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SOME REACTIONS IN THE D-RING OF THE STEROIDS

Thesis presented to
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by

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TO THE MEMORY OF MY DEAR PARENTS

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CONTENTS

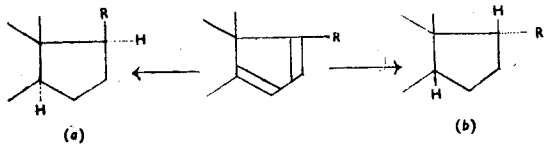
GENERAL INTRODUCTION

The physiological activity of the steroids depends on the various substituents having the " natural " configuration at different asymmetric centres in the cyclopentanoperhydrophenanthrene skeleton. Keeping the rest of the molecule intact, the activity is remarkably changed by altering the configuration at C_{14} and C_{17} in the D-ring of the steroids.

In the case of naturally occurring compounds like testosterone, progesterone and corticosterone, the rings C and D are in the trans position and the substituents, the hydrogen atom and the side chain, respectively at C_{14} and C_{17} are in α—and β — positions. An unique exception to this type of configuration are the cardiac aglycones, where the rings C and D are in the cis position, and the substituents at C_{14} and C_{17} are both in the β — position[1].

Compounds with the inverted 17-iso-arrangement like 17-epi-testosterone[2], 17-isoprogesterone[3], 17-isodesoxycorticosterone[4] and the " alloglycoside "[5] have been found physiologically inactive.

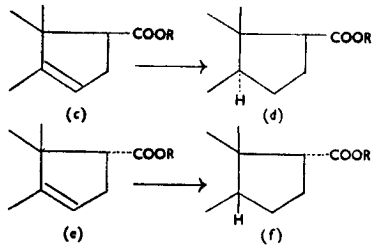
Plattner and Ruzicka[6] have obtained different isomers in the D-ring at C_{14} and C_{17} by the hydrogenation of $\Delta^{14,16}$—doubly unsaturated compounds.



In the case (*a*) hydrogen has attached itself to the two double bonds from behind the plane of the paper, and in the case (*b*) from the front of the plane of the paper.

(1) *L. F. Fieser* and *M. Fieser*, Natural Products Related to Phenanthrene, 3rd Ed., Reinhold, New York, 1949, page 516.
(2) *L. Ruzicka* and *H. Kagi*, Helv. Chim. Acta **19**, 842 (1936).
(3) *A. Butenandt, J. Schmidt-Thome* and *H. Paul*, Ber. **72**, 1112 (1939).
(4) *C. W. Shoppee*, Helv. Chim. Acta **23**, 925 (1940).
(5) *W. A. Jacobs*, J. Biol. Chem. **88**, 519 (1930); *W. A. Jacobs* and *N. M. Bigelow*, ibid. **99**, 521 (1933); *I. D. Lamb* and *S. Smith*, J. Chem. Soc. 442 (1936); *A. Katz* and *T. Reichstein*, Helv. Pharm. Acta **19**, 231 (1944).
(6) *Pl. A. Plattner, L. Ruzicka, H. Heusser, J. Pataki* and *Kd. Meier*, Helv. Chim. Acta **29**, 942 (1946).

With the hydrogenation of Δ^{14}-3β-acetoxy-5-alloetiocholenic acid methyl ester (*c*)[6] and Δ^{14}-3β-acetoxy-5-allo-17-isoetiocholenic acid methyl ester (*e*)[6] it was found that the "normal" 17-compound (*c*) gave, at C_{14} the normal product (*d*) having the rings C and D in the trans position, whereas the 17-iso-compound (*e*) yielded a 14-allo-compound (*f*).



It appears from this evidence that the steric course of hydrogenation is determined to a very great extent by the steric position of the substituent at C_{17}.

Several other modifications have been made at the 17-position in the D-ring of steroids. Amongst the important are the 17-methyl derivatives—17-methyltestosterone[7], 17-methylprogesterone[8] and the D-homosteroids.[9]

The present work which is divided into three parts mainly concerns itself with some reactions which were carried out in the D-ring of steroids.

Part I is concerned with the introduction of a 17-methyl group by the Faworsky re-arrangement of α-halogeno-ketosteroids.

Part II deals with (*a*) base catalysed reaction with Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester and (*b*) with base and acid catalysed reactions with Δ^{16}-3β-acetoxy-14, 15β-epoxy-20-keto-5-allopregnene.

Part III describes the introduction of the 17α-hydroxy group in the steroid nucleus by the reduction of a 16,17α-epoxide with lithium aluminium hydride.

(7) *L. Ruzicka, M. W. Goldberg* and *H. R. Rosenberg*, Helv. Chim. Acta **18**, 1487 (1935); *K. Fujii* and *T. Mataukawa*, J. Pharm. Soc. Japan, **55**, 1333 (1935); *G. I. Kiprianov* and *B. E. Frenkel*, J. Gen. Chem. (U.S.S.R.) **9**, 1682 (1939) or C.A. **34**, 3756 (1940).

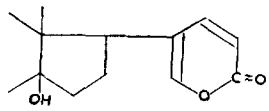
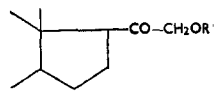
(8) *Pl. A. Plattner, H. Heusser* and *P. Th. Herzig*, Helv. Chim. Acta **32**, 270 (1949).

(9) *H. Heusser, P. Th. Herzig, A. Furst* and *Pl. A. Plattner*, Helv. Chim. Acta **33**, 1093 (1950); *M. W. Goldberg* and *E. Wydler*, ibid. **26**, 1142 (1943); *M. W. Goldberg, J. Sicé, H. Robert*, and *Pl. A. Plattner*, ibid. **30**, 1441 (1947).

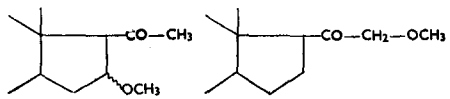
PART I

REARRANGEMENT OF α-HALOGENO-20-KETOSTEROIDS

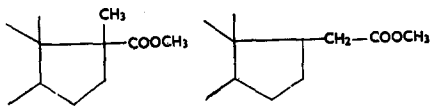
Theoretical Part

For building up the doubly unsaturated six membered lactone ring of toad venoms,  and of the aglycone of the Scilla maritima glycoside[1], it is convenient to have as the starting material, ketolethers of the type  . There seemed a possibility of preparing them from the 20-keto-21-halogenosteroids by a reaction with alcoholates.

In the present investigation the action of potassium methoxide on 21-bromo and 21-chloro pregnenolone[2] was studied. The crude reaction product after acetylation gave a crystalline compound with the desired empirical formula $C_{24}H_{34}O_4$. The Zeisel estimation showed the presence of one methoxyl group. But this compound did not show any characteristic absorption in the U.V.—spectrum for a keto group ; it also did not react with either hydroxylamine or semicarbazide. This clear absence of a keto group ruled out the possibility of the following formulae



The elimination of the above formulae of a 20-ketosteroid left the third possibility of a rearrangement to a 17-methyletiocholenic acid methyl ester (*a*) or to a pregnene-21-acid ester (*b*).



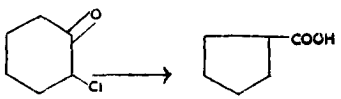
(1) *L. F. Fieser* and *M. Fieser*, Natural Products Related to Phenanthrene, 3rd Ed., Reinhold, New York, 1949, page 552.

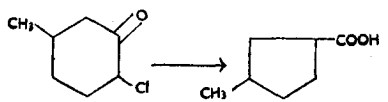
(2) Cf. *T. Reichstein* and *W. Schindler*, Helv. Chim. Acta **23**, 669 (1940); *T. Reichstein* and *H. G. Fuchs*, ibid. **23**, 658 (1940); *H. Reich* and *T. Reichstein*, ibid. **22**, 1128 (1939).

The possibility of these structures is brought out rather well by the presence of a methoxyl group and the absence of a keto group. By the saponification, with 5% methanolic potassium hydroxide, only the acetyl group in the three position was hydrolysed. The constitution (*b*) of pregnene-21-acid methyl ester as a rearrangement product could therefore be eliminated. If a rearrangement of the steroid nucleus is also eliminated, then there remains as a last possibility the structure (*a*) of a strongly sterically hindered tertiary ester as a reaction product of 21-halopregnenolone with potassium methoxide. This constitution is brought out by the following reactions.

The free acid (VIII) is obtained only when the compound is hydrolysed at 160° C. in a steel bomb, or by refluxing it for two days with 10% alcoholic potassium hydroxide. This acid can be reconverted to the original ester with diazomethane.

This interesting rearrangement with alkali hydroxide and with alkali methoxide on α-halo ketones was first observed by Faworsky and Boschowsky[3] who obtained by the action of alcoholic potassium hydroxide, cyclopentanecarboxylic acid and γ-methylcyclopentanecarboxylic acid from α-monochlorocyclohexanone and δ-methyl-α-monochlorocyclohexanone respectively.





Much later Aston and Greenburg[4] further investigated this rearrangement with 3-bromo-3-methyl-2-butanone with either potassium or sodium methoxide, and found that one of the products was methyl trimethyl acetate, and according to them the reaction was supposed to proceed by the following route.

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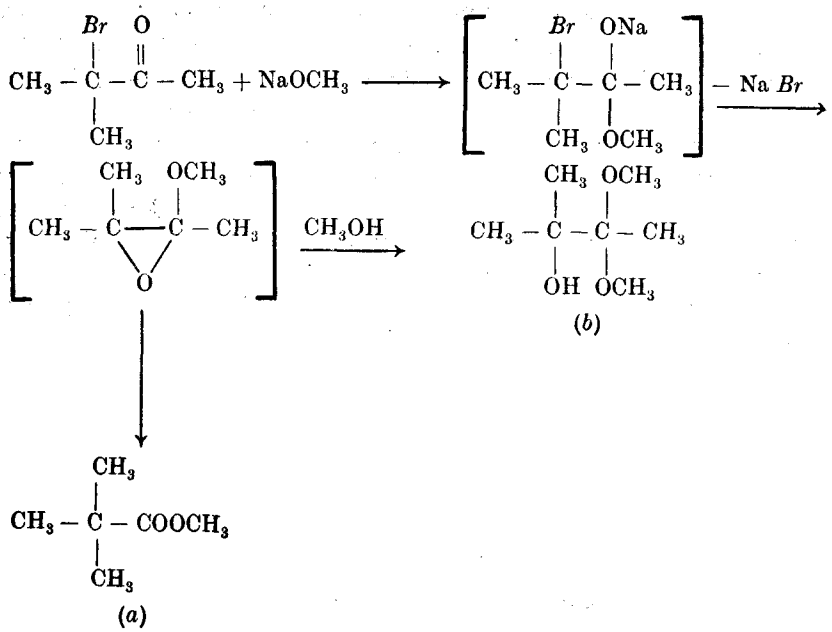
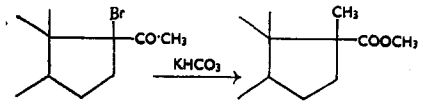
(3) *A. Faworsky* and *W. Boskowsky*,)+(. **46**, 1097 (1914); C. 1915, I, 984,
(4) *J. G. Aston* and *R. B. Greenburg*, J. Am. Chem. Soc. **62**, 2590 (1940).



In the above postulate, the intermediate ethylene oxide rearranges itself to the tertiary acid (*a*), but when there is an excess of methanol the hydroxyacetal (*b*) is also formed.

Following up this work of Aston and Greenburg, Marker and Wagner[5] applied this rearrangement in the steroid series. They found that 17-bromopregnan-3β-ol-20-one, when refluxed with aqueous methanolic potassium bicarbonate, underwent a Faworsky or an Aston-Greenburg rearrangement to give the methyl ester of 3β-hydroxy-17-methyletiocholanic acid.



This substance is the main product of the reaction, and no second substance was isolated.

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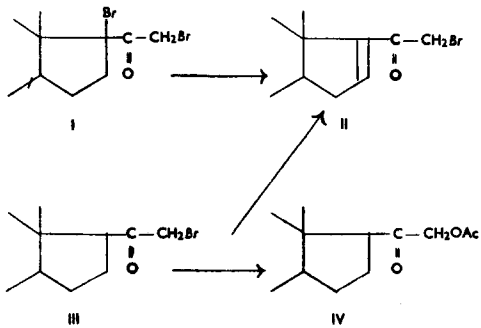
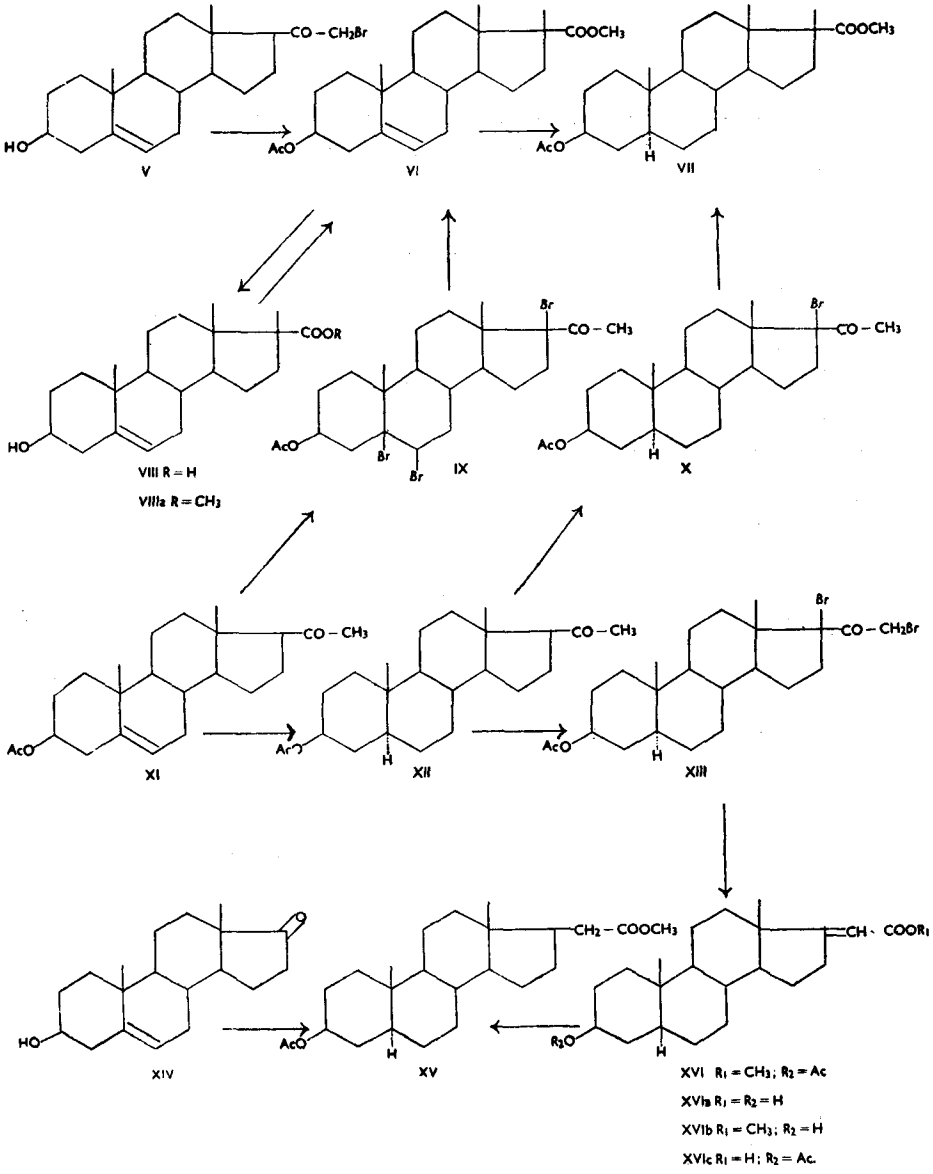
(5) *R. E. Marker* and *R. B. Wagner*, J. Am. Chem. Soc. **64**, 216 (1942).

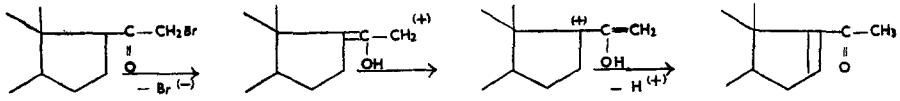
There is, however, a difference in the behaviour of 17-halo-20-ketopregnane (I) type of compounds and its isomeric 21-halo-20-ketopregnane (III) towards potassium acetate in acetic acid. Marker, Crooks and Wagner[7] obtained from 17,21-dihalo compound (I) by this treatment almost only the α,β-unsaturated product (II). But, when they treated in a similar manner the 21-haloketone (III), they got as the main product a ketolacetate (IV) and an α,β-unsaturated ketone (II) as a by-product. It is quite possible that a similar by-product was present in the conversion of the 21-chloro to the 21-acetoxy by Reichstein and Fuchs[6], and therefore chromatography was necessary for the separation of the pure product.



(6) *T. Reichstein* and *H. G. Fuchs*, Helv. Chim. Acta **23**, 658 (1940).



This rearrangement of III to II has been explained by Marker[7] on the basis of allylic rearrangement according to the following scheme :



(7) *R. E. Marker, M. Crooks Jr.* and *R. B. Wagner*, J. Am. Chem. Soc. **64**, 13 (1942).

The wandering of the substituent as well as the double bond from the α-position into α'-position over the keto group has been already observed in the steroid series.[8]

As a final proof of the constitution, the compound (VI) was synthesised by another way from pregnenolone acetate (XI) through the 5,6,17-tribromide (IX). By the method of Marker[5] the substance (IX) gave through the 17-bromoketone the tertiary unsaturated ester (VI) with potassium bicarbonate in methanol (with the simultaneous presence of potassium iodide to remove the bromine from the 5,6 position). So the 21-halo-20-ketosteroid (V) gave under suitable conditions the same 17-methyletioacid ester (VI) as the 17-halo-20-ketosteroid (IX).

In addition it was attempted to connect Δ^5-3β-acetoxy-17-methyletiocholenic acid methyl ester (VI) through hydrogenation with the known 3β-acetoxy-17-methyl-5 alloetiocholanic acid methyl ester m.p. 202° C. of Koechlin and Reichstein[9]. However, this hydrogenation product melted at 125° C. There still remained the possibility that this hydrogenation product as well as (VI) were different from the described compound because of the stereoisomerism at C_{17} or at C_5. Therefore (VII) was prepared from allopregnanolone acetate. The 17-monobromide (X) which contained about 10% of 17,21-dibromide was subjected without purification to an Aston-Greenburg rearrangement according to the method of Marker[5]. The rearrangement yielded about 84% of a neutral product, which was acetylated, and then separated by chromatography. 3β-Acetoxy-17-methyl-5-alloetiocholanic acid methyl ester m.p. 125° C. was obtained as the main product, which agreed with the hydrogenation product of (VI). Another compound with the empirical formula $C_{24}H_{36}O_4$ was isolated from the chromatogram in a smaller yield (about 10%). Now this compound was found to be identical with the sample of the substance of Koechlin and Reichstein of the assumed empirical formula $C_{24}H_{38}O_4$ m.p. 202° C.[9]. The U.V.-spectrum of this compound shows a maximum at 224 mμ(log $\epsilon = 4.22$). As will be seen from later evidence the

(8) Cf. *L. Ruzicka, Pl. A. Plattner* and *R. Aeschbacher*, Helv. Chim. Acta **21** 866 (1938); *L. Ruzicka, Pl. A. Plattner* and *M. Furrer*, ibid. **27**, 727 (1944).

(9) *B. Koechlin* and *T. Reichstein*, Helv. Chim. Acta **27**, 549 (1944).

compound was $\Delta^{17,20}$-3β-acetoxy-5-allopregnene-21-acid methyl ester. This constitution was established by the preparation of different derivatives XVI (*a*), (*b*), (*c*), and by hydrogenation to 3β-acetoxy-5-allopregnane-21-acid methyl ester (XV) which has already been prepared by Plattner and Schreck[10] from dehydro-epiandrostenone-(17) (XIV). The formation of $\Delta^{17,20}$- 3β-acetoxy-5-allopregnene-21-acid methyl ester (XVI) is easily explained by the Faworsky rearrangement[3] of α, α' dibromoketone (XIII) which, as already mentioned, was present in the monobromide employed for the reaction.

(10) *Pl. A. Plattner* and *W. Schreck*, Helv. Chim. Acta 22, 1178 (1939).

Experimental Part*

Δ^5-3β-Acetoxy-17-methyletiocholenic acid methyl ester (VI).

(a) From 21-chloro-pregnenolone (cf. V).

1 g. of 21-chloro-pregnenolone[11] was introduced into a solution of 1.5 g. of potassium in 30 cc. of methanol. The reaction mixture was allowed to stand for 15 minutes at room temperature and then refluxed for 30 minutes on a water bath. The solution was cooled with ice, dilute sulphuric acid was added with stirring, and the methyl ester was taken up in ether. The ether extract was washed with water, then with sodium bicarbonate solution, and again with water, lastly it was dried and evaporated. The amorphous substance was acetylated with 10 cc. of pyridine and 10 cc. of acetic anhydride by keeping it at room temperature for 12 hours. Finally, the reaction mixture was evaporated to dryness, and the residue was chromatographed on 20 g. of aluminium oxide (activity II). The petrol ether-benzene fraction (1.070 g.) gave after crystallization from methanol-water, 0.930 g. of compound (VI) in fine plates melting at 149-156° C. For analysis a sample was crystallized four times and dried under high vacuum at 70° C for 12 hours, m.p. 162-163° C.

$$[\alpha]_D^{17} = -60.4° \text{ (C = 0.531 in chloroform)}$$

3.658 ; 3.756 mg. of the substance gave 9.938 ; 10.207 mg. CO_2 and 3.080 ; 3.130 mg. H_2O

4.963 mg. of the substance required 3.747 cc. 0.02N $Na_2S_2O_3$ solution.

Calculated for

$C_{24}H_{36}O_4$	C 74.19 %	H 9.34 %	OCH_3 7.99 %
Found	C 74.15 %	H 9.42 %	OCH_3 7.81 %
	C 74.16 %	H 9.32 %	

The substance showed no absorption in the U.V.-spectrum and did not react with hydroxylamine or semicarbazide.

*All m.p. are corrected and determined in evacuated capillaries.

(11) Cf. *H. Reich* and *T. Reichstein*, Helv. Chim. Acta **22**, 1128 (1939); *M. Steiger* and *T. Reichstein*, ibid. **20**, 1165 (1937).

(b) From 3β-acetoxy-5,6,17-tribromo-20-ketopregnane (IX.)

(Aston-Greenburg rearrangement)

2.9 g. of the crude tribromide (IX) described on p. 14 was refluxed in 250 cc. of methanol with 5 g. of potassium bicarbonate and 1.6 g. of potassium iodide. After the separation of iodine in the beginning, the solution was again colourless after 10 minutes. The reaction mixture was refluxed for two hours in all, then concentrated under vacuum and acidified with dilute sulphuric acid. Traces of acidic portion in the amorphous residue were methylated in the usual manner with an ether solution of diazomethane. Since the reaction mixture still contained bromine, it was dissolved in 50 cc. of acetic acid with 5 g. of zinc dust and debrominated on a water bath for 18 minutes. After filtering the zinc dust, the filtrate was concentrated under vacuum, water and ether added to it and the ethereal layer well washed with sodium bicarbonate solution and water, then dried and evaporated. The residue was acetylated with a mixture of 10 cc. of pyridine and 10 cc. of acetic anhydride in the usual manner, and the acetylated product was purified by chromatography on 50 g. of aluminium oxide.

The petrol ether—benzene fraction (0.688 g.) yielded after four crystallizations from methanol 0.149 g. of compound (VI) in large plates, which melted at 159—161° C. and the mixed melting point with the above described 17-methyletioacid methyl ester (VI) showed no depression. For analysis, the crystals were once again crystallized from acetone—methanol, and dried under high vacuum at 100° C. for 12 hours.

3.598 mg. of the substance gave 9.777 mg. CO_2 and 2.993 mg. H_2O

Calculated for $C_{24}H_{36}O_4$	C 74.19 %	H 9.34 %
Found	C 74.15 %	H 9.31 %

(c) From Δ^5-3β-hydroxy-17-methyletiocholenic acid methyl ester (VIIIa).

0.045 g. of the 3-hydroxymethyl ester (VIIIa) described below was heated together with a mixture of 3 cc. of pyridine and 3 cc. of acetic anhydride for 2 hours over a water bath. The reaction mixture was evaporated to dryness under vacuum. Crystallization of the residue from methanol gave 0.0428 g. of large plates

which melted at 162-163° C. The mixed melting point with the above described preparations of (VI) showed no depression. The substance was crystallised once again for analysis and sublimed under high vacuum at 156° C., m.p. 163-164° C.

3.418 mg. of the substance gave 9.296 mg. CO_2 and 2.868 mg. H_2O

Calculated for $C_{24}H_{36}O_4$	C 74.19 %	H 9.34 %
Found	C 74.23 %	H 9.40 %

Δ^5-3β-Hydroxy-17-methyletiocholenic acid (VIII).

0.4 g. of Δ^5-3β-acetoxy-17-methyletiocholenic acid methyl ester (VI) was heated in a solution of 1 g. of potassium hydroxide and 10 cc. of methanol for two hours in a steel bomb to 160° C. The solution was concentrated under vacuum, finally acidified with dilute hydrochloric acid and extracted with ether. The acidic constituents were removed from the ether solution with 2N sodium hydroxide. The neutral part (0.027 g.) in ether was not investigated any further. The acidic portion in the sodium hydroxide solution was precipitated by the addition of dilute hydrochloric acid; finally taken up in ether and then worked up as usual. The crude product gave on crystallization from ethanol 0.3 g. of fine needles of the hydroxy acid (VIII) which melted at 230-238° C. For analysis a sample was crystallized till it gave a constant melting point of 245-249° C. and finally it was dried under high vacuum at 100° C. for 40 hours.

$[\alpha]_D^{17} = -50.7°$ (C = 0.414 in ethanol)

3.663 mg. of the substance gave 10.175 mg. CO_2 and 3.232 mg. H_2O

Calculated for $C_{21}H_{32}O_3$	C 75.86 %	H 9.70 %
Found	C 75.81 %	H 9.87 %

After refluxing for 2 days with 10% alcoholic potassium hydroxide, only 70% of the ester group was saponified, and the accumulation of the crude product made its purification rather difficult. (*Cf.* with the analogous saponification by R. E. Marker and R. B. Wagner[5] of 3β-acetoxy-17-methyletiocholanic acid methyl ester).

Anhydride acetate. 0.089 gm. of Δ^5-3β-hydroxy-17-methyletiocholenic acid (VIII) was allowed to stand in a mixture of 0.5 cc. of pyridine and 1 cc. of acetic anhydride for 12 hours, and finally

evaporated to dryness at 80° C. under vacuum. The crystalline residue after washing with a small amount of ether melted at 270-286° C. A sample for analysis was prepared after four crystallizations from ethyl acetate, and finally dried under high vacuum at 100° C. for 12 hours. The neutral substance crystallized in fine needles, which melted at 304-305° C.

3.204 mg. of the substance gave 8.840 mg. CO_2 and 2.632 mg. H_2O

Calculated for $C_{46}H_{66}O_7$	C 75.57 %	H 9.10 %
Found	C 75.29 %	H 9.19 %

Δ^5-3β-hydroxy-17-methyletiocholenic acid methyl ester (VIIIa).

(a) Through partial saponification of the acetyl methyl ester (VI.)

0.500 g. of Δ^5-3β-acetoxy-17-methyletiocholenic acid methyl ester (VI) was warmed in a solution of 80 cc. of methanol, 10 cc. of water, and 10 g. of potassium hydroxide for 2 hours on a water bath. On working up the product in the usual manner it gave 0.440 g. of a neutral portion and 0.040 g. of an acidic portion which was not investigated any further. The neutral portion was crystallized three times from methanol—water and gave 0.370 g. of the hydroxy ester in well-formed plates, which melted at 143-145° C. The mixed melting point with a lower described preparation showed no depression.

(b) Through methylation of Δ^5-3β-hydroxy-17-methyletiocholenic acid (VIII).

0.250 g. of Δ^5-3β-hydroxy-17-methyletiocholenic acid (VIII) was dissolved in absolute dioxane, an excess of an ether solution of diazomethane was added, and the mixture was left in the cold for 3 days. The evolution of nitrogen was very slow. Finally the reaction mixture was evaporated to dryness under vacuum, and the crystalline residue was crystallized once again from benzene-petrol ether, then three times from methanol and finally sublimed under high vacuum at 135° C., m.p. 144-145° C.

$[\alpha]_D^{17} = -53.2°$ (C = 1.012 in chloroform)

3.719 mg. of the substance gave 10.380 mg. CO_2 and 3.296 mg. H_2O

Calculated for $C_{22}H_{34}O_3$	C 76.26 %	H 9.89 %
Found	C 76.17 %	H 9.92 %

3β-Acetoxy-5,6,17-tribromo-20-ketopregnane (IX).

2.5 g. of pregnenolone acetate (XI) was dissolved in 50 cc. of acetic acid; to it was added drop by drop 7.25 cc. of an acetic acid solution containing 1.12 g. of bromine (= 2 equivalents). Finally a few granules of anhydrous aluminium chloride and 2 drops of a 48% hydrobromic acid solution in acetic acid were added and a further 7.98 cc. (= 1.22 g. Br_2 corresponding to 2.2 equivalents bromine) of bromine solution was run in drop by drop (duration 1 hour). The reaction mixture was stirred into 500 cc. of water, the tribromide (IX) which separated was filtered, well washed with water, and dissolved in ether—chloroform. The solution was once again washed with water, dried, and evaporated under vacuum. The crystallized tribromide (IX) (4.2 g.) was used for reaction without purification. A sample was crystallized once from chloroform—benzene and twice from acetone—hexane; it melted at 149-151° C. under decomposition.

3β-Acetoxy-17-methylalloetiocholanic acid methyl ester (VII).

0.072 g. of Δ^5-3β-acetoxy-17-methyletiocholenic acid methyl ester (VI) was hydrogenated in 5 cc. of acetic acid, using 0.0218 g. of platinum oxide (Adams), which was prehydrogenated. After 75 minutes the required 1 mole of hydrogen was absorbed, whereupon the catalyst was filtered off, and the filtrate evaporated to dryness under vacuum. The residue (0.071 g.) after crystallization from methanol gave 0.031 g. of fine flakes which melted at 118-120° C. For analysis the substance was recrystallized twice from methanol and finally dried under high vacuum for 12 hours, m.p. 124-125° C. This product was identical with the later described compound obtained from the 17-monobromide (X) by Aston-Greenburg rearrangement.

$$[\alpha]_D^{17} = +5° \qquad (C = 0.614 \text{ in chloroform})$$

3.596 mg. of the substance gave 9.723 mg. CO_2 and 3.161 mg. H_2O

Calculated for $C_{24}H_{38}O_4$	C 73.80 %	H 9.81 %
Found	C 73.79 %	H 9.84 %

Aston-Greenburg rearrangement of 3β-acetoxy-17-bromo-20-keto-allopregnane (X).

1.215 g. of 3β-acetoxy-17-bromo-20-keto-allopregnane (X)[12] containing about 10 % dibromide was refluxed on a water bath with a solution of 51 cc. of methanol, 9.7 cc. of water and 2.43 g. of potassium bicarbonate for 3 hours. Finally the major part of methanol was evaporated under vacuum, the reaction mixture was poured with stirring into dilute sulphuric acid, and the watery suspension extracted with ether. The usual separation gave 0.793 g. of a neutral and 0.141 g. of an acidic portion.

Working up the neutral portion.

(a) 3β-Acetoxy-17-methylalloetiocholanic acid methyl ester (VII).

The neutral compound was heated on a water bath with a mixture of 5 cc. of pyridine and 5 cc. of acetic anhydride. The reaction mixture was evaporated to dryness under vacuum, and the partially crystalline substance was chromatographed on 24 g. of aluminium oxide. The petrol ether—benzene fraction (0.697 g.) after crystallization from methanol gave 0.349 g. of 3β-acetoxy-17-methyl ester (VII) in the form of fine flakes which melted at 120-121° C. After two more crystallizations from methanol, the melting point remained at 123-124° C. This compound was proved identical with the above mentioned methyl ester (VII) which was prepared by the hydrogenation of the unsaturated ester (VI).

(b) $\Delta^{17,20}$-3β-Acetoxy-allopregnene-21-acid methyl ester (XVI).

The benzene fraction (0.092 g.) of the above mentioned chromatogram melted at 177-183° C. After three crystallizations from acetone—methanol the melting point remained constant at 193-194° C. The substance was dried under high vacuum for 12 hours at 110° C. for analysis.

$[\alpha]_D^{17} = +14.7°$; $+13.1°$ (C = 0.757 ; 0.861 in chloroform)

3.711 mg. of the substance gave 10.071 mg. CO_2 and 3.048 mg. H_2O

Calculated for $C_{24}H_{36}O_4$	C 74.19 %	H 9.34 %
Found	C 74.06 %	H 9.19 %

(12) *R. E. Marker, H. M. Crooks Jr., R. B. Wagner* and *E. L. Wittbecker*, J. Am. Chem. Soc. **64**, 2089 (1942) ; *Pl. A. Plattner, L. Ruzicka, H. Heusser* and *E. Angliker* Helv. Chim. Acta **30**, 386 (1947).

In the U.V.-absorption spectrum in alcohol the substance showed a maximum at 224 mμ (log ε = 4.22). That the substance was $\Delta^{17,20}$-3β-acetoxy-allopregnene-21-acid methyl ester, was proved by the preparation of different derivatives and by hydrogenation (ref. below).

Hydroxy acid-XVIa[13,14]. m.p. 247-289° C.

$[\alpha]_D^{17} = +25.8°$ (C = 0.450 in alcohol)

3.688 mg. of the substance gave 10.244 mg. CO_2 and 3.205 mg. H_2O

Calculated for $C_{21}H_{32}O_3$	C 75.86 %	H 9.70 %
Found	C 75.80 %	H 9.72 %

Hydroxy acid methyl ester-XVIb[14]. m.p. 174-176° C.

Acetoxy acid-XVIc. m.p. 234-236° C.

$[\alpha]_D^{17} = +17.4°$ (C = 0.748 in chloroform)

3.754 mg. of the substance gave 10.123 mg. CO_2 and 3.071 mg. H_2O

Calculated for $C_{23}H_{34}O_4$	C 73.76 %	H 9.15 %
Found	C 73.59 %	H 9.15 %

The mixed melting point of acetoxy methyl ester (XVI) with the already mentioned compound of B. Koechlin and T. Reichstein[9] of m.p. 192-193° C and of the assumed constitution (VII) melted at 192-194° C. Therefore both the compounds are identical and have the constitution (XVI).

Working up of the acidic portion.

0.141 g. of the acidic portion of the above described rearrangement was dissolved in some dioxane and methylated with an ether solution of diazomethane. Finally it was acetylated in a mixture of 5 cc. of acetic anhydride and 5 cc. of pyridine. The partially crystalline crude product (0.145 g.) was chromatographed on 5 g. of aluminium oxide.

(13) *R. E. Marker, H. M. Crooks Jr., R. B. Wagner* and *E. K. Wittbecker* J. Am. Chem. Soc. **64**, 2089 (1942).

(14) Cf. *E. Angliker*, Diss. E.T.H. Zurich 1948.

(a) 3β-Acetoxy-17-methylalloetiocholanic acid methyl ester (VII).

The petrol ether-benzene eluate (0.0745 g.) was crystallized and gave 0.032 g. of fine flakes which melted at 121-122° C. After recrystallization the melting point remained at 123-124° C. The product was proved identical with the above described 3β-acetoxy-17-methylalloetiocholanic acid methyl ester (VII).

(b) $\Delta^{17,20}$-3β-Acetoxyallopregnene-21-acid methyl ester (XVI).

The benzene eluate (0.05454 g.) of the chromatogram after crystallization from methanol gave 0.027 g. of not quite pure $\Delta^{17,20}$-3β-acetoxyallopregnene-21-acid methyl ester of m.p. 177-183° C. The mixed melting point with the pure material of m.p. 193-194° C was 184-189° C.

Hydrogenation of (XVI) to 3β-acetoxyallopregnane-21-acid methyl ester (XV)[15]

0.100 g. of $\Delta^{17,20}$-3β-acetoxyallopregnene-21-acid methyl ester of m.p. 192-193° C. was dissolved in acetic acid and was hydrogenated with 0.020 g. of platinum oxide. After 30 minutes the required one mole of hydrogen was taken up. The catalyst was filtered off, and the filtrate evaporated to dryness under vacuum. The residue was crystallized three times from methanol, and gave the required methyl ester (XV) in the form of coarse cubes which melted at 150-151° C. For analysis the substance was dried for 24 hours at 80° C under high vacuum.

$[\alpha]_D^{17} = +1.3°; +5.8°$ (C = 0.687 in dioxane, 0.956 in chloroform)

3.870 mg. of the substance gave 10.444 mg. CO_2 and 3.355 mg. H_2O

Calculated for $C_{24}H_{38}O_4$	C 73.80 %	H 9.81 %
Found	C 73.66 %	H 9.70 %

The product was identical with 3β-acetoxyallopregnane-21-acid methyl ester (XV) made from dehydroepiandrostenone-(17)[10].

(15) Carried out according to *E. Angliker*, Diss. E.T.H. Zurich 1948

PART II a

BASE CATALYSED REACTION WITH Δ^{16}-3β-ACETOXY-14,15β-EPOXY-5-ALLOETIOCHOLENIC ACID METHYL ESTER

Theoretical Part

The chief characteristic features of some of the known active aglycones of the strophanthus-digitalis group are the α,β-unsaturated γ-lactone ring attached to C_{17}, and a β-orientated 14-hydroxyl group. In periplogenin there is a β-hydroxyl group at C_5 and strophanthidin has an aldehydic group at C_{10} and a β-hydroxyl group at C_5[1].

The problem of partial synthesis of the active natural aglycones resolves itself into the solution of the following 3 points :

(1) Synthesis of the α,β-Unsaturated γ-Lactone Ring.

(2) Introduction of a 5 β-Hydroxyl Group.

(3) Introduction of a 14 β-Hydroxyl Group.

(1) Synthesis of the α,β-Unsaturated γ-Lactone Ring.

The first synthesis of this steroid lactone ring was carried out by Ruzicka, Reichstein and Fürst[2]. They carried out a Reformatzky reaction with bromoacetic ester and Δ^5-3-hydroxy-21-acetoxypregnenone-(20), and obtained a lactone of $\Delta^{5,6;20,22}$-3-acetoxy-21-hydroxynorcholadienic acid. Elderfield[3] and co-workers have synthesised a steroid lactone ring by the same method as that of Ruzicka, Reichstein and Fürst[2] from 21-benzoxypregnanone-(20) which was obtained from etiocholanic acid. Ruzicka, Plattner and Pataki[4] have obtained a lactone by selenium dioxide oxidation of $\Delta^{20,22}$-3-acetoxynorallocholenic acid methyl ester. N-bromosuccinimide oxidation[5] of the same compound resulted

(1) *L. F. Fieser* and *M. Fieser*, Natural Products Related to Phenanthrene, 3rd Ed., Reinhold, New York 1949, page 516.
(2) *L. Ruzicka, T. Reichstein* and *A. Fürst*, Helv. Chim. Acta **24**, 76 (1941).
(3) *J. Fried, R. G. Linville* and *R. C. Elderfield*, J. Org. Chem. **7**, 362 (1942).
(4) *L. Ruzicka, Pl. A. Plattner* and *J. Pataki*, Helv. Chim. Acta **25**, 425 (1942).
(5) *L. Ruzicka, Pl. A. Plattner* and *J. Pataki*, Helv. Chim. Acta **28**, 1360 (1945).

in the described lactone, but at the same time introduced a double bond at the 16,17 position. Plattner and Heusser[6] have synthesised the lactone by an improved method. This involves the condensation of 21-diazopregnenolone with bromoacetic acid. The bromo compound thus obtained gives an unsaturated lactone on treatment with zinc and ethylbromoacetate.

(2) Introduction of a 5β-Hydroxyl Group.

Plattner, Heusser and Kulkarni[7] have achieved the introduction of the 5β-hydroxyl group by the hydrogenation of Δ^4-4,5β-epoxycholestenone-(3).

(3) Introduction of a 14β-Hydroxyl Group.

The only known method of introducing a hydroxyl group in the 14-position is from Plattner, Ruzicka, Heusser, Pataki and Meier[8]. By catalytic hydrogenation of Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester, they were able to obtain the desired 3β-acetoxy-14-hydroxy-14-iso-5-alloetiocholanic acid methyl ester in 20% yield.

The repetition of the above method on the etiocholanic acid series[9] gave only 10% of the wanted 3β-acetoxy-14-hydroxy-14-isoetiocholanic acid ester.

Compounds especially useful as starting material for the synthesis of the unsaturated lactone ring of the cardiac aglycones are of the pregnanolone type. However, the catalyic hydrogenation of Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene[10] yielded from a mixture only about 2% of the required 3β-acetoxy 14-hydroxy-20-keto-14-iso-5-allopregnane.

It was observed that the 14,15-epoxide ester, (Δ^{16}-3β-acetoxy-14, 15β-epoxy-5-alloetiocholenic acid methyl ester), having

(6) *Pl. A. Plattner* and *H. Heusser*, Helv. Chim. Acta **28**, 1044 (1945).
(7) *Pl. A. Plattner, H. Heusser* and *A. B. Kulkarni*, Helv. Chim. Acta **31**, 1822 (1948).
(8) *L. Ruzicka, Pl. A. Plattner, H. Heusser* and *J. Pataki*, Helv. Chim. Acta **29**, 936 (1946); *Pl. A. Plattner, L. Ruzicka, H. Heusser, J. Pataki* and *Kd. Meier*, ibid., **29**, 942 (1946).
(9) *L. Ruzicka, Pl. A. Plattner, H. Heusser* and *Kd. Meier*, Helv. Chim. Acta **30**, 1342 (1947).
(10) *Pl. A. Plattner, L. Ruzicka, H. Heusser* and *E. Angliker*, Helv. Chim. Acta **30**, 385 (1947).

a double bond in the α,β-position to the ester group, and having the epoxide ring also in conjugation with this double bond, was able to react under relatively mild conditions of room temperature with a methanolic solution of potassium hydroxide. There seemed to be two possibilities by which the reaction could have proceeded.

One of the possible courses was the hydrolysis of the epoxide ring; if this had taken place, it would have given a new starting material for preparing aglycones with a hydroxyl group in both 14 and 15 positions. Further, it would have been interesting to study the steric course of the hydrogenation of the 16,17 double bond with the newly introduced hydroxyl at C_{15}.

The other possible course of the reaction was the base catalysed addition of methanol to the 16,17 double bond, with or without the breaking of the epoxide ring.

The present work was undertaken to determine from the products obtained by which of the two ways the reaction had proceeded.

The starting material Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester was prepared with certain modifications according to the method of Plattner, Ruzicka, Heusser, Pataki and Meier[8], from 3β-acetoxy-17-ketoandrostane.

The hydrogenation and oxidation of dehydroepiandrosterone-(17) to 3β-acetoxy-17-ketoandrostane was carried out according to the method of Wenner and Reichstein[11]. Hydrogenation was also tried out under pressure at 60° C. The hydrogenated product on oxidation with chromic acid gave besides the usual mixture of 17-keto derivatives, 3β-acetoxy-17-ketoandrostane and 3β-acetoxy-17-ketoetiocholane, also the 17-acetoxy derivatives. The separation of the ketoderivatives first by Girard reagent T, then by means of chromatography, made this method unnecessarily laborious, and it was abandoned in favour of the comparatively simpler method of Wenner and Reichstein[11].

Δ^{16}-3β-Acetoxy-5-alloetiocholenic nitrile was prepared from 3β-acetoxy-17-ketoandrostane according to the method of Ruzicka,

(11) *V. Wenner* and *T. Reichstein*, Helv. Chim. Acta **27**, 24 (1944).

Plattner, Heusser and Pataki[8]. On chromatography of the crude Δ^{16}-3β-acetoxy-5-alloetiocholenic nitrile, a substance melting at 228-230° C. was obtained from the petrol ether fraction. This compound was not further investigated.

The Δ^{16}-3β-acetoxy-5-alloetiocholenic nitrile was brominated with N-bromosuccinimide and, the 15-bromo derivative was directly converted by splitting off hydrobromic acid into the $\Delta^{14;16}$-3β-acetoxy-5-alloetiocholadienic nitrile[12]. This nitrile was hydrolysed under pressure with sodium hydroxide in ethanol water at 165° C.

This acid was now esterified with diazomethane and then acetylated with acetic anhydride and pyridine in the usual manner. First esterifying the acid and then acetylating it avoided the formation of an anhydride which was encountered by Plattner, Meier and Heusser[12] when the acid was first acetylated and then esterified.

Lastly $\Delta^{14;16}$-3β-acetoxy-5-alloetiocholadienic acid methyl ester was kept with perbenzoic acid for two hours in the dark at room temperature. The double bond conjugated with a carbonyl or a carboxyl group does not, or only with difficulty, react with peracids[13]; therefore the above diene gives only the Δ^{16}-3β acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester.

In the present work Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester (I) on keeping at room temperature with a 5% solution of methanolic potassium hydroxide gave in rather poor yield a crystalline substance (V) melting at 241-243° C. and with the probable empirical formula $C_{24}H_{38}O_6$ or $C_{24}H_{36}O_6$; the substance was not worked on any further. The oily mother liquor failed to crystalize. This oil was acetylated in the usual manner with acetic anhydride and pyridine at room temperature. The acetylated product was chromatographed, and a separation of three substances was effected, namely (II), (III) and (IV).

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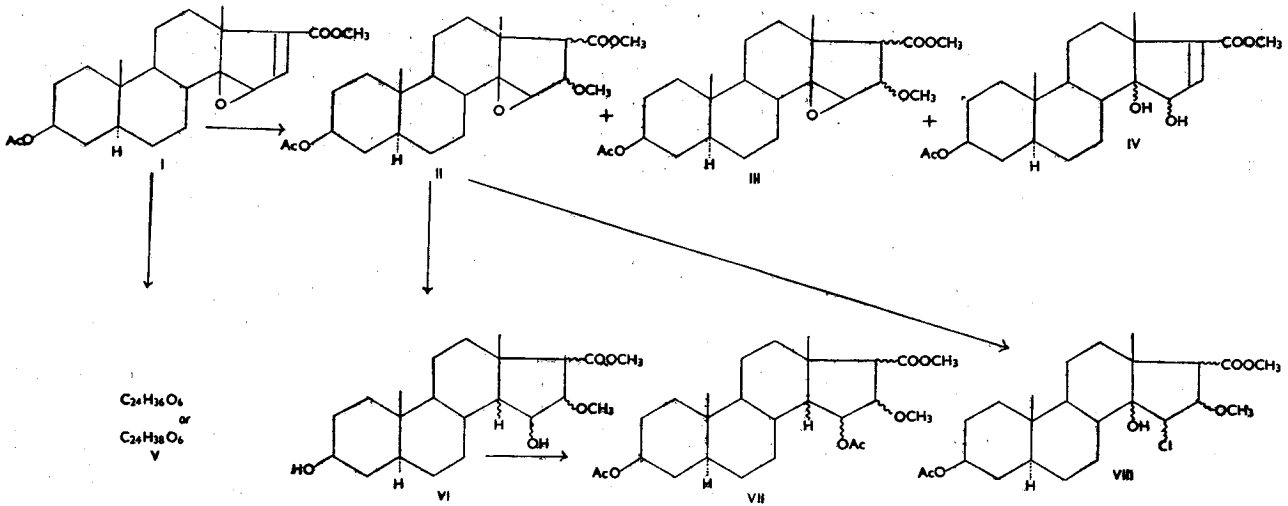
(12) *Pl. A. Plattner, Kd. Meier* and *H. Heusser*, Helv. Chim. Acta **30**, 905 (1947).

(13) Cf. *E. Weitz* and *A. Scheffer*, Ber. **54**, 2327 (1921); *S. Boeseker*, R. **45**, 838(1926); *K. Bodendorf*, Arch. Pharm. **268**, 491 (1930); *D. Swern*, J. Am. Chem. Soc. **69**, 1692 (1947).


COOCH3
AcO
H
O
I
OCH3
II
+
III
OH
IV
C24H36O6
or
C24H38O6
V
HO
VI
OAc
VII
Cl
VIII

Substance (II)

The substance (II) on micro-analysis showed two methoxyl groups and had the empirical formula $C_{24}H_{36}O_6$.

The infra-red spectrum of (II) showed the following

(*a*) Absence of a hydroxyl group

(*b*) Absence of any double bonds

(*c*) Presence of an acetyl group

(*d*) Presence of an epoxide ring

(*e*) Presence of a methoxyl group.

The ultra-violet spectrum did not show any characteristic bands.

Further reactions were carried out to throw more light on the structure of (II).

(1) Substance (II) was reacted with glacial acetic acid and concentrated hydrochloric acid at 40° C. for 40 minutes[18]; on working up, an oil was obtained which was acetylated, chromatographed, and then some of the fractions of the chromatogram were taken together and distilled in a " bulb tube. " The fractions of the above chromatogram which were eluted with petrol ether—benzene, were distilled in the above manner and gave a good analysis for a chlorohydrine derivative of the empirical formula $C_{24}H_{37}O_6Cl$. This compound is believed to be 3β-acetoxy-14ξ-hydroxy-15ξ-chloro-16ξ-methoxy-5-allo-17ξ-etiocholanic acid methyl ester (VIII). The chromatograph fraction eluted with benzene also gave the same analysis as the above mentioned chlorohydrine and could have been an isomer of it. Under these conditions it was not possible to observe the isomerisation of the epoxide (II) to a ketone.

(2) The product of lithium aluminium hydride reduction of substance (II) appeared from the empirical formula $C_{22}H_{36}O_5$ (VI) obtained on analysis of the unacetylated product to have converted the acetyl group in the 3 position to a hydroxyl, and reduced the epoxide ring, but probably due to steric hindrance, does not seem to have attacked the ester group at C_{17}.

The acetylated compound of the above product was an oil and could not be purified enough to give a good analysis. However, the infra-red spectrum of this oil showed the absence of a hydroxyl group ; this indicated that the epoxide ring was broken between C_{14} and the epoxide bridge to a secondary hydroxyl in the 15-position which could be acetylated, and not as usual to a tertiary hydroxyl in the 14-position. The infra-red spectrum also vaguely indicated the presence of a methyl ester group. From this evidence it seemed likely that the reduced product was 3β-15ξ-dihydroxy-16ξ-methoxy-5-allo-17ξ-etiocholanic acid methyl ester (VI).

It would be interesting to study the effect of lithium aluminium hydride on other similarly hindered esters. The carrying out of the lithium aluminium hydride reduction on the isomers (II) and (III) might throw some light on the orientation of the groups at C_{16} and C_{17}.

(3) Pyrolysis of substance (II) was unsuccessful and the substance distilled unchanged.

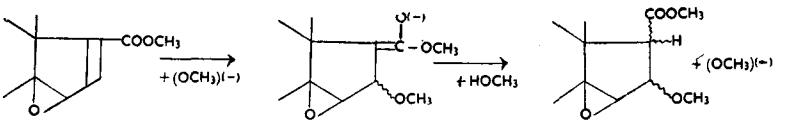
From the infra-red and ultra-violet spectra, and the chemical evidence of substance (II), it appears that the double bond has been attacked with the addition of CH_3OH, and the rest of the molecule has been left undisturbed. The formation of chlorohydrine and the lithium aluminium hydride reduction give evidence of an epoxide ring in (II).

Ruzicka and co-workers[14] obtained from the saponification of $\Delta^{5;16}$-3β-acetoxycholadiene nitrile with methanolic sodium hydroxide under pressure a side product which they believed from the U.V.-absorption spectrum and from Zeisel estimation to be a compound with methanol introduced at the 16,17 double bond, and to which they gave the constitution Δ^5-3β-acetoxy-16ξ-methoxy-etiocholenic acid methyl ester. Gallagher[15] has shown that the product obtained by Marker[16] from $\Delta^{5;16}$-3β-acetoxypregnadiene-20-one upon treatment with methanolic potassium hydroxide is Δ^5-3β-hydroxy-16α-methoxypregnene-20-one.

(14) *L. Ruzicka, E. Hardegger* and *C. Kauter*, Helv. Chim. Acta **27**, 1164 (1944).
(15) *D. K. Fukushima* and *T. F. Gallagher*, J. Am. Chem. Soc. **72**, 2306 (1950); *D. K. Fukushima* and *T. F. Gallagher*, ibid. **73**, 196 (1951).
(16) *R. E. Marker*, J. Am. Chem. Soc. **71** 4149 (1949).

Koelsch[17] also found that methyl acrylate and acrylonitrile formed stable addition products with alcohols very easily.

In the formation of (II) the methoxyl group has very likely attached itself at the 16-position giving 3β-acetoxy-14,15β-epoxy-16ξ-methoxy-5-allo-17ξ-etiocholanic acid methyl ester (II). The course of the reaction could be given as follows.



Substance (III)

Substance (III) appears to be an isomer of (II) as it has the same empirical formula and Zeisel estimation also showed 2 methoxyl groups; also because the infra-red spectrum showed the presence and absence of the same groups as the infra-red spectrum of (II); and lastly it follows immediately after (II) in the chromatogram.

As seen from the already mentioned reaction mechanism there are two asymmetric centres namely at C_{16} and C_{17} which could give rise to 4 isomers, of which (III) may be one.

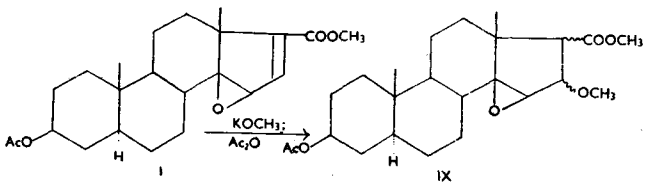
Substance (IV)

In the case of substance (IV) the structure had entirely to be deduced from the infra-red data since the amount of the substance was very small and hence analysis could not be relied upon very much.

The infra-red spectrum of this compound showed the presence of a hydroxyl group, but the absence of an epoxide ring or a methoxyl group. It also gave absorption for an acetyl group and α,β-unsaturated ester. From this data the structure could be Δ^{16}-3β acetoxy-14ξ, 15ξ-dihydroxy-5-alloetiocholenic acid methyl ester (IV). This structure seems probable in view of the fact that some of the unreacted starting material may have got itself changed on the chromatograph column of aluminium oxide to a 14,15 dihydroxy compound with the breaking up of the epoxide ring.

(17) *C. F. Koelsch*, J. Am. Chem. Soc. **65**, 437 (1943).

In a further reaction, instead of potassium hydroxide the base potassium methoxide was used, and the reaction mixture was refluxed for 2 hours. The neutral product thus obtained was acetylated with pyridine and acetic anhydride at room temperature. The acetylated product crystallized well, without resort to chromatographic separation.

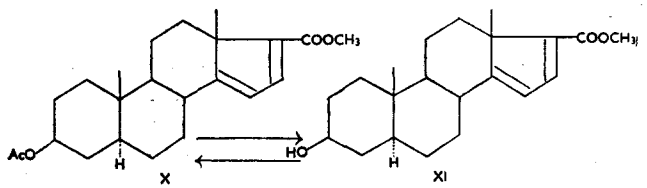


I IX

The empirical formula $C_{24}H_{36}O_6$, (IX,) and the presence of two methoxyl groups pointed strongly to its being a sterio isomer of the compound (II) and (III). The infra-red spectrum of this compound seemed to agree with the above speculation as it showed again a very similar absorption to that of (II) and (III).

The configuration of these 3 stereo isomers (II), (III) and (IX) was not determined.

Having reacted Δ^{16}-3β-acetoxy-14,15 β-epoxy-5-alloetiocholenic acid methyl ester (I) with bases like methanolic potassium hydroxide and methanolic potassium methoxide, it was thought interesting to observe the effect of methanolic potassium hydroxide on $\Delta^{14;16}$-3β-acetoxy-5-alloetiocholadienic acid methyl ester (X) which has only a double bond instead of an epoxide bridge in 14,15 position.



X XI

After the usual working up a neutral product was obtained, which was found to be $\Delta^{14;16}$-3β-hydroxy-5-alloetiocholadienic acid methyl ester (XI). This free hydroxyl derivative on acetylation gave back the starting material $\Delta^{14;16}$-3β-acetoxy-5-alloetiocholadienic acid methyl ester (X).

Experimental Part*

Reaction of Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester (I) with a 5% methanolic solution of KOH.

0.700 g. of Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester (I) was dissolved in 20 cc. of ethanol and 30 cc. of 5% methanolic KOH was added to this solution. The reaction mixture was kept overnight at room temperature. It was then poured into a beaker containing broken ice and neutralised with dil. H_2SO_4 till it reacted blue to Congo paper. This solution was extracted with ethyl acetate (3 times) and shaken with dil. NaOH to remove the acidic part, then washed with water, neutralised with HCl, and finally washed free of HCl. The extract was dried over Na_2SO_4 and evaporated to give 0.656 g. of a neutral oil.

From this oil about 0.013 g. of crystals (V) separated which were crystallized four times from ethyl acetate to give crystals melting at 241.5-243° C. They were dried for analysis under high vacuum at 100° C for 72 hours.

2.993 mg. of the substance gave 7.530 mg. CO_2 and 2.412 mg. H_2O

Calculated for $C_{24}H_{36}O_6$	C 68.54 %	H 8.63 %
$C_{24}H_{38}O_6$	C 68.22 %	H 9.07 %
Found	C 68.66 %	H 9.02 %

Several attempts to crystallize the oily mother liquor (0.6056 g.) failed. It was acetylated at room temperature with 18 cc. of acetic anhydride and 16 cc. of pyridine; the acetylated product was 0.7406 g. of an oil. This was chromatographed over 23 g. of aluminium oxide (activity II).

Fract. No.	Solvent	Eluate in g.	Melting point	Subst. No.
1—4	Petrol ether	0		
5	Petrol ether	0·0667	106·5—108° C.	II
6—7	Benzene	0·3053	99—101° C.	

* All m.p. are corrected and determined in evacuated capillaries.

8	Benzene	0·0251	107—110° C.	
9 –13	9-10 Benzene 11-13 Benzene-ether 9:1	0·0590	112—114·5° C.	III
14	Benzene-ether 9 :1	0·0089	116—118° C.	
15	Benzene-ether 4 :1	0·0030	108—111·5° C.	
16	Benzene-ether 4 :1	0·0065	106—108° C.	
17—18	Ether	0·0376	Oil	
19	Ethyl acetate	0·1060	224—228·5° C.	IV
20	Methanol	0·0018	224—228·5° C.	

The chromatogram fractions 5-7, (II), were collected and crystallized four times from petrol ether to give crystals melting at 103.5-105° C. The crystals were dried for analysis under high vacuum at 70° C. for 80 hours.

$$[\alpha]_D^{24} = +13.5° \qquad (C = 0.525 \text{ in chloroform})$$

3.776 mg. of the substance gave 9.464 mg. CO_2 and 2.928 mg. H_2O

1.320 mg. of the substance required 1.907 cc. $Na_2S_2O_3$ 1/5N

Calculated for $C_{24}H_{36}O_6$	C 68.54 %	H 8.63 %	OCH_3 14.76 %
Found	C 68.40 %	H 8.67 %	OCH_3 14.94 %

The same crystals on sublimation melted at 102-104° C. and gave the following analysis.

1.938 mg. of the substance gave 4.865 mg. CO_2 and 1.494 mg. H_2O

Calculated for $C_{24}H_{36}O_6$	C 68.54 %	H 8.63 %
Found	C 68.51 %	H 8.63 %

The infra-red absorption spectrum of the compound showed the presence of an acetyl group (1725 cm^{-1} and 1240 cm^{-1}), a methoxyl group (1130 cm^{-1}) and an epoxide ring (1280 cm^{-1}).

The U.V.-absorption spectrum did not show any characteristic bands.

The chromatogram fractions 9-14, (III), were collected and also crystallized several times from petrol ether giving crystals melting at 118-119° C. The compound was dried for analysis under high vacuum at 70° C. for 30 hours.

$[\alpha]_D^{24} = 0°$ (C = 0.834 in chloroform)

3.574 mg. of the substance gave 9.000 mg. CO_2 and 2.756 mg. H_2O
2.140 mg. of the substance required 3.050 cc. of $Na_2S_2O_3$ 1/5N

Calculated for $C_{24}H_{36}O_6$	C 68.54 %	H 8.63 %	OCH_3 14.76 %
Found	C 68.72 %	H 8.63 %	OCH_3 14.73 %

The infra-red absorption spectrum of the compound showed the presence of an acetyl group (1729 cm^{-1} and 1250 cm^{-1}), a methoxyl group (1137 cm^{-1}) and an epoxide ring (1284 cm^{-1}).

The U.V.-absorption spectrum did not show any characteristic bands.

The chromatogram fractions 19-20, (IV), were crystallized three times from a mixture of ether—petrol ether giving crystals melting at 231-232° C. The compound was dried for analysis under high vacuum at 100° C. for 50 hours.

0.539 mg. of the substance gave 1.329 mg. CO_2 and 0.454 mg. H_2O

Calculated for $C_{23}H_{34}O_6$	C 67. 95 %	H 8.43 %
Found	C 67.29 %	H 9.43 %

The infra-red absorption spectrum of the compound showed the presence of a hydroxyl group (3380 cm^{-1}), an acetyl group (1720 cm^{-1} and 1235 cm^{-1}) and an α,β-conjugated double bond (1623 cm^{-1}).

Reaction of compound (II) with glacial acetic acid and conc. HCl[18].

0.0510 g. of compound (II) was dissolved in 1.0 cc. of chloroform in a flask fitted with a condenser with a ground glass joint ; 1.0 cc.

(18) cf. *L. Ruzicka, O. Jeger, J. Redel* and *E. Volli*, Helv. Chim. Acta **28**, 199 (1945).

of glacial acetic acid was also added together with 0.075 cc. of pure concentrated hydrochloric acid. The reaction was carried out for 40 minutes with the temperature between 40-45° C.

The reaction mixture was poured over pieces of cracked ice and extracted with ether (3 times). The ether extract was washed with water, shaken with dil. NaOH, and again washed with water, then with dil. HCl and lastly with water till it was neutral. It was dried over Na_2SO_4 and the ether evaporated to give 0.0540 g. of a neutral oily product.

This 0.0540 g. of the oil was acetylated with 1.7 cc. of acetic anhydride and 1.6 cc. of pyridine to yield 0.0589 g. of an acetylated white oil.

0.0589 g. of this oil was chromatographed over 1.8 g. of aluminium oxide (activity II).

Fract. No.	Solvent	cc. of solvent	Eluate in g.	Melting point
1—3	Petrol ether	20	0·0048	oil
4—8	Petrol ether-Benzene 9 : 1	45	0·0175	oil
9	Petrol ether-Benzene 9 : 1	10	0·0030	oil
10—14	Petrol ether-Benzene 4 : 1	45	0·0142	oil
15	Benzene	10	0·0113	oil

Chromatogram fractions 4-8 of the above chromatogram were collected and put in a " bulb tube " with ether as solvent. Ether was gently evaporated. The " bulb tube " was now connected to a high vacuum line and gradually heated to 160-163° C. at 0.003 mm. pressure with a slight rotation from time to time; a yellow oil began to creep over. This was twice distilled into the following bulbs. The tube was sealed under vacuum and given for analysis.

3.760 mg. of the substance gave 8.694 mg. CO_2 and 2.704 mg. H_2O

1.860 mg. of the substance gave 0.552 mg. AgCl

Calculated for			
$C_{24}H_{37}O_6Cl$	C 63.08 %	H 8.10 %	Cl 7.77 %
Found	C 63.10 %	H 8.05 %	Cl 7.34 %

Chromatogram fractions 9-14 were collected and distilled in a " bulb tube " in the above manner at 160-170° C. at 0.005 mm. pressure under high vacuum. The tube was sealed under vacuum and the light yellow oil was given for analysis.

1.739 mg. of the substance gave 3.909 mg. CO_2 and 1.246 mg. H_2O

1.421 mg. of the substance required 1.877 cc. $Na_2S_2O_3$ 1/50N

Found	C 61.34 %	H 8.02 %	OCH_3 13.66 %

Chromatogram fraction 15 was also put in a " bulb tube " and distilled at 160-170° C. at 0.005 mm. pressure under high vacuum. The oil which collected in the bulb was sealed in the tube under vacuum and given for analysis.

2.900 mg. of the substance gave 6.751 mg. CO_2 and 2.073 mg. H O

Calculated for $C_{24}H_{37}O_6Cl$	C 63.08 %	H 8.10 %
Found	C 63.53 %	H 8.00 %

Reaction of compound (II) with $LiAlH_4$.

About 0.064 g. of pulverised $LiAlH_4$ was taken with 2 cc. of ether in a round bottom flask fitted with a ground glass joint condenser, to which was attached a calcium chloride tube and a dropping funnel. 0.0534 g. of compound (II) was dissolved in 4 cc. of ether, and added drop by drop from the dropping funnel into the flask containing $LiAlH_4$ which was kept agitated with a magnetic stirrer.

The reaction mixture was stirred at room temperature for 1 hour, and at 40° C. for 1/2 hour. After the end of this period the round bottom flask was surrounded with a freezing mixture, and water was added drop by drop till there was no more effervescence. Then dil. H_2SO_4 was added till the precipitate dissolved and formed two separate layers of ether and of a watery solution.

The above treated reaction mixture was extracted with ether and the ether extract was shaken with dil. NaOH. After this, the ether extract was washed with water, dil. HCl, Na_2CO_3 solution and finally with water. It was dried over Na_2SO_4 and evaporated to give 0.0424 g. of a neutral substance. This substance after five alternate crystallizations with ether—petrol ether and with ethyl

acetate—petrol ether gave crystals of (VI) melting at 157.5-158° C. These crystals were dried for analysis under high vacuum at 90° C. for 72 hours.

3.313 mg. of the substance gave 8.449 mg. CO_2 and 2.832 mg. H_2O

Calculated for	$C_{22}H_{36}O_5$	C 69.44 %	H 9.54 % (*a*)
	$C_{21}H_{36}O_4$	C 71.55 %	H 10.30 % (*b*)
Found		C 69.60 %	H 9.57 %

0.039 g. of the above substance from the $LiAlH_4$ reaction was acetylated with 1.5 cc. of acetic anhydride and 1 cc. of pyridine at room temperature for 72 hours. The yield of the crude product was 0.0556 g. This crude product was boiled with animal charcoal and benzene and filtered over a small quantity of aluminium oxide. The oil obtained crystallized badly from acetone giving needles melting at 53-56° C. This oil, (VII), was distilled in a " bulb tube " at about 0.01 mm. pressure and at 110-112° C. and then given for analysis.

3.242 mg. of the substance gave 8.408 mg. CO_2 and 2.768 mg. H_2O

2.439 mg. of the substance required 1.301 cc. $Na_2S_2O_3$ 1/50N

Calculated for

$C_{24}H_{38}O_6$	C 68.22 %	H 9.07 %	OCH_3 14.69 % (*c*)
$C_{24}H_{38}O_5$	C 70.90 %	H 9.42 %	OCH_3 15.26 % (*d*)
$C_{23}H_{38}O_5$	C 70.01 %	H 9.71 %	OCH_3 7.86 % (*e*)
$C_{23}H_{34}O_5$	C 70.74 %	H 8.78 %	OCH_3 7.94 % (*f*)
Found	C 70.78 %	H 9.55 %	OCH_3 5.52 %

The infra-red absorption spectrum of the compound showed the presence of an acetyl group (1728 cm^{-1} and 1248 cm^{-1}), a methyl ester (1719 cm^{-1}) and a methoxyl group (1126 cm^{-1}).

(*a*) *Formula for 3β-14ξ or 15ξ-dihydroxy-16ξ-methoxy-5-allo-17ξ-etiocholanic acid methyl ester.*

(*b*) *Formula for 3β-14ξ or 15ξ, 20-trihydroxy-16ξ-methoxy-5-allo-21-norpregnane.*

(*c*) *Formula for 3β-14ξ or 15ξ-dihydroxy-16ξ-methoxy-5-allo-17ξ-etiocholanic acid methyl ester with one free OH group acetylated.*

(*d*) *Formula for 3β-14ξ or 15ξ-dihydroxy-16ξ-methoxy-5-allo-17ξ-etiocholanic acid methyl ester with one free OH group acetylated and with one oxygen atom less.*

(*e*) *Formula for a 3β-14ξ or 15ξ, 20-trihydroxy16ξ-methoxy-5-allo-21-nor pregnane with one free OH group acetylated.*

(*f*) *Formula for a lactone of 3β-acetoxy-14 or 15ξ-dihydroxy-16ξ methoxy 5-alloetiocholanic acid.*

Attempted pyrolysis of compound (II).

0.0365 g. of compound (II) was placed inside a specially shaped test tube. This test tube was attached to a water pump vacuum line and heated red hot with a micro-burner. Then the substance was distilled over. The test tube was cut and the oil drop which had collected was removed with ether. The ether was evaporated to give 0.0359 g. of an oil. This oil was crystallized from petrol ether. The melting point of the crystals was 99-101° C. and the mixed melting point with starting material showed no depression. Therefore, no pyrolysis had taken place and the starting material had distilled over unchanged.

Reaction of Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester (I) with $KOCH_3$ in absolute methanol.

About 0.19 g. of potassium metal was weighed out in a weighing bottle containing xylene. These pieces were rapidly dried between pieces of filter paper, and added to 1 cc. of absolute methanol in a round bottom flask with a reflux condenser fitted with a calcium chloride tube. 0.2508 g. of Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester (I) dissolved in about 3 cc. of absolute ethanol was now added, and the reaction mixture was refluxed for 2 hours, after which it was allowed to stand overnight at room temperature.

The contents of the flask were now poured into a slight excess of dil. H_2SO_4 and extracted with ether. The ether extract was washed with Na_2CO_3 solution and finally with water till it was neutral. This ether extract was dried over Na_2SO_4 and evaporated to give 0.2764 g. of a neutral product.

This neutral product (0.2764 g.) was acetylated at room temperature with 9 cc. of acetic anhydride and 6 cc. of pyridine. The acetylated product was dissolved in benzene and filtered over aluminium oxide to give 0.2513 g. of an oil which on crystallization from methanol gave about 0.1107 g. of crystals (IX). These were recrystallized three times from ether-petrol ether till they gave a constant melting point of 189-190.5° C. The crystals were dried for analysis under high vacuum at 70° C. for 72 hours.

$$[\alpha]_D^{24} = +39.6° \qquad (C = 1.21 \text{ in chloroform}).$$

3.558 mg. of the substance gave 8.947 mg. CO_2 and 2.770 mg. H_2O
1.198 mg. of the substance required 1.680 cc. $Na_2S_2O_3$ 1/50 N

Calculated for			
$C_{24}H_{36}O_6$	C 68.54 %	H 8.63 %	OCH_3 14.76 %
Found	C 68.62 %	H 8.71 %	OCH_3 14.51 %

The infra-red absorption spectrum of the compound showed the presence of an acetyl group (1728 cm^{-1} and 1248 cm^{-1}), a methoxyl group (1135 cm^{-1}) and an epoxide ring (1285 cm^{-1}).

There was no absorption whatever in the U.V.-region.

Reaction of $\Delta^{14;16}$-3β-acetoxy-5-alloetiocholadienic acid methyl ester (X) with a 5% methanolic solution of KOH.

0.0497 g. of $\Delta^{14;16}$-3β-acetoxy-5-alloetiocholadienic acid methyl ester (X) was dissolved in 2.5 cc. of ethanol and was kept overnight at room temperature together with 2.0 cc. of a 5% methanolic solution of KOH.

The reaction mixture was neutralized with dil. H_2SO_4 and extracted with ethyl acetate. The ethyl acetate extract was shaken with dil. NaOH to remove the acidic portion. The ethyl acetate extract was now treated with dil. HCl, Na_2CO_3 solution, and finally with water till it was neutral. It was dried over Na_2SO_4, and ethyl acetate evaporated to yield 0.441 g. of a neutral substance (XI). After crystallizing four times from methanol it gave crystals melting at 172-174° C. The substance was dried at 80° C. for 72 hours under high vacuum for analysis.

2.704 mg. of the substance gave 7.556 mg. CO_2 and 2.212 mg. H_2O

Calculated for $C_{21}H_{30}O_3$	C 76.32 %	H 9.15 %
Found	C 76.26 %	H 9.15 %

The above crystals (0.0385 g.) were acetylated at room temperature by keeping them overnight with 1 cc. of acetic anhydride and 1 cc. of pyridine. The acetylated product was crystallized from methanol and had the m.p. 141-144° C. Mixed melting point with $\Delta^{14;16}$-3β-acetoxy-5-alloetiocholadienic acid methyl ester (X) showed no depression.

PART II b

BASE AND ACID CATALYSED REACTIONS WITH Δ^{16}-3β-ACETOXY-14-15β-EPOXY-20-KETO-5-ALLOPREGNENE

Theoretical Part

A similar type of work as that carried out in Part IIa with Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloeticholenic acid methyl ester (I) was also tried with a compound of the pregnane series, namely Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene (XII).

The studies made on this compound were the action of a methanolic solution of potassium hydroxide which acted as a basic catalyst, and that of acids like sulphuric acid and aluminium oxide which could promote acid catalysed reactions.

The starting material Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene (XII) was prepared according to the method of Plattner, Ruzicka, Heusser and Angliker[10].

BASE CATALYSED REACTION

Action of a methanolic solution of potassium hydroxide.

Δ^{16}-3β-Acetoxy-14,15β-epoxy-20-keto-5-allopregnene was kept for 24 hours at room temperature with a 5% methanolic solution of potassium hydroxide. The product (XIII) with mother liquor obtained from this reaction was acetylated, and the acetylated compound was chromatographed. The chromatogram gave as a major portion compound (XIV) and a trace of compound (XV).

On analysis compound (XIV) gave an empirical formula $C_{24}H_{36}O_5$, and showed the presence of a methoxyl group. From the infra-red absorption spectrum and an analogy of similar previous experiments with Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloeticocholenic acid methyl ester (I) which is discussed in Part IIa, it was concluded that the new compound (XIV) was Δ^{16}-3β-acetoxy-14, 15β-epoxy-16ξ-methoxy-20-keto-5-allopregnene.

Compound (XIV) was hydrogenated with platinum oxide in acetic acid and then oxidised with chromic acid; the product obtained was the starting material (XIV). This experiment showed

the absence of a double bond, or any primary or secondary hydroxyl group. The 14,15-epoxide ring was isomerised to a ketone (XVI) at C_{15} with hydrochloric acid (conc.) and acetic acid; the ketone was identified as a 5-ring ketone from the infra-red absorption spectrum; an increased proportion of conc. hydrochloric acid gave a chlorohydrine (XVII) at the position 14,15. These reactions showed the presence of an epoxide ring.

Compound (XV) which was separated from the ether and ethyl acetate fraction of the chromatogram was not investigated any further.

ACID CATALYSED REACTIONS

Action of aluminium oxide.

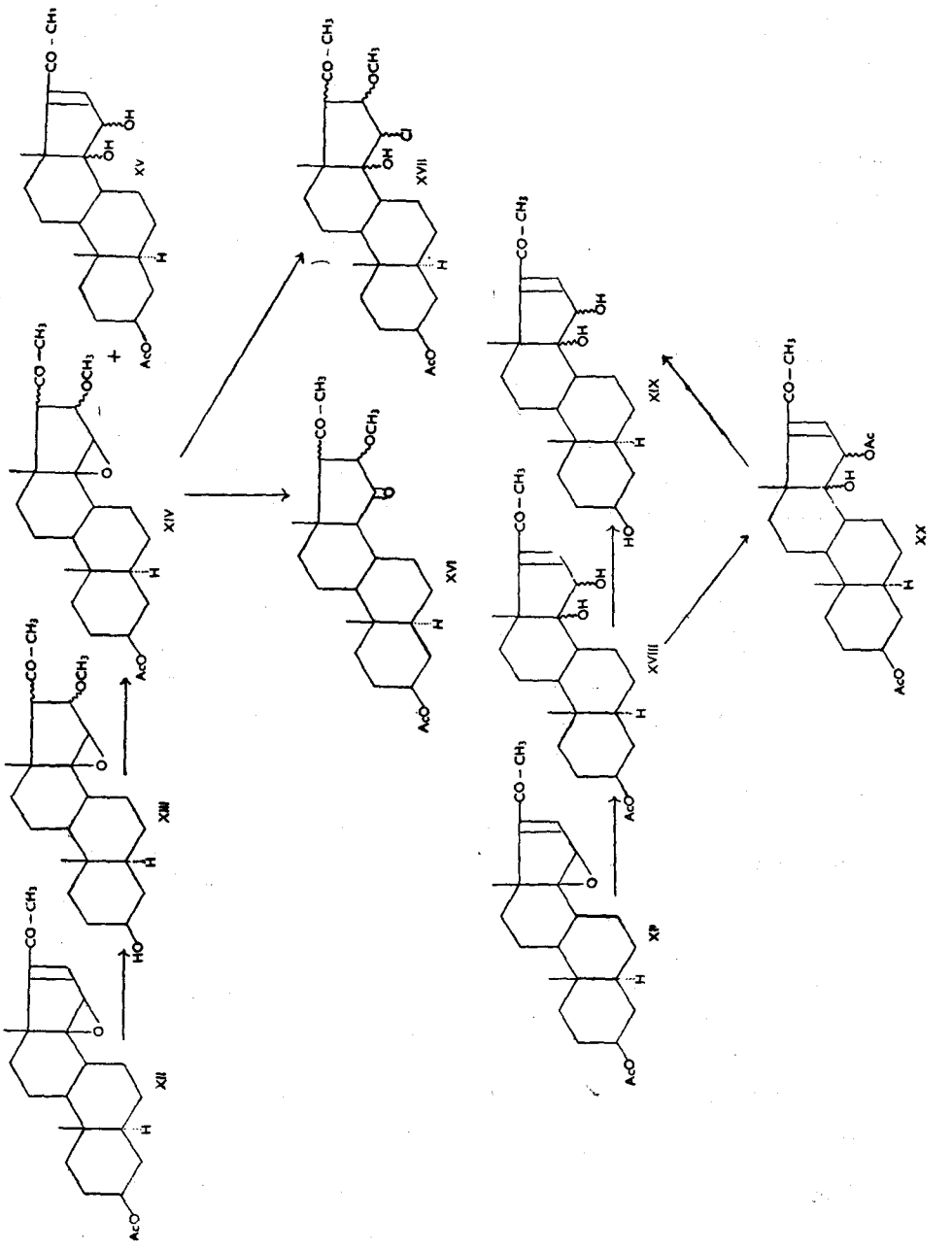
An acid according to G. N. Lewis[19] definition is any compound which is able to accept one or more pairs of electrons to form covalent bonds. By this definition aluminium oxide which can receive a pair of electrons is considered as an acid.

To carry out a reaction which could be catalysed by aluminium oxide the Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene was adsorbed on a column of aluminium oxide and left there for four days. After removing the unreacted starting substance with a 2 : 8 mixture of benzene and petrol ether, the reaction product was eluted with ether and ethyl acetate.

Although the percentage error in the analysis of this reaction product was slightly beyond the usually permitted limit, it was concluded from the infra-red absorption spectrum (which clearly showed the presence of an α, β-unsaturated ketone, an acetyl and a hydroxyl group, and the absence of an epoxide bridge) that this compound should be a monoacetate of the structure Δ^{16}-3β-acetoxy-14ξ,15ξ-dihydroxy-20-keto-5-allopregnene (XVIII). The U.V.-absorpotion spectrum of this compound also showed a maximum absorption at 232 mμ ($\log \epsilon = 3.79$). †

† This extinction is very low and perhaps the substance was not quite pure.

(19) *G. N. Lewis*, Valency and the Structure of Atoms and Molecules, New York 1923.


XII
XIII
XIV
XV
XVI
XVII
XVIII
XIX
XX
AcO
HO
OH
OAc
OCH3
CO-CH3
O
H

For further proof of structure of the monoacetate, the compound was hydrolysed to a trihydroxy derivative (XIX); the monoacetate was also acetylated to a diacetate (XX) and this diacetate was again hydrolysed to the trihydroxy compound. Once again the diacetate did not give a satisfactory analysis, but the analysis of the trihydroxy compound agreed very well in one case. The infra-red absorption spectra of the trihydroxyl compounds and the diacetate agreed very well with the given structures.

Action of sulphuric acid.

Δ^{16}-3β-Acetoxy-14,15β-epoxy-20-keto-5-allopregnene (XII) was this time kept with 20% sulphuric acid in ethanol for nearly 3 days. The reaction product was directly acetylated to the diacetate (XX), and was proved to be identical with the diacetate (XX) obtained from the reaction with aluminium oxide by taking the mixed melting point which showed no depression.

Experimental Part*

Reaction of Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene (XII) with 5% methanolic KOH.

0.1005 g. of Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene (XII) was dissolved in 5 cc. of ethanol and added to 4 cc. of 5% methanolic KOH (5 g. in 100 cc.), and kept at room temperature for 24 hours.

The reaction mixture was neutralised with dil. H_2SO_4 and extracted with ether. The ether extract was washed in turn with water, with Na_2CO_3 solution and again with water. It was dried over Na_2SO_4 and evaporated to give 0.0980 g. of a neutral white crystalline solid (XIII). A part of this substance was crystallized from methanol and then twice from a mixture of acetone and benzene. The crystals obtained melted at 245-248° C. They were dried under high vacuum for analysis at 90° C. for 72 hours.

3.758 mg. of the substance gave 9.996 mg. CO_2 and 3.103 mg. H_2O

Calculated for $C_{22}H_{34}O_4$	C 72.89 %	H 9.46 %
Found	C 72.37 %	H 9.22 %

* All m.p. are corrected and determined in evacuated capillaries.

The U. V.-absorption spectrum showed a maximum at 285 mμ (log $\epsilon = 1.39$).

0.0970 g. of the above reaction product (XIII) was acetylated with 3.4 cc. of acetic anhydride and 3.2 cc. of pyridine by keeping it overnight at room temperature. On working up in the usual manner it gave an yield of 0.1049 g. of a substance.

0.1049 g. of the above acetylated substance was dissolved in benzene, and chromatographed over 3.5 g. of aluminium oxide (activity II).

Fract. No.	Solvent	Eluate in g.	Melting point	Subst. No.
1— 4	Petrol ether	0	——	
5— 6	Petrol ether-Benzene 4 : 1	0·0043	——	
7—11	Petrol ether-Benzene 4 : 1	0·0263	185-187° C.	XIV
12—16	Petrol ether-Benzene 1 : 1	0·0312	183-185° C.	
17—19	Benzene	0·0128	183-184° C.	
20	Benzene-ether 4 : 2	0·0051	183-184° C.	
21—23	Benzene-ether 4 : 2	0·0057	Bad crystals	
24—25	Ether	0·0055	248-251° C.	XV
26	Ethyl acetate	0·0054	246-250° C.	

The chromatogram fractions 7-20, (XIV) were collected together and crystallized several times from ethanol. The melting point of the crystals was 185-187° C. They were dried under high vacuum for analysis at 90° C. for 70 hours.

$$[\alpha]_D^{22} = +31° \qquad (C = 1.00 \text{ in chloroform})$$

3.318 mg. of the substance gave 8.648 mg. CO_2 and 2.627 mg. H_2O

0.870 mg. of the substance required 0.640 cc. $Na_2S_2O_3$ 1/50 N

Calculated for

$C_{24}H_{36}O_5$	C 71.25 %	H 8.97 %	OCH_3 7.67 %
Found	C 71.13 %	H 8.86 %	OCH_3 7.61 %

The infra-red absorption spectrum of the substance showed the presence of an acetyl group (1725 cm^{-1}, and 1245 cm^{-1}), an epoxide ring (1283 cm^{-1}), a methoxyl group (1128 cm^{-1}) and a keto group (1702 cm^{-1}).

The chromatogram fractions 24-26, (XV) were also collected and then crystallized several times; the melting point of the crystals was 250.5-252° C. They were dried under high vacuum for analysis at 80° C. for 50 hours.

1.676 mg. of the substance gave 4.342 mg. CO_2 and 1.391 mg. H_2O

Calculated for $C_{23}H_{34}O_5$	C 70.74 %	H 8.78 %
Found	C 70.70 %	H 9.29 %

Reaction of compound (XIV) with acetic acid and conc. HCl[18].

0.0765 g. of compound (XIV) was dissolved in about 1.5 cc. of chloroform; to this 1.5 cc. of acetic acid and 0.080 cc. of conc. HCl were added. The mixture was refluxed for 40 minutes at 40-45° C. (temperature of reaction mixture).

The reaction mixture was worked up as in the previous experiment, and 0.0882 g. of an oil was obtained.

This oil (0.0785 g.) was acetylated on keeping it overnight with 2 cc. of acetic anhydride and 1.8 cc. of pyridine at room temperature, and then treated in the usual manner to give 0.0839 g. of an oil.

This acetylated oil was chromatographed over aluminium oxide. The petrol ether—benzene 1 : 1 fraction was crystalized 3 times from methanol. The crystals melted at 184-186° C. Mixed melting point with compound (XIV) was 172-178° C. The crystals were dried under high vacuum for analysis at 70° C. for 72 hours.

2.352 mg. of the substance gave 6.114 mg. CO_2 and 1.875 mg. H_2O

Calculated for $C_{24}H_{36}O_5$	C 71.25 %	H 8.97 %
Found	C 70.94 %	H 8.92 %

The infra-red absorption spectrum of the compound showed the presence of an acetyl group (1725 cm^{-1} and 1250 cm^{-1}) and a 5-membered ring ketone (1742 cm^{-1}).

Reaction of compound (XIV) with acetic acid and with a slight excess (50%) of conc. HCl.

0.050 g. of the compound (XIV) was dissolved in 1 cc. of chloroform together with 1 cc. of acetic acid and 0.075 cc. of pure conc. hydrochloric acid, and heated for 40 minutes at 40-45° C. (temperature of the reaction mixture).

The reaction mixture was poured over cracked ice, and Na_2CO_3 solution was added till it was slightly alkaline, and then extracted with ether. The ether extract was washed with water, dried over Na_2SO_4 and evaporated giving 0.0665 g. of an oil.

0.0665 g. of this oil was acetylated by keeping it overnight at room temperature with 1.6 cc. of pyridine and 1.7 cc. of acetic anhydride; by working up in the usual manner it gave an yield of 0.0689 g. of a solid. It was crystallized three times from ethanol, giving crystals (XVII) melting at 186-188° C. These were dried under high vacuum for analysis at 90° C. for 48 hours.

3.284 mg. of the substance gave 7.886 mg. CO_2 and 2.421 mg. H_2O

1.038 mg. of the substance gave 0.377 mg. AgCl

Calculated for $C_{24}H_{37}O_5Cl$	C 65.36 %	H 8.46 %	Cl 8.05 %
Found	C 65.53 %	H 8.25 %	Cl 9.00 %

The infra-red absorption spectrum of this substance showed the presence of a hydroxyl group (3480 cm^{-1}) and an acetyl group (1720 cm^{-1} and 1248 cm^{-1}).

Hydrogenation and oxidation of 3β-acetoxy-14,15β-epoxy-16ξ-methoxy-20-keto-5-allopregnane.

0.125 g. of the compound (XIV) was dissolved in about 10 cc. of acetic acid and was hydrogenated with 0.0205 g. of platinum oxide which was prehydrogenated. After about 15 minutes the substance had taken up 1 mole of hydrogen and on further hydrogenation for a longer period the substance did not absorb any

more. The solution was filtered through " Celite " and evaporated, giving 0.132 g. of a residue. This substance was dissolved in 5 cc. of an acetic acid solution of chromic acid (4.5 mg. O/cc.). After 24 hours the excess of chromic acid was destroyed by the addition of methanol and on usual treatment 0.125 g. of long crystalline needles were separated. After several crystallizations from acetone—hexane the substance melted at 187.5-189° C. Mixed melting point with the starting material showed no depression.

Reaction of Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene with Al_2O_3.

0.200 g. of the epoxide (XII) was dissolved in a small quantity of benzene, and poured over a column of aluminium oxide (6.5 g.) of activity I—II containing petrol ether. The substance was moved towards the centre of the column with a mixture of benzene—petrol ether (2 : 8) and was allowed to remain there for four days. Then it was well eluted with benzene and ether, this gave about 0.145 g. of a substance which was the starting material. Later the column was eluted with a mixture of ether and ethyl acetate (8 : 2), and then with ethyl acetate alone; 0.060 g. of a substance (XVIII) was obtained which was crystallized several times from a mixture of methanol and ether and which melted at 247-248° C. The crystals were dried for analysis under high vacuum at 111° C. for 77 hours.

$[\alpha]_D^{24} = +27.3°$ (C = 0.917 in chloroform)

3.800 mg. of the substance gave 9.763 mg. CO_2 and 2.904 mg. H_2O

Calculated for $C_{23}H_{34}O_5$	C 70.74 %	H 8.78 %
Found	C 70.11 %	H 8.55 %

In the U.V.—absorption spectrum in alcohol the substance showed a maximum at 232 mμ ($\log \epsilon = 3.79$). The infra-red absorption spectrum of the substance showed the presence of a hydroxyl group (3330 cm^{-1}), an acetyl group (1729 cm^{-1} and 1250 cm^{-1}) and an α, β-unsaturated ketone (1649 cm^{-1} and 1612 cm^{-1}).

Hydrolysis of the monoacetate (XVIII.)

0.130 g. of the monoacetate (XVIII) was allowed to stand for two days at room temperature (20° C.) with 10 cc. of 5 %

methanolic KOH and then neutralized. The solution was concentrated under vacuum, water was added to it, and the last traces of methanol were evaporated. It was made acidic to congo red reagent, and the crystals were filtered off. 0.051 g. of these crystals (XIX) were crystallized from methanol and then twice with a mixture of acetone and benzene, m.p. 245-248° C. They were dried under high vacuum for analysis at 90° C. for 48 hours.

3.731 mg. of the substance gave 9.820 mg. CO_2 and 3.905 mg. H_2O

Calculated for $C_{21}H_{32}O_4$	C 72.38 %	H 9.26 %
Found	C 71.83 %	H 9.01 %

Hydrolysis of the diacetate (XX.)

0.0475 g. of the diacetate (XX) was taken in 4 cc. of 5 % methanolic KOH, and left overnight at room temperature. Then the solution was neutralised with 1N H_2SO_4, and the precipitate filtered off, giving 0.015 g. of needles. The filtrate was extracted with ethyl acetate, washed with water, dried and evaporated, to give 0.023 g. of a solid. This was crystallized several times from a mixture of acetone and hexane, crystals (XIX) were obtained melting at 240-242° C. Mixed melting point with the hydrolysis product of the monoacetate (XVIII) showed no depression. The crystals were dried under high vacuum for analysis at 90° C. for 60 hours.

3.498 mg. of the substance gave 9.260 mg. CO_2 and 2.870 mg. H_2O

Calculated for $C_{21}H_{32}O_4$	C 72.38 %	H 9.26 %
Found	C 72.24 %	H 9.18 %

The infra-red absorption spectrum of this compound showed the presence of a hydroxyl group (3380 cm^{-1}), and an α,β-unsaturated ketone (1670 cm^{-1} and 1620 cm^{-1}).

Acetylation of the monoacetate (XVIII.)

0.130 g. of the monoacetate (XVIII) was dissolved in 5 cc. of pyridine with 2 cc. of acetic anhydride and left overnight at room temperature. Then some water was added to the reaction mixture, and the latter was extracted with ether. The ether extract was washed with water, HCl, $NaHCO_3$ solution and finally again

with water, then it was dried and evaporated. The 0.120 g. of the substance (XX) thus obtained was crystallized several times from methanol, m.p. 247-250° C. For analysis the substance was sublimed at 200° C. under high vacuum.

3.788 mg. of the substance gave 9.552 mg. CO_2 and 2.806 mg. H_2O

Calculated for $C_{25}H_{34}O_6$	C 69.42 %	H 8.39 %
Found	C 68.82 %	H 8.29 %

The infra-red absorption spectrum of this compound showed the presence of a hydroxyl group (3520 cm^{-1}), an acetyl group (1725 cm^{-1} and 1245 cm^{-1}) and an α,β-unsaturated ketone (1660 cm^{-1} and 1618 cm^{-1}).

Reaction of the Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene (XII) with dilute 20% H_2SO_4.

0.305 g. of the epoxide (XII) was dissolved in 50 cc. of ethanol, and 10 cc. of 20% H_2SO_4 was added to it with development of turbidity. It was slightly warmed to 35° C. so that it was again clear, and left for 24 hours at room temperature. At the end of this period 5 cc. more of 20% H_2SO_4, was added, and the mixture was allowed to stand another 48 hours. 50 cc. of water was added and ethanol was removed in vacuum at 20° C. 0.162 g. of needle-like crystals which came down on evaporation were filtered off. The filtrate was extracted with ethyl acetate and the extract well washed with water, dried and evaporated yielding 0.055 g. of a crystalline substance.

This substance, together with 0.162 g. needlelike crystals, was acetylated with 5 cc. of acetic anhydride in pyridine by keeping it overnight at room temperature. 0.234 g. of the acetylated substance obtained on evaporating to dryness was chromatographed on 7 g. of aluminium oxide.

The benzene-ether (1:1) fraction of the chromatogram gave about 0.080 g. of a substance melting at 242-245° C. (XX). This was crystallized alternately from alcohol and alcohol-acetone mixture to a constant melting point 241-245.5° C.

The mixed melting point with the diacetate (XX) obtained from the aluminium oxide reaction product showed no depression.

PART III

THE PREPARATION OF 3β,20-DIACETOXY-17α-HYDROXY-21-NORPREGNANE

Theoretical Part

Of the 29 known steroids extracted from the adrenal cortex only six compounds are known to be active in prolonging the life of adrenalectomized animals. Three of these six have a 17α-hydroxyl substituent; they are 17α-hydroxydesoxycorticosterone, 17α-hydroxycorticosterone and 17α-hydroxy-11-dehydrocorticosterone.

Recently the last mentioned 17α-hydroxy-11-dehydrocorticosterone, or cortisone, has acquired great importance due to its use as a chemotherapeutical agent in the treatment of rheumatoid arthritis, leukemia and other disorders.

Since the supply from the adrenal glandular extracts is insufficient, different methods have been adopted for its partial synthesis, in particular for the introduction of the oxygen function at C_{11} and C_{17} in the proper spatial configuration. In this work only the introduction of the C_{17}α-hydroxyl group will be discussed.

One of the general methods for the introduction of a 17α-hydroxyl group in the natural configuration has been through osmium tetroxide oxidation of 17,20-unsaturated steroids[1]. This method proved very useful for the partial synthesis of a series of adrenal cortex hormones[2]. Sarett[3] also used this osmium tetroxide oxidation of the 17,20 double bond in another method in which the 20-ketopregnane is converted to the 17α-hydroxy-20-ketosteroid, through the cyanohydrine of the 20-keto-pregnane derivative, which is dehydrated to the 17,20 unsaturated nitrile; this is oxidised with osmium tetroxide. The intermediate hydroxylation product with the loss of HCN forms the 17α-hydroxy-20-ketsoteroid.

(1) *A. Serini, W. Logeman* and *W. Hildebrand*, Ber. **72**, 391 (1939); *B. Koechlin* and *T. Reichstein*, Helv. Chim. Acta, **26**, 1328 (1943); *L. H. Sarett*, J. Biol. Chem. **162**, 601 (1946).

(2) *H. G. Fuchs* and *T. Reichstein*, Helv. Chim. Acta **24**, 804 (1941); *P. Hegner* and *T. Reichstein*, ibid. **24**, 828 (1941); *D. A. Prins* and *T. Reichstein*, ibid. **24**, 945 (1941).

(3) *L. H. Sarett*, J. Am. Chem. Soc. **70**, 1454 (1948).

Kritchevsky and Gallagher[4] effected the hydroxylation at C_{17} in the α-configuration by the treatment of the enol acetate of a 20-ketosteroid with either peracid or chromic acid followed by saponification.

The 17α- hydroxy compounds were prepared by Julian[5] from the 16,17 α-epoxy steroids by the cleavage of the epoxy compound with hydrobromic acid and the removal of bromine from the bromohydrine with Raney nickel.

Recently the reduction of 16,17 α-epoxy-20-ketosteroids with lithium aluminium hydride[6] resulted in the formation of 17α-hydroxy steroids. In this reduction, if the 20-keto is to be left unreduced, it may be protected by forming a cyclic ketal[6].

The object of the present work was to investigate the formation of a 16,17-epoxide from Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester (I), and the lithium aluminium hydride reduction of the epoxide ring and the ester group, with the object of obtaining a 3β,20-diacetoxy-17α-hydroxy-5-allo-21-norpregnane (III) derivative.

The starting material Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester (I) was prepared by the method of Ruzicka, Plattner, Heusser and Pataki[7].

According to Weitz and Scheffer[8], in general the α,β-unsaturated ketones and esters are not easily attacked by perbenzoic acid. Ruzicka, Hardegger and Kauter[9] got on treating $\Delta^{5;16}$-3 β-acetoxy-etiocholadienic acid methyl ester with perbenzoic acid only the 5,6 α-and β-epoxides. However it must be mentioned

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(4) *T. H. Kritchevsky* and *T. F. Gallagher*, J. Biol. Chem. **179**, 507 (1949); *B. A. Koechlin, D. L. Garmaise, T. H. Kritchevsky* and *T. F. Gallagher*, J. Am. Chem. Soc. **71**, 3262 (1949).

(5) *P. L. Julian, E. W. Meyer, W. S. Karpel* and *I. Ryden*, J. Am. Chem. Soc. **71**, 3574 (1949); *P. L. Julian, E. W. Meyer, W. S. Karpel* and *W. Cole*, J. Am. Chem. Soc. **73**, 1982 (1951).

(6) *Pl. A. Plattner, H. Heusser* and *M. Feurer*, Helv. Chim. Acta **31**, 2210 (1948); *P. L. Julian, E. W. Meyer* and *I. Ryden*, J. Am. Chem. Soc. **71**, 756 (1949).

(7) *L. Ruzicka, Pl. A. Plattner, H. Heusser* and *J. Pataki*, Helv. Chim. Acta **29**, 936 (1946).

(8) *E. Weitz* and *A. Scheffer*, Ber. **54**, 2327 (1921).

(9) *L. Ruzicka, E. Hardegger* and *C. Kauter*, Helv. Chim. Acta **27**, 1164 (1944).

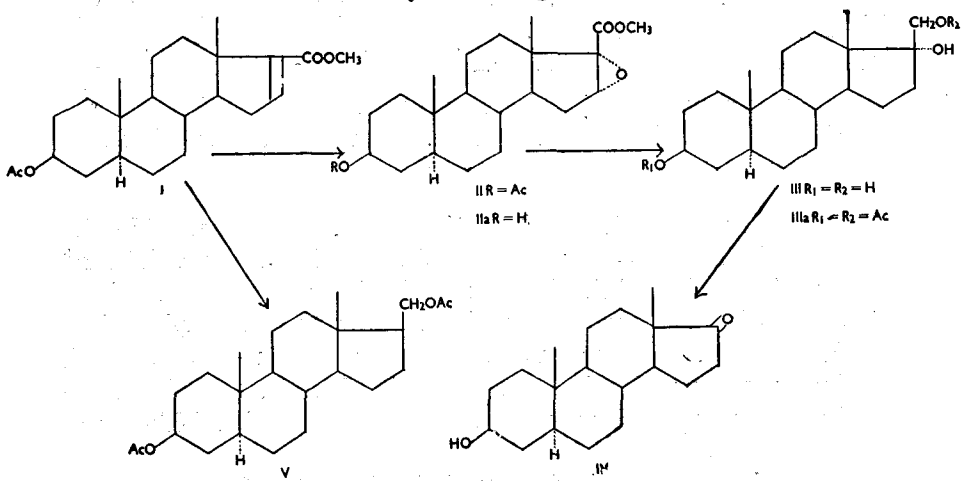
that on treating the same compound with a 30% excess of perbenzoic acid, they did obtain a product which had an oxygen atom more and could have been the 5, 6 and 16,17 diepoxide.

This difficulty in the formation of an epoxide of an unsaturated ester may be because of the stability due to the resonance energy of the α, β unsaturated ester. This mesomerism would be destroyed to a certain extent in the formation of a three membered epoxide ring, so that this addition would be hindered; whereas in the case of an isolated double bond there arises no such question of mesomerism at all.

Swern[10] believes that the introduction of an electron attracting group such as the carbonyl in an ethylenic system diminishes the nucleophilic properties of the double bond, thereby causing a reduction in the reaction rate. Conversely electron-releasing groups cause an increase in the rate of the reaction.

So the solution for the formation of an epoxide lies either in removing the conjugation or using more drastic conditions of experiment.

Lithium aluminium hydride reduction of Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester ought to leave the 16,17 double



bond without any conjugation, and make it susceptible to perbenzoic acid oxidation. However, this compound (Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester) on reduction gave an oil, which

(10) *D. Swern*, J. Am. Chem. Soc. **69**, 1692 (1947).

on acetylation and chromatography gave as the only crystalline fraction 3β-20-diacetoxy-5-allo-21-norpregnane (V) with the reduction of the 16, 17 conjugated double bond.

The reduction of the α,β-conjugated double bond with lithium aluminium hydride was already observed by Nystrom and Brown[11] in the case of the reduction of cinnamic acid to hydrocinnamyl alcohol; by Karrer and Bannerjea[12] in the reduction of o-hydroxy cinnamic acid to o-hydroxyphenylpropanol; and by Dornow, Messwarb and Frey[13] in the reduction of isoamylidene-α-cyano-acetic ester to 3-amino-2-isoamylpropanol. Besides the α,β-conjugated esters, Uffer and Schlittler[14] noticed that the α,β-unsaturated amides also got reduced by lithium aluminium hydride with the reduction of the α,β-conjugated double bond as in the case of α-ethylcrotonamide to α-ethylbutylamine.

The other alternative was to treat the compound with a large excess of perbenzoic acid for a comparatively long period of time to force the introduction of the epoxide group. Δ^{16}-3β-Acetoxy-5-alloetiocholenic acid methyl ester (I) was treated with about 100% excess of perbenzoic acid in chloroform solution at room temperature for about 42 hours, giving the desired 16,17 epoxide (II) in fairly good yields. It was found that if the chloroform used is not pure, then there is the hydrolysis of the 3β-acetoxy to a 3β-hydroxy compound (IIa,) which may be due to the action of some dissolved hydrochloric acid, formed from the decomposition of chloroform.

Now regarding the configuration of the 16,17 epoxide the steric position of C_{17} must be considered. Fieser and Fieser[15] from an inspection of the Stuart models observed that " the front side of C_{17} is the same distance from the angular methyl carbon as the rear side is from the carbon atom 12, but the vibrating methyl group can dominate more space than the restricted 12-methylene group and so exert a short-range or bond-hindrance

(11) *R. F. Nystrom* and *W. G. Brown*, J. Am. Chem. Soc. **69**, 2548 (1947).
(12) *P. Karrer* and *P. Bannerjea*, Helv. Chim. Acta **32**, 1692 (1949).
(13) *A. Dornow*, *G. Messwarb* and *H. H. Frey*, Ber. **83**, 445 (1950).
(14) *A. Uffer* and *E. Schlittler*, Helv. Chim. Acta **31**, 1397 (1948).
(15) *L. F. Fieser* and *M. Fieser*, Natural Products Related to Phenanthrene, 3rd Ed., Reinhold, New York 1949, page 411.

effect " ; this brings about an attack of the entering group readily from the rear or the α-position as seen in the reactions with lithium aluminium hydride, R-magnesium bromide, potassium acetylide, and osmium tetroxide. Gallagher and Kritchevsky[16] have deduced from these results " the rule of the rear " which states " that when there is a plane of symmetry at C_{17} the entering group always attaches to C_{17} in the α-configuration". From the above argument it seems almost certain that the epoxide is the 16, 17 α-epoxide.

Lastly Heusser, Feurer, Eichenberger and Prelog[17] by the hydrogenation with lithium aluminium hydride of $\Delta^{1;3;5,(10)}$-3-benzoxy-16,17 α-epoxy-oestratrien and Δ^4-3-keto-16,17 α-epoxy-androstene obtained $\Delta^{1;3;5,(10)}$-3,17α-dihydroxy-oestratriene and Δ^4-3β-17α-dihydroxyandrostene. This experimental evidence clearly showed that the epoxide bridge was in the 16, 17 α-position.

It has been found that the 11,12-[18] 4,5-[19] and the 16,17-epoxides[20] are extremely resistant to catalytic hydrogenation, but are rather easily reduced by the action of lithium aluminium hydride. The reduction of 3β-acetoxy-16,17α-epoxy-5-alloetiocholanic acid methyl ester (II) was carried out with lithium aluminium hydride; a large excess of this reagent had to be used to give a good yield of 3β-17α-20-trihydroxy-5-allo-21-norpregnane (III). This (III), on acetylation, gave 3β-20-diacetoxy-17α-hydroxy-5-allo-21-norpregnane (IIIa).

Finally, to confirm the structure of this 3β-17α-20-trihydroxy-5-allo-21-norpregnane, it was oxidised with periodic acid[21] to the known 3β-hydroxy-17-ketoandrostane (IV).

(16) *T. F. Gallagher* and *T. H. Kritchevsky*, J. Am. Chem. Soc. **72**, 882 (1950).
(17) *H. Heusser, M. Feurer, K. Eichenberger* and *V. Prelog*, Helv. Chim. Acta **33**, 2243 (1950).
(18) *M. Feurer*, Diss. E.T.H. Zurich 1951.
(19) *Pl. A. Plattner, H. Heusser* and *A. B. Kulkarni*, Helv. Chim. Acta **31**, 1885 (1948); ibid. **32**, 265 (1949); ibid. **32**, 1070 (1949).
(20) *Pl. A. Plattner, H. Heusser* and *M. Feurer*, Helv. Chim. Acta **31**, 2210 (1948).
(21) *Cf. H. E. Stavely*, J. Am. Chem. Soc. **63**, 3127 (1941).

Experimental Part*

Reduction of Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester (I) with lithium aluminium hydride.

0.506 g. of Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester (I) was dissolved in 65 cc. of absolute ether, and added drop by drop to a solution of 0.400 g. of lithium aluminium hydride in 40 cc. of ether under constant stirring at room temperature. After the addition the stirring was continued for another half an hour. Then 10 cc. of water and 30 cc. of H_2SO_4(2N) were added. The ether-water solution was extracted with ether, and then treated in the usual manner to give 0.430 g. of a partially crystalline product.

This product was acetylated with acetic anhydride and pyridine at room temperature; on working up it gave 0.435 g. of the acetylated substance.

The acetylated product was chromatographed over a column of activated alumina. The petrol ether-benzene fraction 4 : 1 was crystallized several times from methanol and gave a substance (V), melting at 138-140° C. The substance was dried for analysis under high vacuum at 90° C. for 60 hours.

2.453 mg. of the substance gave 6.623 mg. CO_2 and 2.069 mg. H_2O

Calculated for	$C_{24}H_{38}O_4$	C 73.80 %	H 9.81 %
Found		C 73.68 %	H 9.43 %

The substance gave a negative reaction with tetranitromethane, thus showing the absence of a 16,17 double bond.

The rest of the chromatogram fractions were oily and could not be crystallized.

Preparation of 3β-acetoxy-16,17α-epoxy-5-alloetiocholanic acid methyl ester.

0.400 g. of Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester (I) was taken in a round bottom flask and dissolved by adding 5 cc. of a chloroform solution of perbenzoic acid (6.8 mg./cc.). The flask was kept at room temperature for 42 hours.

* All m.p. are corrected and determined in evacuated capillaries.

After this period chloroform was completely evaporated under vacuum and the solid remaining in the flask was extracted with ether. The ether extract was washed with 2N Na_2CO_3, and later with water. It was dried over Na_2SO_4, and the ether evaporated to give 0.434 g. of a white solid substance. This substance on four crystallizations from CH_3OH gave crystals melting at 153-154° C. (II.) They were dried under high vacuum for analysis at 80° C. for 96 hours.

$[\alpha]_D^{24} = +36.8°$ (C = 0.82 in chloroform)

3.630 mg. of the substance gave 9.372 mg. CO_2 and 2.807 mg. H_2O

Calculated for $C_{23}H_{34}O_5$	C 70.76 %	H 8.78 %
Found	C 70.46 %	H 8.65 %

Preparation of 3β-hydroxy-16,17α-epoxy-5-alloetiocholanic acid methyl ester.

0.051 g. of Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester (I) was just dissolved in chloroform and 1.6 cc. of perbenzoic acid (3.6 mg./cc.) was added to it in the cold. The flask was allowed to stand at room temperature for 24 hours.

The chloroform was evaporated under vacuum and the substance was extracted with ether, washed with cold 2N. sodium carbonate solution, and rewashed with water till neutral. The ether extract was dried over Na_2SO_4 and evaporated to give 0.056 g. of an oily crystalline product. This was crystallized several times from methanol giving 3β-hydroxy-16,17α-epoxy-5-alloetiocholanic acid methyl ester (IIa) melting at 176-178° C. The substance was dried for analysis under high vacuum at 90° C. for 70 hours.

2.062 mg. of the substance gave 5.471 mg. CO_2 and 1.730 mg. H_2O

Calculated for $C_{21}H_{32}O_4$	C 72.38 %	H 9.26 %
Found	C 72.41 %	H 9.40 %

0.035 g. of this substance together with the mother liquor was acetylated with 3 cc. of acetic anhydride and 3 cc. of pyridine, and the acetylated product was chromatographed on activated alumina. The petrol ether and petrol ether-benzene 1:1 fractions were collected together and crystallized from methanol to give

3β-acetoxy-16,17α-epoxy-5-alloetiocholanic acid methyl ester (II) melting at 152-154° C. The substance showed no depression on mixed melting point determination with compound (II.) It was dried for analysis under high vacuum at 85° C. for 72 hours.

3.741 mg. of the substance gave 9.698 mg. CO_2 and 2.958 mg. H_2O

Calculated for $C_{22}H_{34}O_5$	C 70.76 %	H 8.78 %
Found	C 70.75 %	H 8.85 %

Reaction of 3β-acetoxy-16,17α-epoxy-5-alloetiocholanic acid methyl ester (II) with lithium aluminium hydride.

About 0.26 g. of pulverized $LiAlH_4$, was dissolved in about 100 cc. of absolute ether in a suitable round bottom flask fitted with a reflux condenser having a ground glass joint. 0.1246 g. of 3β-acetoxy-16,17α-epoxy-5-alloetiocholanic acid methyl ester (II) was dissolved in about 11 cc. of absolute ether and was added drop by drop with continuous stirring with a magnetic stirrer under moisture free conditions and at room temperature. After the addition was complete the reaction mixture was stirred for 3 hours at room temperature, and later on the reaction mixture was heated and refluxed for about one hour.

The reaction mixture was decomposed by first surrounding the flask with a freezing mixture and then adding water to it, drop by drop, till there was no more effervescence. This solution was now extracted with ether and shaken with a large quantity of dilute NaOH until all the inorganic material was dissolved and two clear layers were formed. The ether extract was washed with water till it was neutral, dried over Na_2SO_4 and evaporated to give 0.0893 g. of a white solid. This was crystallized from a mixture of ethyl acetate and petrol ether to give about 0.035 g. of a substance melting at 202-205° C. and about 0.035 g. of the same substance melting at 206-208° C. The latter portion was once again crystallized from the above solvent and gave crystals (III) melting at 206-208° C. These were sublimed under high vacuum for analysis at 130° C.

$[\alpha]_D^{24} = -11.0°$ (C = 0.766 in dioxane)

3.400 mg. of the substance gave 9.256 mg. CO_2 and 3.236 mg. H_2O

Calculated for $C_{20}H_{34}O_3$	C 74.49 %	H 10.63 %
Found	C 74.29 %	H 10.65 %

0.0409 g. of the above substance (III) was acetylated with 1 cc. of acetic anhydride and 0.5 cc. of pyridine by keeping it overnight at room temperature, and then for 1 hour on a water bath at 80° C. It was worked up in the usual manner and extracted with ether giving 0.0465 g. of a yellow solid. This was purified by boiling it with benzene and a trace of animal charcoal; afterwards this solution was filtered over a small column of aluminium oxide. On evaporation of benzene it gave 0.0303 g. of a white crystalline solid. A further 0.0135 g. was obtained by washing the column with ether. The mixed m.p. of both these fractions remained unaltered. They were crystallized several times from a mixture of ether and petrol ether; the crystals obtained melted at 153.5-156° C. They were dried under high vacuum for analysis at 70° C for 50 hours

$[\alpha]_D^{22} = -6.5°$ (C=0.771 in dioxane)

1.911 mg. of the substance gave 4.948 mg. CO_2 and 1.588 mg. H_2O

Calculated for $C_{24}H_{38}O_5$	C 70.90 %	H 9.42 %
Found	C 70.66 %	H 9.30 %

Periodic acid oxidation[21] of 3β-17α-20-trihydroxy-21-norpregnane (III).

0.0161 g. of 3β-17α-20-trihydroxy-21-norpregnane (III) was dissolved in 3 cc. of methanol, and to this was added about 0.036 g. of crystalline periodic acid dissolved in water. The mixture was left for 24 hours at room temperature, and then after adding water extracted with ether and ethyl acetate. The combined solvents were washed with Na_2CO_3 solution and later with water, dried over Na_2SO_4 and evaporated. The neutral material weighed 0.0179 g. It was crystallized several times from ether-pentane to give 0.007 g. of crystals (IV) melting at 168-170° C. The mixed melting point with 3β-hydroxy-17-ketoandrostane showed no depression. These crystals were sublimed under high vacuum for analysis at 80° C.

2.921 mg. of the substance gave 8.41 mg. CO_2 and 2.66 mg. H_2O

Calculated for $C_{19}H_{30}O_2$	C 78.57 %	H 10.41 %
Found	C 78.57 %	H 10.19 %

SUMMARY

PART I

21-Chloro-and 21-bromo-pregnenolone gave on treatment with potassium methoxide in methanol by Faworsky rearrangement the Δ^5-3β-acetoxy-17-methyletiocholenic acid methyl ester, and on hydrogenation the corresponding 5, 6 saturated ester. Both these compounds can also be obtained through Aston-Greenburg rearrangement of 3β-acetoxy-5, 6, 17-tribromo-20-keto pregnane. The compound described in the literature by Koechlin and Reichstein as 3β-acetoxy-17-methyl-5-alloetiocholanic acid methyl ester, was proved to be the $\Delta^{17,20}$-3β-acetoxy-5-allopregnene-21-acid methyl ester.

PART II a

Base catalysed reactions with methanolic potassium hydroxide and potassium methoxide and subsequent acetylation of Δ^{16}-3β-acetoxy-14,15β-epoxy-5-alloetiocholenic acid methyl ester gave by the addition of methanol to the double bond at the 16,17 position altogether three of the four possible isomers. Two of these isomers were obtained when the reaction was base catalysed with methanolic potassium hydroxide and the third when methanolic potassium methoxide was used as a base catalyst. One of these isomers was reacted with lithium aluminium hydride, and also with a mixture of concentrated hydrochloric acid and acetic acid with the formation of a reduced compound and a chlorohydrine respectively. The infra-red spectra of these four isomers showed the presence of an epoxide ring, of a methoxyl group and the absence of a double bond. From these investigations the structure of these isomers was assumed to be 3β-acetoxy-14,15β-epoxy-16ξ-methoxy-5-allo-17ξ-etiocholanic acid methyl ester.

PART II b

Δ^{16}-3β-Acetoxy-14,15β-epoxy-20-keto-5-allopregnene was also subjected under similar conditions to a base catalysed reaction with methanolic potassium hydroxide and here also there was an addition of methanol to the 16,17 double bond. But in this

case only one of the four possible isomers was obtained. Further, acid catalysed reactions were also studied with Δ^{16}-3β-acetoxy-14,15β-epoxy-20-keto-5-allopregnene. Here it was noticed that only the 14,15 epoxide ring was broken up giving Δ^{16}-3β-acetoxy-14ξ-15ξ-dihydroxy-20-ketopregnene.

PART III

One of the known methods for the introduction of a 17α-hydroxyl group in the steroid nucleus is through the corresponding 16,17α-epoxide. An attempt was made to try out this method on Δ^{16}-3β-acetoxy-5-alloetiocholenic acid methyl ester. Since in this ester the 16,17 double bond is conjugated with an electron attracting carbonyl group the epoxide formation is difficult. Reducing the carbonyl group with lithium aluminium hydride also reduced the double bond thus making the epoxide formation impossible. Therefore the above Δ^{16}-etiocholenic acid methyl ester was treated with a 100% excess of perbenzoic acid for 42 hours ; this gave the desired 3β-acetoxy-16, 17α-epoxy-5-alloetiocholanic acid methyl ester. This epoxide on reduction with lithium aluminium hydride yielded 3β-17α, 20-trihydroxy-5-allo-21-norpregnane. The structure of this compound was proved by periodic acid oxidation to the known 3β-hydroxy-17-ketoandrostane.

Zusammenfassung

TEIL I

21-Chlor-und 21-Brom-pregnenolon ergaben nach Behandlung mit Kalium-methylat in Methanol durch Faworsky'sche Umlagerung den Methylester der Δ^5-3β-Acetoxy-17-methyl-ätiocholensäure, und nach Hydrierung den entsprechenden gesättigten Ester. Man kann diese beiden Verbindungen auch durch Aston-Greenburg'sche Umlagerung des 3β-Acetoxy-5,6,17-tribrom-20-ketopregnans erhalten. Es wurde bewiesen, dass die in der Literatur durch Koechlin und Reichstein als 3β-Acetoxy-17-methyl-5-ällo-atiocholansäure-methylester beschriebene Verbindung, den $\Delta^{17,20}$-3β-Acetoxy-5-allopregnen-21-säure-methylester darstellt.

TEIL IIa

Bei der durch Basen katalysierten Addition von Methanol an die Doppelbindung des Δ^{16}-3β-Acetoxy-14, 15-oxydo-5-allo-ätiocholensäure-methylesters konnten nach anschliessender Acetylierung drei der insgesamt vier möglichen stereoisomeren Additionsprodukte isoliert werden; zwei dieser Isomeren wurden bei der Reaktion in Gegenwart von Kalium-hydroxyd erhalten, und das dritte Isomere bildete sich bei der in Gegenwart von Kaliummethylat durchgeführten Addition. Auf Grund des Verlaufs verschiedener an diesen Verbindungen durchgeführten Umsetzungen sowie nach den IR.-Spektren wird angenommen, dass in den Additionsprodukten stereoisomere 3β-Acetoxy-14,15-oxydo-16ξ-methoxy-5-allo-17ξ-ätiocholansäure-methylester vorliegen.

TEIL IIb

Unter ähnlichen Reaktionsbedingungen wurde auch Δ^{16}-3β-Acetoxy-14,15β-oxydo-20-keto-5-allopregnen der Behandlung mit Kaliumhydroxyd in Methanol unterworfen, wobei auch hier Methanol sich an die Doppelbindung anlagerte. In diesem Fall aber wurde nur eines der vier möglichen isomeren Additions-produkte erhalten. Es wurden ferner säurekatalysierte Reaktionen an Δ^{16}-3β-Acetoxy-14,15β-oxydo-20-keto-5-allopregneu studiert. Hier

stellte man fest, dass nur der Oxydring aufgespalten wurde, und zwar unter Bildung von Δ^{16} 3β-Acetoxy-14ξ-15ξ-dihydroxy-20-ketopregnen.

TEIL III

Eine der bekannten Methoden für die Einführung einer 17α-Hydroxylgruppe im Steroidgerüst führt über das entsprechende 16, 17α-Oxyd. Es wurde versucht, diese Methode bei Δ^{16}-3β-Acetoxy-5-allo-ätiocholensäure-methylester anzuwenden. Da in diesem Ester die 16,17-Doppelbindung mit der Carbonylgruppe konjugiert ist, verläuft die Epoxydbildung nur sehr langsam. Reduktion der Carbonylgruppe mit Lithiumaluminiumhydrid hatte auch die Reduktion der Doppelbindung zur Folge. Die Umsetzung des Δ^{16}-ätiocholensäure-methylesters während 42 Stunden, mit einem 100-proz. Überschuss von Perbenzoesäure, ergab den gewünschten 3β-Acetoxy-16,17α-oxydo-5-allo-ätiocholansäure-methylester und dessen Reduktion mit Lithiumaluminiumhydrid lieferte 3β, 17α, 20-trihydroxy-5-allo-21-norpregnen. Mit Hilfe des oxydativen Abbaus mittels Perjodsäure zum bekannten 3β-Hydroxy-17-ketoandrostan wurde die Struktur dieser Verbindung bewiesen.

CURRICULUM VITAE

I, Sam Framroze Boyce, was born on 9th December, 1918, at Bombay, India. I passed the Matriculation Examination of the University of Bombay, from the St. Xavier's High School, Bombay, in 1935. I joined the St. Zavier's College and obtained the degree of Bachelor of Science of the University of Bombay in 1941, with Chemistry as my principle subject, and Physics as a subsidiary subject. I carried out research on " Synthesis of Some Quinoline Derivatives " at the Royal Institute of Science, Bombay, for which I was awarded the Master of Science degree of the University, of Bombay in 1947. During the period I was working for my M.Sc., I was employed part-time by my father who was an industrial chemist. During 1940-41 I was in the University Training Corps, and during 1944-45, in the Indian Air Training Corps.

In 1947 I joined the Swiss Federal Institute of Technology and undertook the foregoing research work, which was finished in June, 1951. This work was interrupted due to my illness and also because of my visit to Bombay on account of the illness of my father.