXV)

CH_2N_2



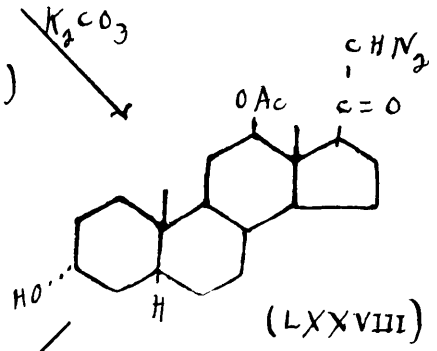
(LXXVI)

KOH



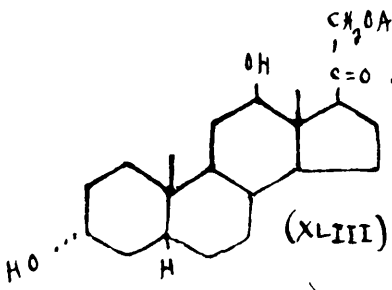
(LXXVII)

K_2CO_3



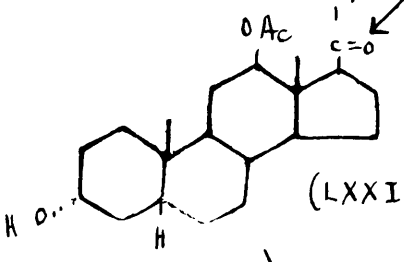
(LXXVIII)

CH_3COOH



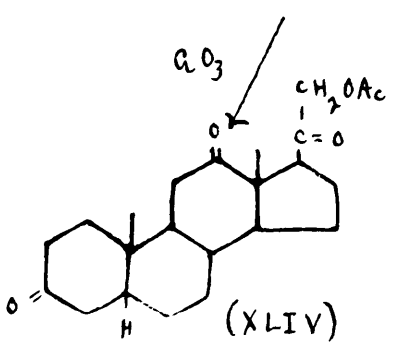
(XLIII)

CH_3COOH



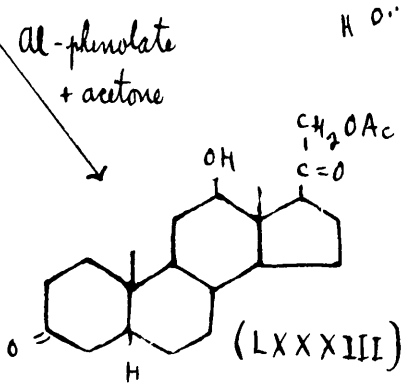
(LXXIX)

CrO_3



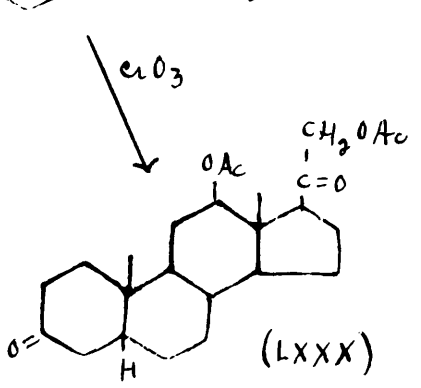
(XLIV)

Al-phenolate + acetone



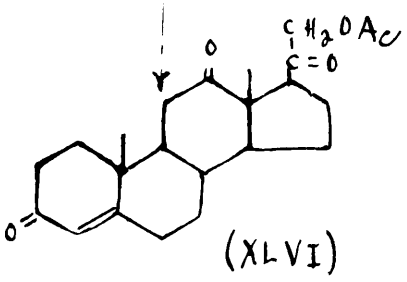
(LXXXIII)

CrO_3



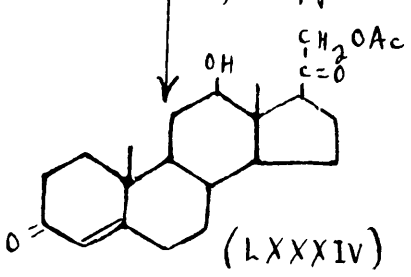
(LXXX)

Br_2, then pyridine



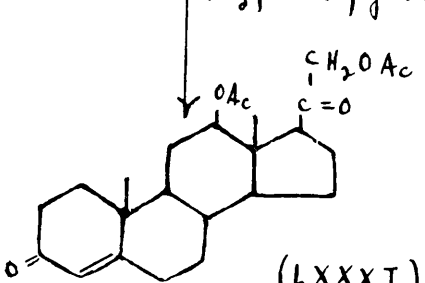
(XLVI)

Br_2, then pyridine



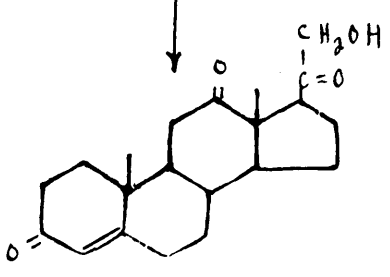
(LXXXIV)

Br_2, then pyridine



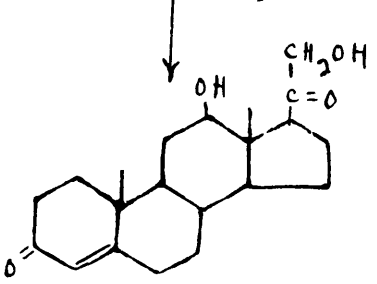
(LXXXI)

$KHCO_3$



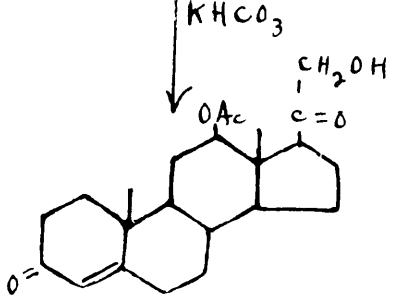
(LXXXVI)

$KHCO_3$

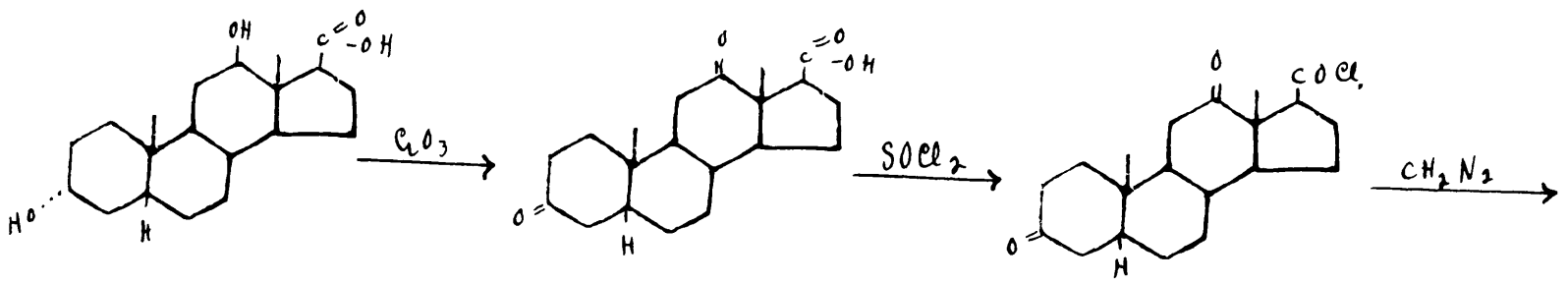


(LXXXV)

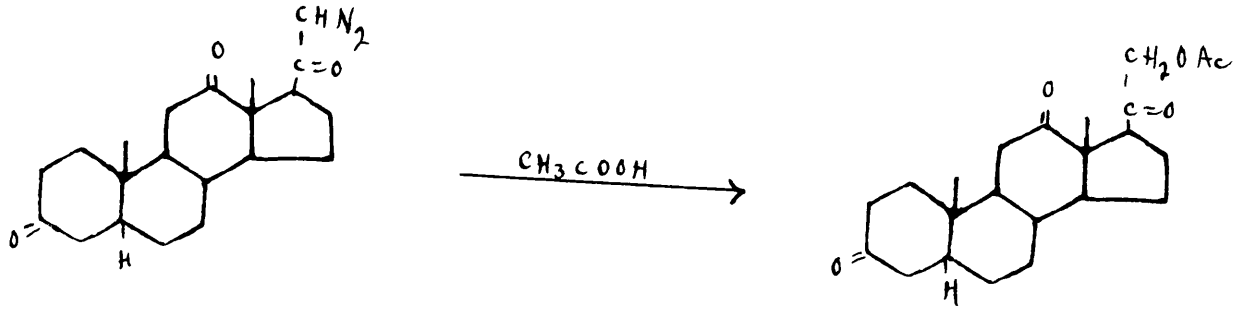
$KHCO_3$



(LXXXII)



(XXIV) (XXVII) (LXXXVII)



(LXXXVIII) (XLIV)