[Contribution from the George S. Cox Medical Research Institute, University of Pennsylvania]

INVESTIGATIONS ON STEROIDS. XI. NEW TRANSFORMATION PRODUCTS OF STROPHANTHIDIN: STUDIES ON ETHYL 3(β),5,19-TRIHYDROXYETIOCHOLANATE[1]

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With the aim of preparing steroids structurally related to progesterone and the adrenal cortical hormones, strophanthidin (I) has been transformed into a number of new compounds which will serve as intermediates towards this goal. It is intended to prepare ultimately substances which are derived from progesterone, 11-desoxycorticosterone, and 17-hydroxy-11-desoxycorticosterone in that the angular carbon atom 19 between rings A and B is either missing (19-nor compounds) or is present in an oxygenated form, *e.g.* as a primary alcohol, aldehyde or carboxyl group. Not only compounds with "normal" configurations, but also those which possess "iso" configurations in particular at carbon atoms 14 and 17 will be included in our studies. The present investigation is dealing essentially with steroids possessing the "normal" configurations at these carbon atoms.

The previously (1) reported conversion of strophanthidin (I) into ethyl 3(β), 5,19-trihydroxyetiocholanate (VIII) and the corresponding free acid (IX) has been simplified and improved in a number of respects, details of which are given in the experimental section. The yield of crystalline ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate (VI) as obtained by direct crystallization is 33–35 per cent. In addition, there were isolated small amounts of a by-product, $C_{20}H_{28}O_4$, tentatively formulated as a lactone (1). The substance $C_{20}H_{28}O_4$ apparently does not arise from an impurity in 3(β),5,14,19-tetrahydroxy-14-isoetiocholanic acid (V)[3] since very pure samples of V also gave the so-called lactone. It is a rather labile substance and is altered on attempted recrystallization. On subjecting

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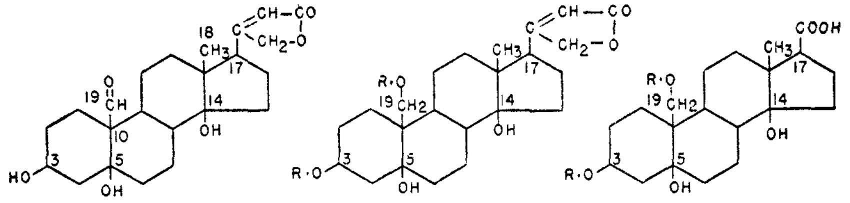
[2] Misses A. R. Johnson and V. I. Vivian as well as Mrs. P. Comegys Olmsted participated in particular in the preparation of larger quantities of ethyl 3(β),5,19-trihydroxyetiocholanate in accordance with an improved method. Miss M. A. Wagner was in charge of all experiments dealing with the products resulting from the Raney nickel dehydrogenation as well as the tritylation of ethyl 3(β),5,19-trihydroxyetiocholanate. Capable assistance was also rendered by Miss Mary G. Conroy.

[3] In our previous publication (1) compounds V and IV had been designated 3,5,14,19-tetrahydroxyetiocholanic acid and 3,19-diacetoxy-5,14-dihydroxyetiocholanic acid respectively. The new names are in stricter agreement with the nomenclature (2).

it to chromatographic purification it was recovered from the early eluates. A new substance melting at 167–168°, tentatively assigned the formula $C_{22}H_{32}O_5$, was found in some of the later eluates. Chromatography of the mother liquors from the crystallization of VI gave some ethyl 3(β),5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate (*cf.* 1) and additional amounts of VI which was characterized as the diacetate (VII). Attempts to transform IV into VI by simultaneous dehydration and alcoholysis resulted in an impractically low yield.



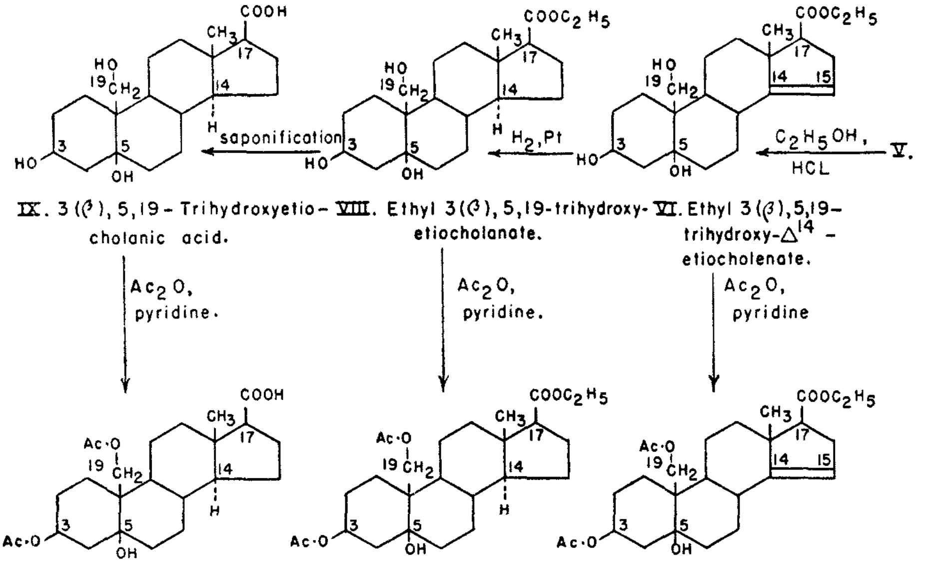
I. Strophanthidin

II. (R=H) Strophanthidol

III. (R=Ac) Strophanthidol 3,19-diacetate.

V. (R=H) 3(β),5,14,19-Tetrahydroxy-14-isoetiocholanic acid.

IV. (R=Ac) 3(β),19-Diacetoxy-5,14-dihydroxy-14-isoetiocholanic acid.



IX. 3(β),5,19-Trihydroxyetiocholanic acid.

VIII. Ethyl 3(β),5,19-trihydroxy-etiocholanate.

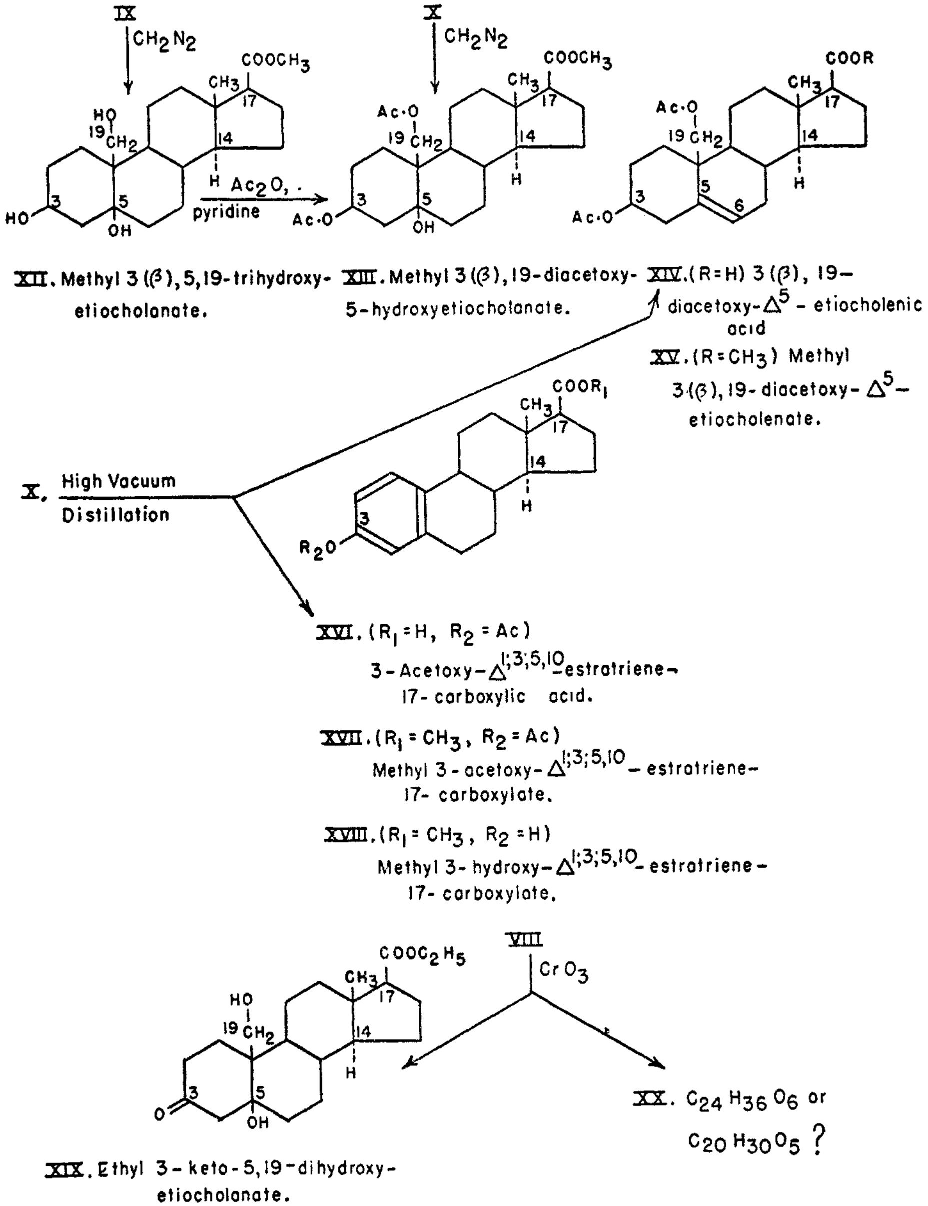
VI. Ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate.

X. 3(β),19-Diacetoxy-5-hydroxy-etiocholanic acid.

XI. Ethyl 3(β),19-diacetoxy-5-hydroxyetiocholanate

VII. Ethyl 3(β),19-diacetoxy-5-hydroxy-Δ^{14}-etiocholenate.

Acetylation of 3(β),5,19-trihydroxyetiocholanic acid (IX) (1) gives the amorphous 3(β),19-diacetate (X). The latter yields with diazomethane the amorphous methyl ester (XIII) which can also be obtained by acetylation of crystalline XII (1). It may be noted that compounds analogous to X and XIII having an additional hydroxyl group at carbon atom 14, *i.e.* IV and its methyl ester, are likewise amorphous (1, 2).



XII. Methyl 3(β),5,19-trihydroxy-etiocholonate.

XIII. Methyl 3(β),19-diacetoxy-5-hydroxyetiocholonate.

XIV. (R = H) 3(β), 19-diacetoxy-Δ^5-etiocholenic acid

XV. (R = CH_3) Methyl 3(β),19-diacetoxy-Δ^5-etiocholenate.

XVI. (R_1 = H, R_2 = Ac) 3-Acetoxy-$\Delta^{1,3,5,10}$-estratriene-17-carboxylic acid.

XVII. (R_1 = CH_3, R_2 = Ac) Methyl 3-acetoxy-$\Delta^{1,3,5,10}$-estratriene-17-carboxylate.

XVIII. (R_1 = CH_3, R_2 = H) Methyl 3-hydroxy-$\Delta^{1,3,5,10}$-estratriene-17-carboxylate.

XIX. Ethyl 3-keto-5,19-dihydroxy-etiocholonate.

XX. $C_{24}H_{36}O_6$ or $C_{20}H_{30}O_5$?

Ethyl 3(β),19-diacetoxy-5-hydroxyetiocholanate (XI) can be distilled in a high vacuum without decomposition. As reported earlier (1) it also remains unchanged on treatment with pyridine-phosphorus oxychloride at room temperature. It was not attempted to enforce a dehydration at a higher temperature, because treatment of the methyl ester of IV with pyridine-phosphorus oxychloride at room temperature did not proceed in a uniform fashion (2).

Contrary to expectations based on previous experience (3) (4, p. 1048) distillation of 3(β),19-diacetoxy-5-hydroxyetiocholanic acid (X) led to a mixture of substances from which only a small amount of the anticipated 3(β),19-diacetoxy-Δ^5-etiocholenic acid (XIV) could be isolated as the methyl ester (XV). The major part of the material was the product of a more extensive decomposition. The portion which crystallized from ether was converted into the methyl ester. After purification by chromatography and deacetylation, methyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate (XVIII) was identified by comparison with a sample of the material recently synthesized from cholesterol by Djerassi and Scholz[5] (6, *cf.* also 7). The absorption spectrum of XVIII[4] showed a maximum at 281 mμ and a minimum at 249 mμ which is characteristic of natural estrogens in which ring A is benzenoid (5). It must have arisen from 3-acetoxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylic acid (XVI) by way of methyl 3-acetoxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate (XVII).

It was attempted to subject ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) to selective oxidation either in position 3 or 19. Oxidation of VIII at room temperature with one equivalent of chromic acid yielded 80% of neutral material. Crystallization from ether gave a substance, probably $C_{22}H_{34}O_5$, which was characterized by its oxime. From its infrared spectrum (obtained through the courtesy of Drs. K. Dobriner and R. Norman Jones of the Sloan-Kettering Institute for Cancer Research in New York) the presence of a 3-keto group was indicated.[5a] The substance is tentatively designated as ethyl 3-keto-5,19-dihydroxyetiocholanate (XIX). From the lower-melting and resinous fractions resulting from the isolation of XIX another substance, possibly $C_{24}H_{36}O_6$ or $C_{20}H_{30}O_5$ (XX), was obtained after chromatography. It still awaits chemical identification.[5b] Attempts to prepare an oxime were of no avail.

An effort was made to dehydrogenate ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) according to the Oppenauer method. In general, the dehydrogenation of secondary alcohol groups in position 3 does not present any difficulties, and the participation in the reaction of the primary alcohol group in position 19 was not expected. As is known from the literature, primary alcohols will react only in case they contain an activated hydroxyl group (8, 9). On treating ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) in the usual fashion with acetone and aluminum *tert*-butoxide in benzene solution, there was an almost quantitative recovery

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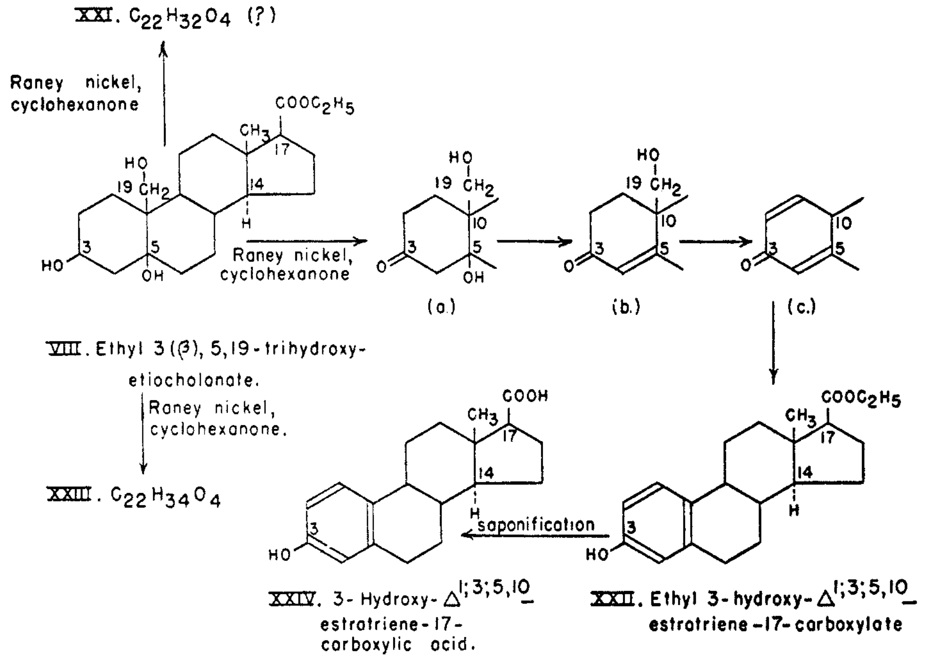
[4] Determination by Messrs. J. L. Ciminera and K. B. Streeter of the Sharp & Dohme Research Laboratories.

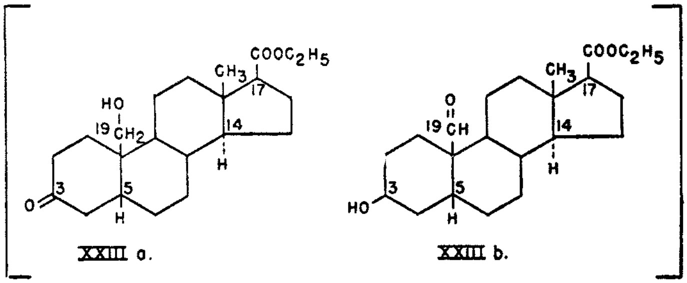
[5] Private communication; published while this paper was in press (6).

[5a] Position of bands ($CHCl_3$): 1723 cm.$^{-1}$ (probably $COOC_2H_5$); 1710 cm.$^{-1}$ (probably 3-ketone or possibly, but less likely an aldehyde).

[5b] Determination of the infrared spectrum at the Sloan-Kettering Institute gave the following bands ($CHCl_3$): 1723–1719 cm.$^{-1}$ (probably $COOC_2H_5$ and/or aldehyde); 1703 cm.$^{-1}$ (could be 3-ketone; too low for aldehyde).

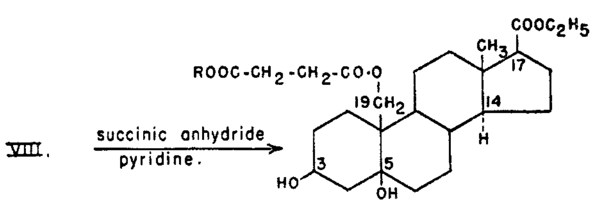
of the starting material. Aluminum phenoxide has been used successfully for the dehydrogenation of secondary alcohol groups in position 3 of saturated





XXIII a.

XXIII b.



XXV. (R = H) Ethyl 19-hemisuccinyloxy-3(β),5-dihydroxyetiocholanate.

XXVI. (R = CH_3) Methyl ester of ethyl 19-hemisuccinyloxy-3(β), 5-dihydroxyetiocholanate.

steroids, especially where aluminum *tert*-butoxide had failed (10, *cf.* also 11, 12). Substituting aluminum phenoxide for aluminum *tert*-butoxide with ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) did not produce any result; under these experimental conditions ketonic material failed to appear. It would be interesting to determine whether there is any definite group in the molecule which prevents the reaction from proceeding in the normal fashion. In particular, the hydroxyl group in position 5 should be considered. In the present instance the hydroxyl groups at carbon atoms 3 and 5 are in *cis*-position. The question arises, whether an intermediary aluminum complex is formed in which both of these hydroxyl groups are involved, and whether such a complex would block the normal course of the reaction. It is proposed therefore, to study the Oppenauer reaction with various 3,5-dihydroxy steroids. Compounds having these two hydroxyl groups in *cis*-position as well as those having them in *trans*-position should be included.

Kleiderer, *et al.* (13, 14) recently described the dehydrogenation with Raney nickel (15) of 3-hydroxy steroids to the corresponding 3-keto compounds in the presence of cyclohexanone as hydrogen acceptor. This reaction was applied to ethyl 3(β),5,19-trihydroxyetiocholanate (VIII). In this instance the possibility of the formation of an aldehyde group at carbon atom 10 had to be considered. This might be followed by subsequent condensation of the aldehyde group with cyclohexanone.[6] The experiment was performed under conditions similar to those of Kleiderer and Kornfeld (14). The reaction yielded three products on chromatography.

The least polar substance (XXI) may possess the formula $C_{22}H_{32}O_4$. It is probably saturated. The amount obtained did not permit further investigation.

The second substance, as isolated in the process of chromatographic separation, was identified as ethyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate (XXII). The melting point was 176–178° (remelting at 184–186°). It is to be noted that, though this substance is a phenol, it is not soluble in a solution of 2 *N* sodium hydroxide. The ultraviolet absorption curve, plotted as molecular extinction coefficients, is practically identical with that of a reference sample of estradiol (Figure 1). With tetranitromethane the substance gave an orange color. Saponification of this compound (XXII) yielded 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylic acid (XXIV) with the melting point 266–270°. The identical acid has been prepared by Djerassi and Scholz[5] (6) from methyl 3-keto-$\Delta^{1;4}$-etiocholadienate which in turn has been synthesized from cholesterol (7). The mixed melting point of the samples prepared in the two laboratories did not show a depression. It appears noteworthy, especially from the viewpoint of stereochemical considerations, that an identical product has been obtained from strophanthidin and cholesterol.

Furthermore it should be pointed out that Djerassi and Scholz[5] (6) have transformed XXIV into 3-methoxy-17-(β-acetoxyacetyl)-$\Delta^{1;3;5,10}$-estratriene, a compound which they could also prepare from 17-ethinylestradiol which is accessible from estrone. Hence, a correlation between the cardiac aglycones and the hormones of the estrogen series, which had been attempted by Butenandt and Gallagher (17), has been established.

It is not intended to present a detailed discussion of the reaction mechanism of the conversion of VIII into XXII. It is reasonable to assume that first dehy-

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[6] Lit. *cf.* (9, p. 1266); for additional example *cf.* (16).

drogenation occurs at carbon atom 3 leading to (a) which is easily dehydrated to (b). The mechanism of the loss of carbon atom 19 is not clear. It is uncertain whether one molecule of methanol is split off directly or whether there is first a dehydrogenation to an aldehyde group followed by the elimination of one molecule of formaldehyde. The intermediate (c), a dienone, will rearrange to the phenol XXII.

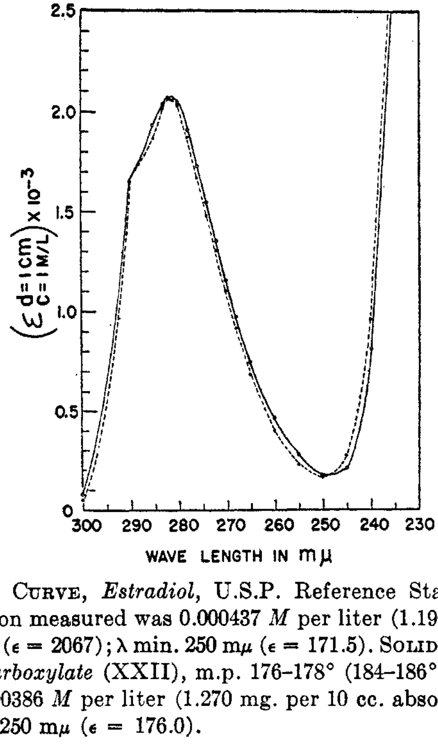

FIGURE 1.[7] BROKEN CURVE, *Estradiol,* U.S.P. Reference Standard, m.p. 174.5–176°; concentration of solution measured was 0.000437 *M* per liter (1.190 mg. per 10 cc. absolute alcohol). λ max. 282 mμ (ϵ = 2067); λ min. 250 mμ (ϵ = 171.5). SOLID CURVE, *Ethyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate* (XXII), m.p. 176–178° (184–186°); concentration of solution measured was 0.000386 *M* per liter (1.270 mg. per 10 cc. absolute alcohol). λ max. 282 mμ (ϵ = 2075); λ min. 250 mμ (ϵ = 176.0).

The ethyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate (XXII) and the 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylic acid (XXIV) have been assayed for estrogenic activity through the courtesy of Drs. E. A. Doisy and S. A. Thayer at St. Louis University School of Medicine. The material was dissolved in water with the aid of a small amount of alcohol and injected into spayed mice according to a standard procedure, using estrone as the standard. Twenty mice were used at each dose level. The standard gave the usual 50% response at a level of 0.05 micrograms. Both XXII and XXIV were inactive at 0.05 micrograms and 10 micrograms. The ethyl ester (XXII) was also inactive at a level of 20 micrograms. The latter dosage represents the maximum solubility of this compound in water.

The third substance (XXIII) isolated from the chromatogram probably possesses the formula $C_{22}H_{34}O_4$ and, in view of its polarity, the structures XXIIIa or XXIIIb may be considered. It appears to be saturated and gives a resinous acetate from which the original material may be recovered by saponification.

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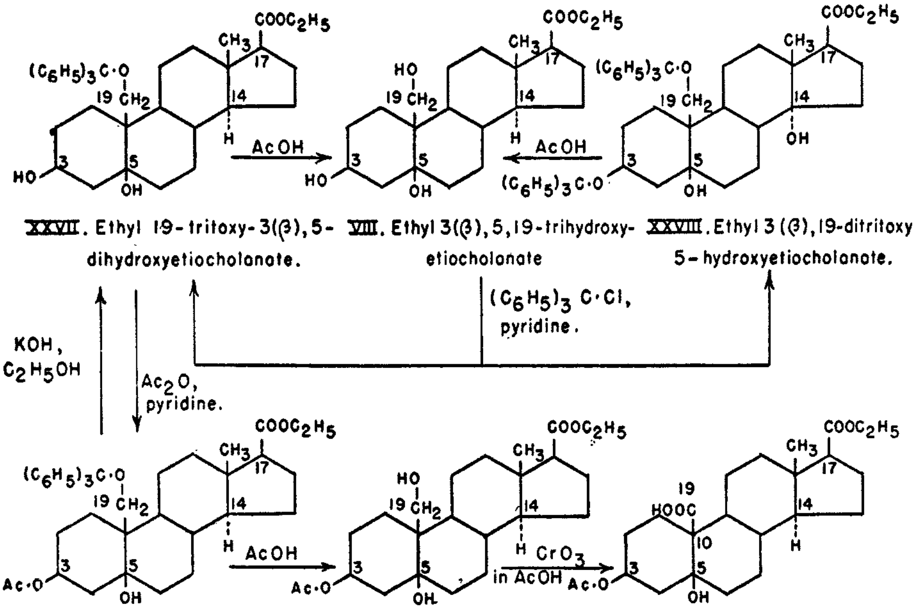
[7] The absorption curves were kindly determined by Dr. D. L. Drabkin (Dept. of Physiological Chemistry, Graduate School of Medicine, University of Pennsylvania) with a Beckman DU Spectrophotometer with a 1 cm. depth silica cuvette.

Another approach to achieving partial oxidation of VIII would have consisted in performing a partial deacetylation of XI followed by oxidation of the resulting monoacetate. Various attempts to bring about such a partial deacetylation failed. Partial succinoylation (*cf. e.g.*, 18, 18a, 19, 20, 21, 21a) of VIII furnished a small yield of a resinous acid succinate interpreted to possess formula XXV. It was characterized as the crystalline methyl ester XXVI.

As is known from sugar chemistry, primary alcohol groups react much more rapidly with triphenylchloromethane than secondary ones. If one uses stoichiometric proportions the primary group or groups can be tritylated, whereas the reaction with secondary alcohol groups is negligible. There are almost no instances of the use of this reagent in steroid chemistry (*cf.*, *e.g.* 22). Ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) represented a test case, in that it contains a primary alcohol group at carbon atom 19, and a secondary one at carbon atom 3. On treating it with one mole of triphenylchloromethane, ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (XXVII) was obtained as main reaction product. It was accompanied by a small amount of ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate (XXVIII) and a fair quantity of unchanged starting material, (VIII). A regular by-product was triphenylcarbinol. These four components of the reaction mixture may be separated quantitatively by chromatography. Considering the fact that part of the triol VIII is recovered unchanged and that the ditrityl ether XXVIII can be smoothly hydrolyzed to the triol VIII, no steroid material is lost.



XXVII. Ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate.

VIII. Ethyl 3(β),5,19-trihydroxyetiocholanate

XXVIII. Ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate.

XXIX. Ethyl 3(β)-acetoxy-19-tritoxy-5-hydroxyetiocholanate.

XXX. Ethyl 3(β)-acetoxy-5,19-dihydroxyetiocholanate.

XXXI. 3(β)-Acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester.

A complete cleavage of the 19-monotrityl ether XXVII to the triol VIII is best achieved by treating it at room temperature with slightly diluted acetic acid overnight. Attempts to prepare under similar conditions ethyl 3(β)-tritoxy-5,19-dihydroxyetiocholanate by partial hydrolysis of the ditrityl ether XXVIII failed. Only the triol VIII resulted.

On acetylating ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (XXVII) in the usual fashion, the resinous ethyl 3(β)-acetoxy-19-tritoxy-5-hydroxyetiocholanate (XXIX) was obtained. The uniformity of this resinous substance was assured by chromatographic purification. On saponifying XXIX in an alkaline medium (KOH, absol. ethanol), ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (XXVII) was recovered. On performing the hydrolysis of XXIX in slightly diluted acetic acid, cleavage of the ether linkage occurred leading to ethyl 3(β)-acetoxy-5,19-dihydroxyetiocholanate (XXX). This monoacetate could easily be subjected to an oxidation with chromic acid yielding the 20-ethyl ester of 3(β)-acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid (XXXI).[8] The dicarboxylic acid from which the latter ester (XXXI) is derived, *i.e.* 3(β),5-dihydroxy-21-norpregnane-19,20-dioic acid is a stereoisomer of a compound prepared in Butenandt's laboratory (17) and by Ehrenstein (3). The latter possesses the iso configurations at carbon atoms 14 and 17.

According to preliminary experiments XXVII can be oxidized to the corresponding 3-keto compound by N-bromoacetamide (25, 26, 27). Treatment of XXVII with chromium trioxide in acetic acid is connected with simultaneous cleavage of the ether linkage and, therefore, apparently a mixture of oxidation products results.

EXPERIMENTAL

The melting points were determined with the Fisher-Johns melting-point apparatus. The readings are sufficiently near the true melting points so that no corrections have been made. Unless stated otherwise the microanalyses were carried out by Mr. Joseph F. Alicino, Metuchen, N. J. (J.F.A.), Dr. E. W. D. Huffman, Denver 2, Colo. (E.W.D.H.), and Mr. James Rigas, Brooklyn 25, N. Y. (J.R.).

Further observations on the preparation of the starting material: Ethyl 3(β),5,19-trihydroxyetiocholanate (VIII)

a. *Strophanthidol* (II). The melting point of this substance, when crystallized from ethyl acetate, was usually between 216 and 222°.

b. *Strophanthidol 3,19-diacetate* (III). After the termination of the acetylation of strophanthidol (1), the reaction product is poured into water. The following day the crystalline material is washed with 0.5 *N* hydrochloric acid and with water. The subsequent treatment remains unchanged; recrystallization of the crude diacetate from acetone-petroleum ether or acetone-ether.

c. *3(β),19-Diacetoxy-5,14-dihydroxy-14-isoetiocholanic acid* (IV). Since publication of the earlier paper (1) the oxidation of a total of 494.03 g. of III yielded 206.64 g. (41.8% of the invested material) of acid-ether extract. Reoxidations of a total of 69.82 g. of neutral oxidation product gave 22.21 g. (31.8%) of acid-ether extract. On reoxidizing (ratio of $KMnO_4$ one-half that of an original oxidation) neutral material which had been oxidized twice, a yield of about 12% of acid-ether extract resulted. In any instance the subsequent

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[8] This nomenclature is based on recent proposals by Reichstein (23). A different nomenclature has been suggested by Fieser (24, page 522; footnote 15).

extraction with ethyl acetate furnished additional acid fractions. However, on saponification of such material either no, or only small, amounts of V resulted.

d. *3(β),5,14,19-Tetrahydroxy-14-isoetiocholanic acid* (V). Since the last report (1) a total of 148.35 g. of acid-ether extract was converted into 63.60 g. of crystalline tetrahydroxy acid (V).[9] The resinous products (45.51 g.) still contain appreciable amounts of V (1). The saponification of acid-ether extracts originating from reoxidations of neutral material furnished considerably smaller yields of crystalline V.

e. *Dehydration of 3(β),5,14,19-tetrahydroxy-14-isoetiocholanic acid* (V): *Crystalline ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate* (VI) *and other products.* A solution of 3.5 g. of 3(β),5,14,19-tetrahydroxy-14-isoetiocholanic acid in 102 parts of approximately 0.1 *N* absolute alcoholic hydrogen chloride was kept just at the boiling point (bath temperature 81–87°) for a period of two hours. A slow distillation was subsequently performed under such conditions that within 1½ hours the solution was reduced to about ⅙ of its volume. Addition of water until the first appearance of turbidity and subsequent heating gave a clear solution. On standing overnight needles of the compound $C_{20}H_{28}O_4$, tentatively designated a lactone (1) separated. Any adhering resinous material may be removed by careful leaching with 50% alcohol or with ether. After removal of alcohol from the combined filtrates *in vacuo*, the mixture was extracted first with 245 cc. of ether and then with five portions of 122 cc. of ether. The combined ether extracts were washed with two 7-cc. portions of water, then extracted with 25.2 cc. and 12.6 cc. of a solution of 5% sodium carbonate and finally washed with seven 7-cc. portions of water. After drying with sodium sulfate, removal of the ether left a neutral residue. Crystallization of this from acetone gave successive crops (33–35% yield) of ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate. After further crystallization from acetone it melted at 187–191.5°. Analyses and optical rotation have been reported previously (1).

The combined carbonate extracts and aqueous washings were acidified with 6.3 cc. of conc'd hydrochloric acid and extracted with six portions of ether. After washing the ether extracts with water and drying with sodium sulfate, removal of the solvent left a resinous acid residue which has not been investigated in detail (*cf.* 1).

The combined acetone mother liquors were treated as previously described (1, footnote 5a).

From a total of 67.06 g. of 3(β),5,14,19-tetrahydroxy-14-isoetiocholanic acid in 25 experiments, 2.89 g. of the so-called lactone, 5.27 g. of resinous acid material, 32.94 g. of resinous neutral material, and 24.30 g. of VI were obtained.

When the resinous acid from the saponification of 3(β),19-diacetoxy-5,14-dihydroxy-14-isoetiocholanic acid was treated similarly, none of the lactone was obtained, and 7–14% of VI was isolated. A total of 57.18 g. of resinous acid yielded in 24 experiments 8.55 g. of resinous acid material, 5.26 g. of VI, and 35.62 g. of resinous neutral material.

The dehydration of resinous material which had been obtained by saponification of the acid ethyl acetate extracts resulting from the oxidation of strophanthidol diacetate (*vide supra*) did not yield any VI by direct crystallization.

f. *Dehydration of 3(β),19-diacetoxy-5,14-dihydroxy-14-isoetiocholanic acid* (IV). One gram of the crude 3(β),19-diacetoxy-5,14-dihydroxy-14-isoetiocholanic acid (*i.e.*, acid-ether extract from the oxidation of strophanthidol 3,19-diacetate) was treated exactly according to the procedure given above for the dehydration of the crystalline 3(β),5,14,19-tetrahydroxy-14-isoetiocholanic acid. This yielded 0.063 g. of the lactone (?) fraction; m.p. 75–77°. In addition 0.090 g. of resinous acid material and 0.626 g. of a resinous neutral fraction was

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[9] In a recent comparison Dr. Helmut C. Neumann varied the saponification in carrying it out in a solution of methanol with three moles rather than ten moles of potassium hydroxide. In addition, the solution was not refluxed, but allowed to stand at room temperature (25–30°) for eighteen hours. The yield of the crystalline acid (V) as obtained from ethyl acetate was 44.4% rather than 39.6%. Also the purity was somewhat superior (m.p. 213–217° rather than 209–214°).

obtained. The latter did not crystallize when it was treated with acetone. The late eluates of a subsequent chromatographic purification produced some crystalline material, which by recrystallization from acetone yielded 0.032 g. of a compound melting at 189–191°. It gave no depression in melting point when mixed with an authentic sample of ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate.

Anal. Calc'd for $C_{22}H_{34}O_5$ (378.27): C, 69.79; H, 9.06.
Found: C, 69.80; H, 8.93 (J.F.A.).

g. *Ethyl 3(β),5,19-trihydroxyetiocholanate* (VIII) *by hydrogenation of crystalline ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate* (VI). Reduction of 1.00 g. of VI in a total of 15 cc. of glacial acetic acid over 400 mg. of previously reduced platinum oxide (room temperature; atmospheric pressure) resulted in the uptake of one equivalent of hydrogen in 2½ hours. The solution was filtered from the platinum and quickly brought to dryness *in vacuo* (45°). The sirupy residue was repeatedly dissolved in a small volume of absolute alcohol and taken to dryness in order to remove the last traces of acetic acid. Recrystallization of the residue from acetone gave ethyl 3(β),5,19-trihydroxyetiocholanate as prisms. First crop: wt., 0.8231 g.; m.p. 187–188°, remelt. 190–191°; no depression of the m.p. when mixed with an authentic sample of VIII (1, page 842). Second crop: wt., 0.0858 g.; m.p. 178–181°. Third crop: wt., 0.0173 g.; m.p. 178–181°.

The mother liquors from the above gave low-melting or resinous material. A solution of 2.53 g. of this (from several runs) in 200 cc. of benzene was chromatographed over 65 g. of Brockmann aluminum oxide (Merck & Co., Rahway). A fraction resulting from a benzene-ether (1:1) eluate (0.2247 g.) yielded by repeated recrystallizations from acetone 0.0257 g. of a substance melting at 210–213°. It gave a depression of the melting point when mixed with a reference sample of ethyl 3(β),5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate. The analysis is in agreement with the formula $C_{20}H_{32}O_4$.

Anal. Calc'd for $C_{20}H_{32}O_4$ (336.25): C, 71.37; H, 9.59.
$C_{22}H_{36}O_4$ (364.28): C, 72.47; H, 9.96.
Found: C, 71.43, 71.78; H, 9.63, 9.50 (J.F.A.).

Elution with ether and ether-chloroform (3:1) gave 0.2665 g. of crystalline material which after crystallization from acetone yielded 0.0780 g. of ethyl 3(β),5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate, m.p. 211–214°, which was identified by mixed m.p. with a known sample.

Anal. Calc'd for $C_{22}H_{34}O_5$ (378.27): C, 69.79; H, 9.06.
Found: C, 69.56; H, 9.36 (J.F.A.).

Successive elution with a mixture of 198 c. of chloroform and 2 cc. of methanol and a mixture of 195 cc. of chloroform and 5 cc. of methanol gave 0.8575 g. of material from which, after recrystallization from acetone, 0.1887 g. of VIII was obtained.

Compound (*Lactone?*) $C_{20}H_{28}O_4$. Various attempts were made to purify and identify the compound tentatively (1) interpreted to be a lactone. The pooled material resulting from several dehydrogenation experiments (melting points between 90 and 100°) was purified by chromatographic adsorption on aluminum oxide. The major part was recovered from the early eluates (benzene and benzene-ether) which yielded crystalline residues. The crystalline residues (m.p. 103–104°)[9a] were analyzed as such as well as after washing them with a mixture of acetone and petroleum ether.

Anal. Calc'd for $C_{20}H_{28}O_4$ (332.22): C, 72.24; H, 8.49.
Found: C, 72.44, 71.57; H, 9.14, 8.82 (J.F.A.).

Treatment of the residues of some of the late eluates (ether-methanol) with acetone furnished material which was purified by crystallization from acetone; m.p. 167–168°. There was a pronounced depression (mixed m.p. 143–150°) of the melting point when mixed with ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate (m.p. 191–192°). In chloroform solution the substance gave a yellow color with tetranitromethane.

Anal. Calc'd for $C_{22}H_{34}O_5$ (378.27): C, 69.79; H, 9.06.
$C_{22}H_{32}O_5$ (376.25): C, 70.17; H, 8.57.
Found: C, 70.24, 70.41; H, 8.70, 8.49 (J.F.A.).

[9a] Determination of the infrared spectrum at the Sloan-Kettering Institute gave a band (CS_2) at 1739 cm.$^{-1}$, probably indicating an alkyl ester or a δ-lactone.

The compound $C_{20}H_{28}O_4$ proved to be rather labile. In unsuccessful attempts at recrystallization it was treated with a number of solvents. When some of these solutions were brought to dryness, the previously crystalline material refused to crystallize. Renewed purification by chromatography proved that the major part of the material had become considerably more polar, since it was now eluted in the later fractions of the chromatogram. A crystalline sample of compound $C_{20}H_{28}O_4$ (m.p. 98–100°) was subjected to catalytic hydrogenation in the presence of platinum in a solution of glacial acetic acid. No crystalline hydrogenation product could be obtained.

Ethyl 3(β),19-diacetoxy-5-hydroxy-Δ^{14}-etiocholenate (VII). To a solution of 30 mg. of ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate (m.p. 191–192°) in 0.2 cc. of pyridine was added 0.2 cc. of acetic anhydride and the mixture allowed to stand at room temperature overnight. The solvents were removed *in vacuo* and the resinous residue taken up in 12.5 cc. of ether which was subsequently washed twice with 0.4-cc. portions of 2 *N* hydrochloric acid, twice with 0.2-cc. portions of *N* sodium carbonate, and finally five times with 0.1-cc. portions of water. After drying with sodium sulfate, the ether solution was concentrated. Crystalline material separated and, on further concentrating, another crystalline crop was obtained. First crop: wt., 18.5 mg.; m.p. 118–120°. Second crop: wt., 13.0 mg.; m.p. 112–113°. Resinous residue: wt., 5.5 mg. (Total: 37.0 mg.). Recrystallization of the first crop from ether yielded 11.6 mg., m.p. 115–117°.

Anal. Calc'd for $C_{26}H_{38}O_7$ (462.30): C, 67.49; H, 8.28.
Found: C, 67.17; H, 8.32 (J.F.A.).

3(β),19-Diacetoxy-5-hydroxyetiocholanic acid (X). To a solution of 0.307 g. of 3(β),5,19-trihydroxyetiocholanic acid (m.p. between 265 and 270°) in 1.5 cc. of pyridine was added 0.9 cc. of acetic anhydride and the mixture allowed to stand at room temperature for a period of about two days. After the addition of 0.9 cc. of water it was heated on the water-bath for 2½ hours and then concentrated to a syrupy consistency *in vacuo* (60°). The residue was taken up in 150 cc. of ether and this solution washed free of pyridine with 5 cc. of N hydrochloric acid (acid to Congo) and with three 1-cc. portions of water. The ether phase was extracted with five 3 cc.-portions of *N* sodium carbonate and subsequently washed three times with 1 cc. of water. After drying, the ether solution yielded 0.015 g. of resinous neutral material. The combined carbonate extracts and aqueous washings were cooled with ice, transferred into a separatory funnel, and made acid to Congo by the addition of 2 cc. of conc'd hydrochloric acid which produced a thick colorless precipitate. This was extracted with three 50-cc. portions of ether. The combined ether extracts were washed five times with 1 cc. of water, dried, and brought to dryness *in vacuo*. Since the sticky colorless residue contained some acetic acid, it was dried in a vacuum desiccator over pellets of potassium hydroxide, then taken up in acetone and again brought to dryness *in vacuo*, which yielded a colorless brittle foam. The treatment with acetone was repeated a few times and the foam eventually dried over potassium hydroxide. Constant weight: 0.368 g. (Calc'd 0.380 g.). This material resisted all attempts at crystallization. In chloroform solution no yellow color was obtained with tetranitromethane, indicating that no simultaneous dehydration had occurred. $[\alpha]_D^{26}$ +68.1° (20.0 mg. in 2.0 cc. of chloroform; l = 1.51 dm., α +1.03°).

Anal. Calc'd for $C_{24}H_{36}O_7$ (436.28): C, 66.01; H, 8.32.
Found[10]: C, 65.40, 65.20; H, 8.37, 8.22.

Methyl 3(β),19-diacetoxy-5-hydroxyetiocholanate (XIII). (a) *From 3 (β),19-diacetoxy-5-hydroxyetiocholanic acid* (X). To a solution of 61 mg. of 3(β),19-diacetoxy-5-hydroxyetiocholanic acid in 5 cc. of ether was added at 0° a slight excess of an ethereal solution of diazomethane. The mixture was kept at 0° for 15 minutes and at room temperature for an additional 10 minutes. The excess diazomethane was boiled off on a water-bath. This yielded a colorless solution which was transferred into a separatory-funnel by means of 20 cc. of ether. After washing with 1.5 cc. of *N* hydrochloric acid, 1 cc. of water, 11.5 cc. of 5% sodium carbonate, and three times with 1 cc. of water, the solution was dried and subsequently brought to dryness. Yield, 54 mg. of a colorless glass which resisted all attempts at crystallization.

Anal. Calc'd for $C_{25}H_{38}O_7$ (450.30): C, 66.62; H, 8.50.
Found: C, 67.00, 66.93; H, 8.73, 8.67 (E.W.D.H.).

(b) *From methyl 3(β),5,19-trihydroxyetiocholanate* (XII). To a solution of 82 mg. of methyl 3(β),5,19-trihydroxyetiocholanate (m.p. 218°) in 0.35 cc. of pyridine was added 0.35 cc. of acetic anhydride and the mixture allowed to stand at room temperature overnight. It was then brought to dryness *in vacuo* (65°), the resinous residue taken up in 25 cc. of ether, and the latter solution washed with 2 cc. of *N* hydrochloric acid, 2 cc. of 5% sodium carbonate, and four times with 1-cc. portions of water. The ethereal solution was subsequently dried and brought to dryness. Yield, 90 mg. of a resin; attempts at crystallization were unsuccessful. The material was dissolved in a mixture of 5 cc. of benzene and 10 cc. of petroleum ether and chromatographed over 3.0 g. of aluminum oxide (diam. of column: 8 mm.). The adsorbate was successively eluted with 15-cc. portions of benzene-petroleum ether (benzene content gradually increasing), benzene, benzene-ether (ether content gradually increasing), ether, ether-acetone (acetone content gradually increasing), acetone, acetone-methanol (methanol content gradually increasing), and methanol. All residues obtained from these eluates were resinous. The main fraction (34 mg.) was obtained from the benzene phase. It resisted all attempts at crystallization.

Anal. Calc'd for $C_{25}H_{38}O_7$ (450.30): C, 66.62; H, 8.50.
Found: C, 66.75, 66.86; H, 8.71, 8.77 (E.W.D.H.).

Distillation in a high-vacuum of 3(β),19-diacetoxy-5-hydroxyetiocholanic acid (X). This is an abbreviated account of two experiments. First experiment: A total of 0.287 g. of dry 3(β),19-diacetoxy-5-hydroxyetiocholanic acid was heated in a vacuum (oil-pump) to 170° and the temperature gradually raised to 205°. During this time there was a persistent gas evolution in the retort. After this reaction had come to a standstill, the reaction product was distilled in a high-vacuum (oil and mercury vapor pumps combined) at a temperature of 240–265°. The distillate was a slightly greenish glass; wt., 0.234 g.; $[\alpha]_D^{26}$ +3.3° (20.0 mg. in 2.0 cc. of chloroform; l = 1.51 dm., α +0.05°). The residue in the retort was a light brown resin; wt., 0.005 g.

Total loss of weight: 0.048 g. (16.7%).

Calc'd loss of weight: $C_{24}H_{36}O_7$ (436.28) $\xrightarrow{-H_2O}$ $C_{24}H_{34}O_6$ (418.27): 4.1%

$C_{24}H_{36}O_7$ (436.28) $\xrightarrow[-CH_3COOH]{-H_2O}$ $C_{22}H_{30}O_4$ (358.23): 17.9%

$C_{24}H_{36}O_7$ (436.28) $\xrightarrow[-CH_3COOCH_3]{-H_2O,\ -2\ H}$ $C_{21}H_{26}O_4$ (342.20): 21.6%

Anal.[10] Calc'd for $C_{24}H_{34}O_6$ (418.27); C, 68.86; H, 8.19.
$C_{22}H_{30}O_4$ (358.23): C, 73.69; H, 8.44.
$C_{21}H_{26}O_4$ (342.20): C, 73.64; H, 7.66.
Found: C, 71.17; H, 7.81.

The distillate resisted attempts at crystallization. A sample was subjected to a separation into acid and neutral material; only small amounts of neutral material were obtained. The distillate was transformed into the methyl ester with diazomethane; 0.215 g. of distillate yielded 0.225 g. of methyl ester. The latter was a colorless glass which was dissolved in a mixture of 25 cc. of benzene and 7.5 cc. of petroleum ether and chromatographed over 10.0 g. of aluminum oxide (diam. of column: 20 mm.). The adsorbate was successively eluted with 25-cc. portions of benzene-petroleum ether (benzene content gradually increasing), benzene, benzene-ether (ether content gradually increasing), ether, ether-methanol (methanol content gradually increasing). A substantial amount (0.052 g.) was recovered from the benzene-petroleum ether fractions. One of them (0.023 g.) was crystalline. Recrystallization from ether yielded several crystalline crops, totalling 12.4 mg. with melting points between 133 and 144°. The melting point of the purest fraction (4.9 mg.) was 143–144°. A solution in chloroform yielded a yellow color with tetranitromethane.

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[10] Microanalysis by Mr. William Saschek, Department of Biochemistry, Columbia University, New York.

Anal.[11] Calc'd for $C_{25}H_{36}O_6$ (432.28): C, 69.40; H, 8.39.
[Methyl 3,19-diacetoxy-Δ^5-etiocholenate (XV)]
Found: C, 69.77; H, 8.98.

Second experiment:[12] A total of 0.657 g. of 3(β),19-diacetoxy-5-hydroxyetiocholanic acid was quickly heated in a high-vacuum (oil and mercury vapor pumps combined) to 153°, then slowly raised to 176°. After the gas evolution had ceased the material was distilled at about 250°. Total loss of weight, 0.124 g. (18.8%). The glassy distillate was recrystallized from ether. This furnished several crops of rosette arrangements of crystals totalling 0.138 g., m.p. between 144 and 150°. The weight of the non-crystalline part was 0.372 g. Recrystallization of the combined crystalline fractions from ether furnished several crops of crystals with varying melting points; wt. of the first crop, 0.041 g.; m.p. 158–161°.

Anal.[11] Calc'd for $C_{24}H_{34}O_6$ (418.27): C, 68.86; H, 8.19.
$C_{22}H_{30}O_4$ (358.23): C, 73.69; H, 8.44.
$C_{21}H_{26}O_4$ (342.20): C, 73.64; H, 7.66.
Found: C, 72.43; H, 8.75.

The further working up of this experiment was delayed for a period of more than one year. During the course of this time, there occurred a slight weight increase of the non-crystalline fraction. In addition, it had also become very sparingly soluble in ether (autoxidation?) Treatment with diazomethane yielded the methyl ester which was subsequently subjected to a chromatographic fractionation. No uniform material resulted from this treatment. The crystalline part which was still readily soluble in ether, was likewise transformed into the methyl ester. The resulting resinous product was purified by chromatographic fractionation. From a total of 100 mg. of crude methyl ester, about 36 mg. could be recovered from the early eluates (benzene-petroleum ether combinations). This material, which was resinous, was subjected to solvolysis with 0.5 equiv. of potassium hydroxide in methanol at room temperature, to transform any acetoxy groups into hydroxy groups. The resulting non-crystalline product was purified by chromatographic fractionation. As expected, it had become more polar. After first eluting with benzene-petroleum ether combinations and with benzene, approximately 11 mg. could be secured from the benzene-ether eluates. On recrystallizing from a mixture of acetone and petroleum ether 1.6 mg. of feather-shaped crystals (methyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate), m.p. 217–219° was obtained. A solution in chloroform gave with tetranitromethane a deep golden-yellow color. The ultraviolet absorption spectrum was determined[4] in the region between 200 and 300 mμ. There was a maximum at 281 mμ and a minimum at 249 mμ. There was no depression of the melting point when mixed with an authentic sample of methyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate which was kindly supplied by Drs. C. Djerassi and C. R. Scholz (6).

Partial oxidation of ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) *with chromic acid.* In a preliminary experiment oxidation of VIII with one equivalent of chromium trioxide and subsequent treatment of the neutral fraction with Girard's Reagent T resulted in a substantial loss of material, indicating the possible presence of an aldehydic substance. To avoid this loss, the use of this reagent was excluded in the main experiment.

To a solution of 0.9505 g. (2.5 millimoles) of VIII in 55 cc. of glacial acetic acid there was added at room temperature over a period of seven hours, a solution of 0.1853 g. (1.1 equivalent) of chromium trioxide in 55 cc. of 95% acetic acid. The following day 10 cc. of alcohol was added and the solution brought to dryness *in vacuo*. In order to remove the acetic acid completely the residue was repeatedly dissolved in 5-cc. portions of alcohol and taken to dryness. Thereafter 25 cc. of water was added and the material transferred into a separatory-funnel by means of 500 cc. of ether. The ether phase was washed with 30 cc. of *N* sulfuric acid and four 4-cc. portions of water. Some gummy, probably acid material remained undissolved, but went into the carbonate phase when the ether solution was subsequently extracted with three 10-cc. portions of *N* sodium carbonate. After washing the ether solution

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[11] Microanalysis by Dr. Francine Schwarzkopf, Elmhurst, L. I.

[12] Partly with the assistance of Mr. Charles P. Balant.

four times with 5 cc. of water, it was dried and brought to dryness; wt. of neutral residue, 0.7722 g. The combined carbonate phases and aqueous washings were cooled with ice and acidified to Congo by the addition of an excess (20 cc.) of 4 *N* sulfuric acid. This was followed by thorough extraction with 500 cc. and 25 cc. of ether. The combined ether extracts were washed eight times with 5 cc. of water, dried, and brought to dryness. In order to remove traces of acetic acid, the resinous acid residue was dried overnight in a vacuum desiccator over potassium hydroxide; wt. of the acid material, 0.1013 g.; it has not yet been investigated.

To the neutral residue (0.7722 g.) was added some ether, which caused the separation of crystalline material (1st crop); after drying, 0.1110 g.; m.p. 155–165°. The filtrate was brought to dryness, the brittle foamy residue dissolved in a small amount of ether, and the solution allowed to stand in a refrigerator. This caused the separation of additional crystalline material (2nd crop); wt., 0.0484 g., m.p. 120–138°. The filtrate yielded 0.6135 g. of a resinous residue.

Ethyl 3-keto-5,19-dihydroxyetiocholanate (XIX)? It was possible to purify the first crop by repeated fractional crystallizations from ether. In each instance the material was dissolved in the required amount of ether and this solution concentrated to about one half of its volume. Transparent needles separated on standing in a refrigerator. Thus six fractions (A) were obtained, totalling 0.0326 g.; melting points between 199 and 203°. For additional material of this substance *vide infra*, $[\alpha]_D^{29.5}$ +76.2° (10.6 mg. in 2.0 cc. of chloroform; l = 1.51 dm., α +0.61°). For infrared spectrum see theoretical part.

Anal. Calc'd for $C_{22}H_{34}O_5$ (378.27): C, 69.63; H, 9.04.

Found: C, 69.05; H, 9.21 (J.F.A.); C, 69.04; H, 9.07 (E.W.D.H.).

Two other fractions (B), totalling 0.0216 g., represented the same substance, but were less pure. The melting points were between 181 and 186°. Two other fractions (C), totalling 0.0180 g., had melting points between 140 and 163°. They definitely represented mixtures. The combined resinous fractions (D) resulting from the recrystallizations of the first original crop, weighed 0.0460 g.

CHROMATOGRAPHIC FRACTIONATION

NO. OF FRACTION	SOLVENT	WEIGHT OF RESIDUE, G.	APPEARANCE OF RESIDUE
1	25 cc., benzene (original solution)	0.0037	Colorless resin
2	15 cc., benzene	.0015	Colorless resin
3	10 cc. of benzene + 5 cc. of ether	.0048	Yellow resin
4	5 cc. of benzene + 10 cc. of ether	.0037	Colorless resin
5	15 cc. of ether	.0156	Yellowish, crystalline
6	10 cc. of ether + 5 cc. of chloroform	.0061	White, crystalline
7	5 cc. of ether + 10 cc. of chloroform	.0030	White, crystalline
8	15 cc. of chloroform	.0035	Yellowish, crystalline
9	15 cc. of chloroform + 0.005 cc. of methanol	.0109	White, crystalline
10	15 cc. of chloroform + 0.01 cc. of methanol	.0117	White, crystalline
11	15 cc. of chloroform + 0.01 cc. of methanol	.0093	White, crystalline
12	15 cc. of chloroform + 0.02 cc. of methanol	.0077	White, crystalline
13	15 cc. of chloroform + 0.03 cc. of methanol	.0074	White, crystalline
14	15 cc. of chloroform + 0.04 cc. of methanol	.0072	White, crystalline
15	15 cc. of chloroform + 0.1 cc. of methanol	.0056	Partially crystalline
16	14.5 cc. of chloroform + 0.5 cc. of methanol	.0070	Colorless resin
17	10 cc. of chloroform + 5 cc. of methanol	.0017	Colorless resin
18	15 cc. of methanol	.0017	White, crystalline
Total	....	0.1021	

Recrystallization from ether of the second original crop of crystals (0.0484 g.) gave various fractions, melting over a wide range between 130 and 160°. This material was therefore combined with the above C-series (0.0180 g.) and the resinous D-series (0.0460 g.); total, 0.1124 g. This was subjected to a chromatographic separation by dissolving it in 25 cc. of benzene and passing this solution through a column (diam. 10 mm.) of 4 g. of alkali-free aluminum oxide[13] within a period of 75 minutes. The eluting was done at the rate of 15–20 minutes for each fraction.

No crystals were obtained from fractions 1 to 4. Fractions 5–7 furnished from ether eight crops of crystalline material, totalling 0.0146 g.; melting points between 180 and 199°. They obviously represented impure ethyl 3-keto-5,19-dihydroxyetiocholanate and were therefore pooled with the above B-series. Fraction 8 represented a mixture; only low-melting (between 144 and 164°) material was obtained.

Compound $C_{24}H_{36}O_6$ *or* $C_{20}H_{30}O_5$ (XX)? From fractions 9–15 there were secured twenty-five crops of crystalline material totalling 0.0332 g., range of melting points between 162 and 171°. The combined crops furnished from ether silky, felt-like needles, which sometimes had the appearance of long fibers, such as in asbestos. The melting point of the purest material (0.0170 g.) was 171–173°. For additional material of this substance *vide infra.* $[\alpha]^{29}_{D}$ $-11.2°$ (9.5 mg. in 2.0 cc. of chloroform, $l = 1.51$ dm., α $-0.08°$). For infrared spectrum see theoretical part.

Anal. Calc'd for $C_{24}H_{36}O_6$ (420.28): C, 68.53; H, 8.63.
$C_{20}H_{30}O_5$ (350.23): C, 68.53; H, 8.63.
Found: C, 68.64, 69.06; H, 8.87, 8.91 (E.W.D.H.).[14]

An attempt to secure an oxime of this substance failed. The isolated reaction product contained no nitrogen and was apparently unchanged starting material. In an attempt to prepare an acetyl derivative the acetylation carried out in the usual fashion at room temperature yielded a resinous reaction product, which resisted attempts at crystallization. Seeding with ethyl 3,19-diacetoxy-5-hydroxyetiocholanate was of no avail. There was not enough material for a chromatographic purification.

The resinous residue (0.6135 g.), as obtained after the separation of the original crops of crystals, was chromatographed over 18 g. of alkali-free aluminum oxide.[13] The combination of eluants was similar to those of the chromatogram given above. There resulted a total of only 0.0712 g. of crystalline material. Of these crystalline fractions only 0.0067 g. were obtained from the benzene, benzene-ether, ether, ether-chloroform, and chloroform eluates. The eluate secured with a mixture of 50 cc. of benzene and 0.03 cc. of methanol yielded 0.0096 g. of crystalline material, m.p. 192–198°. There was no depression of the melting point when mixed with the substance believed to be ethyl 3-keto-5,19-dihydroxyetiocholanate. It was therefore pooled with the crystalline B-series mentioned above. The subsequent eluates, resulting from further chloroform-methanol combinations, yielded altogether 0.0549 g. of crystalline material melting between 140 and 176°.

The latter crystalline fractions, including the material contained in their respective mother liquors was pooled with fraction 8 of the chromatogram recorded above (total, 0.1821 g.). A renewed chromatographic separation yielded only impure, if any, of the ethyl 3-keto-5,19-dihydroxyetiocholanate; however, several crops, totalling 0.0325 g., of the pure compound $C_{24}H_{36}O_6$ (or $C_{20}H_{30}O_5$) were obtained; 0.0198 g. of somewhat less pure material of the same substance was identified.

The crystalline B series mentioned above and the material which had been pooled with it during the course of these purification procedures (total 0.0458 g.) was recrystallized from ether and furnished 0.0247 g. of practically pure ethyl 3-keto-5,19-dihydroxyetiocholanate (m.p. 196–198°).

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[13] One part of aluminum oxide (standardized acc. to Brockmann, Merck & Co., Rahway, N. J.) was placed in an adsorption column and slowly washed with two parts of a 9:1 mixture of methanol-glacial acetic acid. The material was subsequently washed acid free with methanol and dried in an oven at a temperature of 200° for four hours.

[14] Two different samples of the same degree of purity.

Summary: The total yield of pure ethyl 3-keto-5,19-dihydroxyetiocholanate was 0.0573 g., that of compound $C_{24}H_{36}O_6$ or $C_{24}H_{38}O_6$ (?) 0.0493 g.

Oxime of ethyl 3-keto-5,19-dihydroxyetiocholanate. To 10.1 mg. of ethyl 3-keto-5,19-dihydroxyetiocholanate dissolved in 1.7 cc. of absolute alcohol was added a mixture of 20 mg. of hydroxylamine hydrochloride and 30 mg. of sodium acetate in 0.15 cc. of water. After refluxing on a water-bath for a period of three hours an ample amount of water was added which caused the immediate precipitation of short white needles. They were filtered after some standing and washed with water; 1st crop: dry wt., 4.6 mg., m.p. 187–189°. It gave a depression of the melting point when mixed with the starting material. An additional crystalline separation was obtained by removing the alcohol *in vacuo*; 2nd crop: dry wt., 4.6 mg., m.p. 185–187°. The combined material was recrystallized from aqueous alcohol, yield 5.9 mg., m.p. 188–189°.

Anal. Calc'd for (monoöxime) $C_{22}H_{35}NO_5$ (393.25): N, 3.56. Found: N, 3.80 (J.F.A.).

Attempts to dehydrogenate ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) *according to the Oppenauer method.* (*a*) *With aluminum tert-butoxide and acetone.* To a solution of 200 mg. of aluminum *tert*-butoxide (Eastman Kodak) in 8 cc. of dry benzene was added 100 mg. of ethyl 3(β),5,19-trihydroxyetiocholanate dissolved in 4 cc. of acetone. This mixture was refluxed on a water-bath for a period of ten hours; after five hours 1 cc. of additional acetone was added. The working up in the usual fashion was followed by a separation with Girard's Reagent T. This furnished practically no ketonic fraction (3.8 mg.). Recrystallization of the non-ketonic fraction (90.8 mg.) furnished 80.2 mg. of pure starting material. (b) *With aluminum phenoxide*[15] *and acetone.* Aluminum phenoxide (800 mg.) was dissolved in 30 cc. of benzene by warming. A solution of 200 mg. of ethyl 3(β),5,19-trihydroxyetiocholanate in 12 cc. of dry acetone was added and the mixture refluxed for a period of 25 hours. It was taken up in 260 cc. of ether and this solution washed with 25 cc. of *N* sulfuric acid. The phenol was removed with three 8-cc. portions of *N* sodium hydroxide. The ether phase was washed neutral with water, dried, and brought to dryness; wt. of the crystalline residue, 206 mg. This was separated by means of Girard's Reagent T. Practically no ketonic material was obtained (2.3 mg.). The weight of the non-ketonic part was 188 mg., from which by recrystallization 169 mg. of pure starting material was secured.

Treatment of ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) *with Raney nickel in the presence of cyclohexanone.*[16] To a total of 0.503 g. of pure ethyl 3(β),5,19-trihydroxyetiocholanate (melting points between 185 and 187°), dissolved in 5 cc. of redistilled cyclohexanone (b.p. 148–150°), was added a suspension of approximately 2.12 g. of Raney nickel[17] in 20.5 cc. of toluene. This mixture was stirred and refluxed continuously for a period of 24 hours, bath temperature 128–132°. Because part of the solvent had evaporated, 5 cc. of toluene each was added after 18 and 22 hours respectively. After the termination of the reaction the solution was filtered from the Raney nickel and the latter washed with acetone. The filtrate was freed from solvents by first distilling it in an ordinary and finally in an oil-pump vacuum (bath temperature, 75°). The oily residue had a strong phenolic odor. The residue was dissolved in 200 cc. of redistilled ether and this solution washed with two 10-cc. portions of 2 *N* sodium hydroxide and six 5-cc. portions of water. After drying with sodium sulfate the ether solution was brought to dryness, eventually in an oil-pump vacuum. The residue was a slightly yellow clear resin which resisted attempts at crystallization, weight 0.498 g.

The residue was dissolved in a mixture of 20 cc. of benzene and 20 cc. of petroleum ether which was chromatographed over a column of 15 grams of alkali-free aluminum oxide[13] (diameter 2.5 cm.). The adsorbed material was eluted successively with 40-cc. portions of mixtures of benzene and petroleum ether (benzene content gradually increasing), benzene,

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[15] (11, page 523).

[16] The initial reaction was carried out by Miss V. I. Vivian.

[17] The Raney nickel was kindly supplied by Drs. E. C. Kleiderer and E. C. Kornfeld of the Eli Lilly & Co. Research Laboratories in Indianapolis. They had used it successfully to catalyze the conversion of dihydrocholesterol to cholestanone in the presence of cyclohexanone, (14). It was applied in our experiment about three weeks after its preparation.

mixtures of benzene and ether, (ether content gradually increasing), ether, mixtures of ether and chloroform (chloroform content gradually increasing), chloroform, mixtures of chloroform and methanol (methanol content gradually increasing), and finally methanol. A total of 0.495 g. was recovered. The major part of the material (0.430 g.) was contained in the eluates of benzene-petroleum ether, benzene, and benzene-ether. Though the residues were partly crystalline, apparently no efficient separation had been achieved. One of the chloroform-methanol (39:1) eluates furnished 7.1 mg. of a crystalline residue, which yielded needle-shaped crystals from acetone; m.p. 209–211°. They were not further investigated. The test with tetranitromethane yielded no yellow color. This substance gave a depression of the melting point when mixed with the compound interpreted to be ethyl 3-keto-5,19-dihydroxyetiocholanate (XIX).

Chromatographic Fractionation

NO. OF FRACTION	SOLVENT	WEIGHT OF RESIDUE, G.	APPEARANCE OF RESIDUE
1	15 cc. of benzene + 25 cc. of petr. ether (original solution)	0.0047	Greasy
2	15 cc. of benzene + 25 cc. of petr. ether	.0361	Resinous
3	20 cc. of benzene + 20 cc. of petr. ether	.0601	Crystalline
4	20 cc. of benzene + 20 cc. of petr. ether	.0222	Crystalline
5	25 cc. of benzene + 15 cc. of petr. ether	.0207	Resinous
6	25 cc. of benzene + 15 cc. of petr. ether	.0295	Partly cryst., partly resinous
7	30 cc. of benzene + 10 cc. of petr. ether	.0361	Essentially crystalline
8	30 cc. of benzene + 10 cc. of petr. ether	.0204	Crystalline
9	35 cc. of benzene + 5 cc. of petr. ether	.0200	Essentially crystalline
10	35 cc. of benzene + 5 cc. of petr. ether	.0224	Few cryst. centers
11	40 cc. of benzene	.0104	Resinous
12	40 cc. of benzene	.0113	Resinous
13	38 cc. of benzene + 2 cc. of ether	.0166	Resinous
14	37 cc. of benzene + 3 cc. of ether	.0175	Partly cryst., partly resinous
15	36 cc. of benzene + 4 cc. of ether	.0147	Essentially crystalline
16	35 cc. of benzene + 5 cc. of ether	.0136	Partly cryst., partly resinous
17	34 cc. of benzene + 6 cc. of ether	.0132	Partly cryst., partly resinous
18	33 cc. of benzene + 7 cc. of ether	.0119	Partly cryst., partly resinous
19	32 cc. of benzene + 8 cc. of ether	.0106	Partly cryst., partly resinous
20	31 cc. of benzene + 9 cc. of ether	.0071	Partly cryst., partly resinous
21	30 cc. of benzene + 10 cc. of ether	.0053	Resinous
22–32	40 cc. of each of benzene-ether mixtures; ether content gradually increasing	.0160	Resinous
33	40 cc. of ether	.0023	Greasy
34–38	40 cc. of each of ether-methanol mixtures; methanol content gradually increasing	.0165	Resinous
39	40 cc. of methanol	.0090	Crystalline
Total		0.4482	

The major part of the material (0.430 g.) was subjected to a renewed chromatographic separation, for which it was dissolved in a mixture of 15 cc. of benzene and 25 cc. of petroleum ether. The solution was filtered through a column (diam. 25 mm.) of 14 g. of alkali-free aluminum oxide[13] within a period of 30 minutes. The eluates were passed through, each within about 15 minutes.

The chromatogram on page 281 suggested the presence of at least three different substances.

Compound $C_{22}H_{32}O_4$ (XXI)? Fractions 1 and 2 resisted attempts at crystallization. Fractions 3 and 4 were separately recrystallized by dissolving them in a small volume of ether to which petroleum ether was added. This caused the immediate separation of rosette arrangements of long, thin needles. Several crystalline fractions, totalling 30.6 mg., with melting points varying between 97 and 114°, were repeatedly recrystallized. Eventually five crystalline fractions resulted, totalling 17.6 mg. and melting between 111 and 116°. The determination of mixed melting points established the identity of these fractions and hence the combined material was subjected to a renewed recrystallization from equal amounts of ether and petroleum ether: 1st crop: wt., 10.6 mg., m.p. 115–116°. 2nd crop: wt., 1.3 mg., m.p. 111–113°. 3rd crop: wt., 0.9 mg., m.p. 114.5–115.5°. Total pure material, 12.8 mg. It is believed that the total yield may be somewhat increased by subjecting the mother liquors, including the chromatographic fractions 2 and 5, to a renewed chromatographic fractionation. The substance (1st crop) was transparent in the region between 200 and 300 mμ.[4] The test with tetranitromethane yielded no yellow color.

Anal. Calc'd for $C_{22}H_{32}O_4$ (360.25): C, 73.28; H, 8.95.
$C_{22}H_{34}O_4$ (362.27): C, 72.87; H, 9.46.
$C_{22}H_{36}O_4$ (364.28): C, 72.47; H, 9.96.
Found: C, 73.10; H, 8.98 (J.R.); C, 71.43; H, 8.72 (J.F.A.).

Molecular Weight (Cryoscopic):
Wt. of solvent (camphor): 4.080 mg., wt. of sample: 0.375 mg., K = 36.0°; Δt: 8.8°, mol. wt. 376 (J.R.).
Wt. of solvent (exaltone): 2.366 mg., wt. of sample: 0.184 mg., K = 21.0°; Δt: 4.8°, mol. wt. 340 (E.W.D.H.).
Wt. of solvent (exaltone): 1.045 mg., wt. of sample: 0.104 mg., K = 21.0°; Δt: 6.3°, mol. wt. 332 (E.W.D.H.).

Ethyl 3-hydroxy-$\Delta^{1;3;5,10}$*-estratriene-17-carboxylate* (XXII). The chromatographic fraction 5 resisted attempts at crystallization. Fractions 6 to 9 were separately recrystallized by dissolving them in a small amount of ether and adding about twice the volume of petroleum ether. This caused the separation of rosettes of stout needles. Eventually 10 crystalline fractions of identical material (mixed m.p.'s), totalling 48.9 mg. with melting points between 175 and 178° (solidification and remelting between 184 and 188°) were obtained. Recrystallization of 43.7 mg. of combined material from ether-petroleum ether furnished several fractions (total, 37.5 mg.) of clusters of stout rectangular crystals which melted between 176 and 178°, then solidified and remelted between 184 and 186°. A solution of the substance in chloroform yielded with tetranitromethane an orange color. For ultraviolet absorption spectrum see the theoretical part. $[\alpha]_D^{26.0}$ +97.0° (12.7 mg. in 2.0 cc. of chloroform; l = 1.51 dm., α +0.93°).

Anal. Calc'd for $C_{21}H_{28}O_3$ (328.22): C, 76.78; H, 8.60.
Found: C, 76.00; H, 8.38 (J.R.); C, 76.88; H, 8.33 (E.W.D.H.);
C, 76.71; H, 8.78 (J.F.A.).

Molecular Weight (Cryoscopic):
Wt. of solvent (camphor): 4.900 mg., wt. of sample: 0.453 mg., K = 36.0°; Δt = 10.0°, mol. wt. 332.8 (J.R.).
Wt. of solvent (exaltone): 1.910 mg., wt. of sample: 0.170 mg., K = 21.0°; Δt = 5.6°, mol. wt. 334 (E.W.D.H.).
Wt. of solvent (exaltone): 4.505 mg., wt. of sample: 0.414 mg., K = 21.0°; Δt = 5.9°, mol. wt. 327 (E.W.D.H.).

The mother liquors resulting from the purification of chromatographic fractions 6–9 were combined with fraction 10. On rechromatographing this material only an additional 2.6 mg. of pure ethyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate was obtained.

3-Hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylic acid (XXIV). Ethyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate (6 mg.), melting at 175–177° (184–188°), was dissolved in 0.6 cc. of 10% potassium hydroxide in methanol. After refluxing this mixture for four hours, 2 cc. of water was added, and the methanol removed *in vacuo*. The solution was thereafter acidified to Congo with conc'd hydrochloric acid, which produced a gelatinous precipitate. This was followed by extracting with four 5-cc. portions of ether and washing the combined ether extracts with 1 cc. of water, 2 cc. of *N* sodium carbonate, and two 1-cc. portions of water. After drying with sodium sulfate the ether phase yielded 0.9 mg. of neutral material. The carbonate phase including the aqueous washings was made acid to Congo with conc'd hydrochloric acid and the resulting precipitate brought into solution by extracting four times with 5 cc. of ether. The combined ether phases were washed three times with 1 cc. of water, dried with sodium sulfate, and brought to dryness. Thus 3.5 mg. of crystalline acid material resulted which was recrystallized from small amounts of ether; immediate crystallization of small cubes. First crop: 1.5 mg., m.p. 266–270° (turning dark brown). Second crop: 0.7 mg., m.p. 266–270° (turning dark brown). This substance did not give a depression of the melting point when it was mixed with another sample of 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylic acid which was kindly furnished by Drs. C. Djerassi and C. R. Scholz (6).

Compound $C_{22}H_{34}O_4$ (XXIII). The chromatographic fractions 11 and 12 were not investigated; fraction 13 resisted attempts at crystallization. Fractions 14–20 yielded identical material which crystallized from ether in rosettes of long stout rectangular prisms. Eleven crystalline fractions, totalling 41.8 mg., had melting points between 134 and 137°. Six fractions, totalling 12.0 mg., yielded melting points between 128–133°. Renewed crystallization of either combined group raised the melting point to 136–137°. Rechromatographing of the combined mother liquors including the chromatographic fraction 13 will probably yield additional amounts of this material. The substance was transparent in the region between 200 and 300 mμ.[4] The test with tetranitromethane gave no yellow color. $[\alpha]_D^{26.0}$ +69.8° (7.4 mg. in 2.0 cc. of chloroform; $l = 1.51$ dm., α +0.39°)

Anal. Calc'd for $C_{22}H_{32}O_4$ (360.25): C, 73.28; H, 8.95.
$C_{22}H_{34}O_4$ (362.27): C, 72.87; H, 9.46.
$C_{22}H_{36}O_4$ (364.28): C, 72.47; H, 9.96.
Found: C, 73.38, H, 10.02 (J.F.A.); C, 72.67; H, 9.52 (J.R.);
C, 72.93; H, 9.42 (E.W.D.H.).

Molecular Weight (Cryoscopic):
Wt. of solvent (camphor): 4.102 mg., wt. of sample: 0.385 mg., K = 36.0°; Δt = 10.9°, mol. wt. 310 (J.R.).
Wt. of solvent (exaltone): 2.414 mg., wt. of sample: 0.168 mg., K = 21.0°; Δt = 4.0°, mol. wt. 365 (E.W.D.H.).
Wt. of solvent (exaltone): 3.061 mg., wt. of sample: 0.391 mg., K = 21.0°; Δt = 7.6°, mol. wt. 353 (E.W.D.H.).

Acetylation: A solution of 8.6 mg. of the above substance in 0.25 cc. of pyridine and 0.25 cc. of acetic anhydride stood overnight at room temperature. After bringing it to dryness *in vacuo*, the ether solution of the residue was washed neutral with *N* hydrochloric acid and *N* sodium carbonate. After drying, the ether solution yielded 8.6 mg. of a resinous residue which resisted attempts at crystallization from various solvent combinations. To a solution of the residue in 1 cc. of ethanol was added 0.22 cc. of 0.1 *N* potassium hydroxide in ethanol (*i.e.* 1 equiv. KOH). After standing overnight at room temperature the mixture was made acid to litmus with glacial acetic acid. It was then brought to dryness *in vacuo*, the residue taken up in ether and washed neutral with *N* sodium carbonate. After drying, the ether solution yielded 7.3 mg. of a resinous residue which was recrystallized from ether, m.p. 134–136°; mixed m.p. with starting material, 136–137°.

Attempts at partial de-acetylation of ethyl 3(β),19-diacetoxy-5-hydroxyetiocholanate (XI).

(*a*) *Ethanolysis in the presence of 0.25 equiv. of potassium hydroxide.* To a solution of 116.1 mg. (0.25 millimole) of ethyl 3(β),19-diacetoxy-5-hydroxyetiocholanate in 10 cc. of absolute ethanol was added at 3° a total of 0.625 cc. of 0.1 *N* absol. alcoholic potassium hydroxide (0.0625 millimoles). After three-days standing at about 3°, the solution was made acid to litmus with acetic acid and then worked up in the customary fashion. Yield, 95.9 mg. (Calc'd for complete solvolysis, $C_{26}H_{40}O_7 \rightarrow C_{22}H_{36}O_5$: 95.1 mg.). Recrystallization furnished at least 88.5 mg. of pure ethyl 3(β),5,19-trihydroxyetiocholanate.

(*b*) *Ethanolysis in the presence of 1 equiv. of hydrogen chloride.* A solution of 92.9 mg. (0.2 millimole) of ethyl 3(β),19-diacetoxy-5-hydroxyetiocholanate in 2.0 cc. of 0.1 *N* absolute alcoholic hydrogen chloride (0.2 millimole) was prepared at room temperature and then allowed to stand in the refrigerator for 46 hours. The solution was quickly brought to dryness *in vacuo* (40°), the resinous residue taken up in ether, and the latter washed with sodium carbonate, and water. After drying and removal of the ether, 84.8 mg. of a glassy residue was obtained. (Calc'd for partial solvolysis: $C_{26}H_{40}O_7 \rightarrow C_{24}H_{38}O_6$: 84.4 mg.) When this material was chromatographed over aluminum oxide, two peaks of the chromatogram were observed. Approximately 36 mg. of crystalline material appeared in the earlier, *i.e.* less polar eluates. One fraction of the latter eluates (15 mg.) resisted all attempts at crystallization. The last eluates gave 29 mg. of crystalline residues. On purifying, the former crystalline material was identified as unchanged ethyl 3(β),19-diacetoxy-5-hydroxy-etiocholanate and the latter as ethyl 3(β),5,19-trihydroxyetiocholanate.

(*c*) *Hydrolysis in aqueous methanol in the presence of potassium bicarbonate.* To 100 mg. of ethyl 3(β),19-diacetoxy-5-hydroxyetiocholanate dissolved in 5 cc. of methanol was added a solution of 100 mg. of potassium bicarbonate in 2.5 cc. of water. After standing at room temperature (28.5°) for 21 hours, the mixture was concentrated to 1.2 cc. *in vacuo* and extracted with chloroform which was subsequently washed with water, dried, and evaporated. This furnished 84.6 mg. of a dry foam which yielded from acetone a total of 60.9 mg. of crystalline material, identified as ethyl 3(β),5,19-trihydroxyetiocholanate. The remaining 23.7 mg. of non-crystalline material furnished by chromatographic separation only 1.6 mg. of ethyl 3(β),5,19-trihydroxyetiocholanate and 1.8 mg. of a less polar, still impure, crystalline substance.

Ethyl 19-hemisuccinyloxy-3(β),5-dihydroxyetiocholanate (XXV) *and its methyl ester* (XXVI). To a solution of 250 mg. of succinic anhydride (Eastman Kodak, m.p. 118–119° in 1.35 cc. of pyridine was added 250 mg. of ethyl 3(β),5,19-trihydroxyetiocholanate. It was allowed to stand at room temperature (28°) for three hours, and was poured on ice, and the precipitate taken up in 50 cc. of ether which was washed with 20 cc. of cold 2 *N* hydrochloric acid, four portions of 1 cc. of water, 5 cc. of cold 10% potassium carbonate, and four times with 1 cc. of water. The ether was dried with sodium sulfate and brought to dryness; wt. of the crystalline residue 202.7 mg. (neutral). The carbonate extract including the subsequent aqueous washings were acidified with 4 cc. of cold 2 *N* hydrochloric acid. The turbid solution was extracted with three portions of 25 cc. of ether. The combined ether was washed five times with 1 cc. of water, dried, and evaporated to dryness; wt. 54.2 mg. (acid).

Recrystallization of the neutral part (202.7 mg.) gave 183.6 mg. of pure ethyl 3(β),5,19-trihydroxyetiocholanate. The acid fraction (54.2 mg.) was a brittle foam which resisted attempts at crystallization. It was transformed into the methyl ester by treating it with a slight excess of diazomethane; yield, 51.9 mg. of resinous methyl ester. It was chromatographed in a mixture of 3.5 cc. of benzene and 6.5 cc. of petroleum ether through a column of 2.0 g. of alkali-free aluminum oxide[13] (diam. 10 mm.). The eluting was done successively with 10-cc. portions of benzene-petroleum ether (benzene content gradually increasing) benzene, benzene-ether (ether content gradually increasing), ether, and ether-methanol (methanol content gradually increasing). The major part of the material (35.0 mg.) was eluted by solvent combinations containing 8 cc. of benzene + 2 cc. of ether and 6 cc. of benzene + 4 cc. of ether. Recrystallization of these fractions from a small volume of ether yielded 16.4 mg. of flat hexagonal crystals, m.p. 103°. The mother liquors were rechromatographed and thus furnished an additional 3.1 mg. of material, m.p. 101.5–102.5° and 1.0 mg. of slightly less pure crystals, m.p. 99–100°. Total yield of pure crystalline ester, 20.5 mg. $[\alpha]^{24}_{D}$ +52.5° (5.3 mg. in 2.0 cc. of chloroform; l = 1.51 dm., α +0.21°).

Anal. Calc'd for $C_{27}H_{42}O_8$ (494.33): C, 65.54; H, 8.56.
(Methyl ester of hemisuccinate)
$C_{32}H_{48}O_{11}$ (608.38): C, 63.12; H, 7.95.
(Dimethyl ester of dihemisuccinate)
Found: C, 65.94; H, 8.62 (E.W.D.H.).

Ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (XXVII) *and ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate* (XXVIII).[18] A total of 760.6 mg. (2 millimoles) of ethyl 3(β),5,19-trihydroxyetiocholanate with melting points between 180 and 186° was dissolved in 8 cc. of anhydrous pyridine (distilled over BaO) to which was added 557.1 mg. (2 millimoles) of triphenylchloromethane (Eastman Kodak, m.p. 102–103°; checked by titration, 97.7% pure). The solution was heated on a steam-bath for 85 minutes and then allowed to stand at 23° for 16 hours. It was poured into 250 cc. of ice and water. After one hour, the white gummy precipitate was taken up in 100 cc. of ethyl acetate and the aqueous phase washed with eight portions of 25 cc. of ethyl acetate. The combined ethyl acetate extracts were successively washed with four portions of 25 cc. of *N* hydrochloric acid, two portions of 25 cc. of *N* sodium bicarbonate, and six portions of 3 cc. of water. After drying with sodium sulfate, the ethyl acetate was evaporated, the residue taken up in acetone and again brought to dryness *in vacuo*. In this fashion 1196 mg. of a brittle foam (theoretical, 1244 mg.) was obtained which was dissolved in a mixture of 10 cc. of ether and 10 cc. of petroleum ether. Overnight 59.9 mg. of clusters of crystals separated, m.p. 182–184°. There was no depression of the melting point when mixed with an authentic sample of ethyl 3(β),5,19-trihydroxyetiocholanate. The filtrate was brought to dryness *in vacuo* and thus yielded 1140 mg. of a white brittle foam. This residue was subjected to chromatographic adsorption in a mixture of 140 cc. of benzene and 60 cc. of petroleum ether. The solution was filtered through a column (diam. 22 mm.) of 45 g. of alkali-free aluminum oxide.[13] The original solution was passed through within 3½ hours, the following eluate within one hour, and all other eluates within about thirty minutes each.

The residue of fraction 1 yielded from ether mushroom-like arrangements of long thin needles, yield 80.0 mg., m.p. 155–166° (decomp.) (representing crude ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate; *vide infra*). An attempt was made to purify this substance further by subjecting the total of fraction 1 to a renewed chromatographic separation (*vide infra*).

Fraction 2 furnished from ether five crops of small cubic crystals; yield 80.6 mg., melting points between 154 and 162°. Fraction 3 gave only one crop of similar crystals; yield, 16.4 mg., m.p. 156–162°. All of these samples were identified as triphenylcarbinol. There was, however, a depression of the melting point, when triphenylcarbinol was mixed with the crystalline crop resulting from fraction 1. No crystalline material resulted from fraction 4.

Fractions 5 to 11 gave by separate treatment with ether thirteen crops of cubic or rectangular crystals; yield 416.3 mg., melting points between 177 and 183°. Mixed melting points within this whole series did not show a depression, (representing fairly pure ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate, *vide infra*). No crystals were secured from fractions 12–14.

By dissolving fractions 15–17 separately in acetone and concentrating to a small volume, six crops of prismatic crystals were obtained; yield 138.3 mg., melting points between 181 and 185°. There was no depression of the melting point of either crop with ethyl 3(β),5,19-trihydroxyetiocholanate.

Fractions 18–20 were not investigated. The following material was combined for a renewed chromatographic separation: the mother liquors of the various crystalline crops

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[18] A preliminary tritylation experiment was carried out by Miss M. G. Conroy.

resulting from fractions 2–10 and fractions 15–17 and the residues of fractions 11–14; total 366.2 mg. The chromatographic scheme differed from the one given above in particular in that the number of the benzene-petroleum ether combinations was doubled. This was done with a view of obtaining additional amounts of the crystalline material which had resulted from the above chromatographic fractions 5–11 (*i.e.* ethyl 19-tritoxy-3 (β), 5-dihydroxyetiocholanate; *vide infra*). The renewed chromatographic treatment yielded only a trace of crystals identical with those which had been obtained from fraction 2 of the previous chromatogram. A total of 15.2 mg. was identified as pure triphenylcarbinol. Three crystalline crops, totalling 70.3 mg. were found identical with the material isolated from fractions 5–11 of the previous chromatogram (*i.e.* ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate; *vide infra*). Only 4.6 mg. of more ethyl 3(β),5,19-trihydroxyetiocholanate resulted from this renewed chromatographic separation.

CHROMATOGRAPHIC FRACTIONATION

NO. OF FRACTION	SOLVENT	WEIGHT OF RESIDUE, MG.	APPEARANCE OF RESIDUE
1	140 cc. of benzene + 60 cc. of petr. ether (original solution)	88.9	Brittle foam
2	140 cc. of benzene + 60 cc. of petr. ether	143.4	Crystalline
3	160 cc. of benzene + 40 cc. of petr. ether	118.4	Brittle foam
4	170 cc. of benzene + 30 cc. of petr. ether	99.1	Brittle foam
5	180 cc. of benzene + 20 cc. of petr. ether	155.4	Partly cryst., partly foam
6	190 cc. of benzene + 10 cc. of petr. ether	127.4	Brittle foam
7	200 cc. of benzene	103.6	Brittle foam
8	200 cc. of benzene	74.8	Brittle foam
9	190 cc. of benzene + 10 cc. of ether	27.7	Brittle foam
10	175 cc. of benzene + 25 cc. of ether	14.6	Partly resin, partly foam
11	150 cc. of benzene + 50 cc. of ether	19.5	Partly resin, partly foam
12	100 cc. of benzene + 100 cc. of ether	5.9	Resinous
13	50 cc. of benzene + 150 cc. of ether	5.1	Resinous
14	200 cc. of ether	4.0	Resinous
15	200 cc. of ether + 1 cc. of methanol	17.2	Partly resin, partly cryst.
16	195 cc. of ether + 5 cc. of methanol	133.0	Crystalline
17	190 cc. of ether + 10 cc. of methanol	19.8	Crystalline
18	175 cc. of ether + 25 cc. of methanol	4.5	Partly resin, partly cryst.
19	150 cc. of ether + 50 cc. of methanol	3.0	Crystalline
20	200 cc. of methanol	29.1	Crystalline
Total		1194.4	

The total yields of the various groups of crystalline materials, as obtained from both chromatograms, were therefore as follows: (a) material later identified as ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate, 80.0 mg.; (b) triphenylcarbinol, 112.2 mg.; (c) material later identified as ethyl 19-tritoxy-3(β), 5-dihydroxyetiocholanate, 486.6 mg.; (d) ethyl 3(β),5,19-trihydroxyetiocholanate, 142.9 mg. (The total of the latter substance recovered was 202.8 mg.; *cf.* the material isolated before chromatography.).

Ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate(XXVII). The combined crystalline material referred to under (c) was recrystallized once more from ether. The first crop represented 391.0 mg. of rectangular crystals, m.p. 179–183°. In addition 56.4 mg. of crystalline material, m.p. 176–179°, was obtained. $[\alpha]_D^{21.5}$ −16.0° (19.6 mg. in 2.0 cc. of chloroform; l = 1.51 dm., α −0.24°).

Anal. Calc'd for $C_{41}H_{50}O_5$ (622.39): C, 79.05; H, 8.10.

Found: C, 78.99, 79.10; H, 8.09, 8.03 (E.W.D.H.); C, 78.91; H, 8.05 (J.R.).

Ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate (XXVIII). With a view of purifying the material referred to under (a) the total of fraction 1 (88.9 mg.) of the above chromatogram was subjected to another chromatographic adsorption. To obtain an efficient separation, the number of the benzene-petroleum ether combinations was raised to twenty-one. The bulk of the material (67.2 mg.) came out in three adjoining eluates (3 cc. of benzene +12 cc. of petr. ether; 3 cc. of benzene + 12 cc. of petr. ether; 4 cc. of benzene + 11 cc. of petr. ether). Separate recrystallization of these three fractions from ether furnished four crops of crystals (total 55.5 mg.), mushroom-like arrangements of long thin needles. They were combined and again recrystallized from ether. First crop, 31.2 mg., m.p. 153–164° (decomp.). Second crop, 6.3 mg., m.p. 153–162° (decomp.). $[\alpha]_D^{23.5}$ +28.0° (20.8 mg. in 2.0 cc. of chloroform, l = 1.51 dm.; α +0.44°).

Anal. Calc'd for $C_{60}H_{64}O_5$ (864.50): C, 83.29; H, 7.46.

Found: C, 82.67; H, 7.77 (E.W.D.H.).

The above preparation of ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate and ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate has been repeated with similar results on a somewhat larger scale (starting material, 1.39 g. of ethyl 3(β),5,19-trihydroxyetiocholanate).

Oxidation of ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (XXVII) *with chromic acid in glacial acetic acid.* A total of 62.2 mg. (0.1 millimole) of ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate was dissolved in 2 cc. of glacial acetic acid. Immediately thereafter 5.5 cc. (the equiv. of 1.1 atoms of oxygen) of a solution of 133.4 mg. of chromium trioxide in 100 cc. of 95% acetic acid was added over a period of 75 minutes at room temperature. After standing overnight, 0.4 cc. of absolute ethanol was added and the solution brought to dryness *in vacuo.* In order to remove the acetic acid completely, the residue was taken up in absol. ethanol and this solution again brought to dryness. To the residue was added 2 cc. of water and 25 cc. of ether. The solution was washed with 2 cc. of *N* sulfuric acid, three times with small amounts of water, once with 1 cc. of *N* sodium carbonate, and four times with 1 cc. of water. After drying and evaporating the ether 53.4 mg. of a neutral residue was obtained. After the addition of some ice, the carbonate phase and the subsequent aqueous washings were acidified with 0.6 cc. of 4 *N* sulfuric acid and extracted four times with 15 cc. of ether. The combined ether extracts were washed with water, dried, and evaporated to dryness. Only a trace of acid material (4.3 mg.) was obtained. The neutral phase was purified by chromatography. From the early eluates (benzene-petr. ether) triphenylcarbinol was isolated. From the later eluates (benzene-ether) several crystalline, but non-identical fractions resulted. One of them had the melting point 202–210°; it did not show a depression of the m.p. when mixed with the compound interpreted to be ethyl 3-keto-5,19-dihydroxyetiocholanate which had been obtained by the partial oxidation of ethyl 3(β),5,19-trihydroxyetiocholanate with chromic acid (*vide supra*).

Cleavage of the ether linkage of ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (XXVII). To a solution of 31.1 mg. (0.05 millimole) of the 19-trityl ether in 1 cc. of glacial acetic acid was added 2.75 cc. of 95% acetic acid and the mixture allowed to stand at 27° for 24 hours (1st experiment) and one hour (2nd experiment) respectively. The solution was quickly brought to dryness *in vacuo* (50°), the residue taken up in ethanol, and this solution again evaporated *in vacuo.* This treatment was repeated until the odor of acetic acid had disappeared. The crystalline residue was dissolved in 15 cc. of ether and this solution successively washed once with 1 cc. of *N* sulfuric acid, four times with 0.5 cc. of *N* sodium carbonate, and four times with 0.5 cc. of water. After drying with sodium sulfate the ether solution was evaporated to dryness. The residue, weighing 28.4 mg. (1st expt.) and 29.5 mg. (2nd expt.) respectively, was recrystallized from a small amount of ether which furnished 8.5 mg. (1st expt.) and 9.4 mg. (2nd expt.) of not quite pure ethyl 3(β),5,19-trihydroxyetiocholanate. The remainder in each experiment was subjected to chromatographic adsorption. The scheme of the chromatogram was analogous to the one given in the preparation of ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (*vide supra*). In either experiment only triphenylcarbinol and additional amounts of ethyl 3(β),5,19-trihydroxyetiocholanate resulted. No unchanged ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate was isolated.

Cleavage of the ether linkages of ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate (XXVIII). To a solution of 171.6 mg. (~0.2 millimole) of fairly pure 3(β),19-ditrityl ether in 5.5 cc. of glacial acetic acid was added 15.1 cc. of 95% acetic acid. After standing at room temperature (30°) for a period of seven hours, the mixture was quickly brought to dryness *in vacuo*. The residue was dissolved in ethanol and evaporated *in vacuo*. This treatment was repeated several times until no more odor of acetic acid was noticeable. The residue was taken up with 5 cc. of water and 75 cc. of ether. The ether was washed with 6 cc. of *N* sulfuric acid, 4 cc. of *N* sodium carbonate, and four portions of 2 cc. of water, dried with sodium sulfate, and evaporated to dryness; wt. of the crystalline residue 156.1 mg. The chromatographic purification (*vide supra*) yielded from the early eluates (benzene-petroleum ether) triphenylcarbinol and from the late eluates (benzene-ether and ether-methanol) ethyl 3(β),5,19-trihydroxyetiocholanate. There was no indication of the presence of any other substance.

Ethyl 3(β)-acetoxy-19-tritoxy-5-hydroxyetiocholanate (XXIX). To a solution of 62.2 mg. (0.1 millimole) of ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate in 1 cc. of pyridine was added 1 cc. of acetic anhydride and the mixture allowed to stand at room temperature (27°) for about 22 hours. It was brought to dryness *in vacuo*, the resinous residue dissolved in 75 cc. of ether and washed twice with 2 cc. of *N* hydrochloric acid, four times with 1 cc. of *N* sodium carbonate, and five times with 3 cc. of water. After drying with sodium sulfate and evaporating the ether 65.0 mg. of a brittle foam was obtained which resisted attempts at crystallization. In order to assure the uniformity of this material, 26.5 milligrams was chromatographed through 1 gram of alkali-free aluminum oxide[13] (diam. of column 10 mm.). The major part of the material (24.6 mg.) was recovered from the earliest eluates, varying from 2 cc. of benzene + 8 cc. of petr. ether to 3.5 cc. of benzene + 6.5 cc. of petr. ether. The chromatogram demonstrated, therefore, that in accordance with expectation, the acetylation had resulted in a decrease of polarity. The residues isolated from these early eluates still resisted attempts at crystallization. They were eventually combined. It is realized that the figures recorded for the optical rotation are of limited value. $[\alpha]_D^{23}$ +20.2° (19.8 mg. in 2.0 cc. of chloroform, l = 1.51 dm., α +0.30°).

Anal. Calc'd for $C_{43}H_{52}O_6$ (664.41): C, 77.66; H, 7.89.
Found: C, 76.27; H, 8.13 (J.R.).

Deacetylation of ethyl 3(β)-acetoxy-19-tritoxy-5-hydroxyetiocholanate (XXIX). To a solution of 10.2 mg. of 3(β)-acetoxy-19-tritoxy-5-hydroxyetiocholanate in 10 cc. of absol. ethanol was added 0.16 cc. of an absolute alcoholic solution of 0.1 *N* potassium hydroxide (1 equiv. of KOH). The mixture was allowed to stand at room temperature for 23 hours. The still alkaline solution was made slightly acid with one droplet of glacial acetic acid and then brought to dryness *in vacuo*. The residue was taken up in 30 cc. of ether and washed once with 0.5 cc. of *N* sodium carbonate and with six 0.5-cc. portions of water. After drying with sodium sulfate the ether phase was evaporated. On recrystallizing the residue from ether, 6.2 mg. of crystalline material was obtained, m.p. 177–179°; no depression of m.p. with ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate.

Ethyl 3(β)-acetoxy-5,19-dihydroxyetiocholanate (XXX) *by cleavage of the ether linkage of ethyl 3(β)-acetoxy-19-tritoxy-5-hydroxyetiocholanate* (XXIX). To a solution of 65.0 mg. of ethyl 3(β)-acetoxy-19-tritoxy-5-hydroxyetiocholanate in 2.0 cc. of glacial acetic acid was added 5.5 cc. of 95% acetic acid and the mixture allowed to stand at room temperature (29°) for two hours. It was brought to dryness *in vacuo* (50–55°) and the residue taken up in ethanol which was subsequently evaporated. This treatment was repeated several times in order to remove all traces of acetic acid. The residue was taken up in 25 cc. of ether and washed with 2 cc. of *N* sulfuric acid, with four 1-cc. portions of *N* sodium carbonate, and with four 1-cc. portions of water. After drying and evaporating the ether, 61.0 mg. of a residue was obtained. No attempt at fractional crystallization was undertaken. This material was chromatographed in a mixture of 5 cc. of benzene and 20 cc. of petroleum ether through a column (diam. 10 mm.) of 4.0 g. of alkali-free aluminum oxide[13] within a period of about one hour. The eluants (25 cc. each) were passed through at the rate of approx. fifteen minutes. A substantial amount of material (21.3 mg.) was obtained from the second and third eluate (ratio, 10 cc. of benzene + 15 cc. of petr. ether). After recrystallization it was identified as triphenylcarbinol. Only traces of residues were secured from the eluates 4–7. Substantial quantities (total 34.2 mg.) were isolated from the eluates 8–14 (ratios, 20 cc. of benzene + 5 cc. of petr. ether; 25 cc. of benzene; 20 cc. of benzene + 5 cc. of ether; and 15 cc. of benzene + 10 cc. of ether). These residues were first separately recrystallized from ether. This furnished 25 crops of long thin rectangular crystals; total 29.2 mg., melting points between 150 and 153°. There were no depressions of the melting points within this whole series. All crystalline crops were therefore combined and recrystallized once more from ether; rosettes of short fine needles, wt. of first crop 14.0 mg., m.p. 149–152°. Additional crystalline crops of identical material were secured from the mother liquor. $[\alpha]_D^{30}$ +50.7° (12.8 mg. in 2.0 cc. of chloroform, l = 1.51 dm., α +0.49°).

Anal. Calc'd for $C_{24}H_{38}O_6$ (422.30): C, 68.20; H, 9.07.
Found: C, 68.30; H, 9.01 (E.W.D.H.).

In another experiment, carried out in the same fashion, the cleavage of the trityl ether linkage did not go to completion. In order to be safe, it is recommended, therefore, to perform the cleavage over 24 hours rather than two hours as stated above.[19]

3(β)-Acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester,[8] (XXXI) *by oxidation with chromic acid of ethyl 3(β)-acetoxy-5,19-dihydroxyetiocholanate* (XXX). To a solution of 35.2 mg. of ethyl 3(β)-acetoxy-5,19-dihydroxyetiocholanate in 2.0 cc. of glacial acetic acid was added at room temperature within a period of 20 minutes, a total of 2.5 cc. (the equiv. of 2.2 atoms of oxygen) of a solution of 136 mg. of chromium trioxide in 25 cc. of 95% acetic acid. The mixture appeared green after the addition of 2.0 cc. of the chromium trioxide solution, but stayed brown after the total of 2.5 cc. had been added. After standing overnight 0.5 cc. of ethanol was added and the mixture brought to dryness *in vacuo.* In order to remove the last traces of acetic acid the residue was repeatedly taken up in ethanol and brought to dryness. Finally 2 cc. of water was added and the resulting precipitate taken up in 25 cc. of ether. The ether was washed successively with 2 cc. of *N* sulfuric acid, small amounts of water, 1 cc. of *N* sodium carbonate, and four 1-cc. portions of water. After drying and evaporating the ether, 14.1 mg. of a resinous neutral residue was obtained. In contradistinction to the starting material it did not crystallize from ether. The carbonate phase, including the subsequent aqueous washings, was cooled with ice, acidified with 1 cc. of 4 *N* sulfuric acid, and extracted with four 15-cc. portions of ether. The combined ether extracts were washed with eight 1-cc. portions of water, dried, and evaporated *in vacuo*; wt. of the acid residue 15.2 mg. On adding ether immediate crystallization of clusters of small rectangles occurred. First crop: wt. 2.2 mg., m.p. 206–212° (decomp.). Second crop: wt. 4.3 mg., m.p. 207.5–209° (decomp.). Third crop: wt. 2.2 mg., m.p. 203–204° (decomp.). These three crystalline fractions were combined and recrystallized once more from ether; rosettes of long thin needles, wt. 6.3 mg., m.p. 206–210° (decomp.). $[\alpha]_D^{24.5}$ +65.7° (22.45 mg. in 2.0 cc. of chloroform, l = 1.51 dm., α +1.11°).

Anal. Calc'd for $C_{24}H_{36}O_7$ (436.28): C, 66.01; H, 8.32.
Found: C, 66.14; H, 8.28 (E.W.D.H.).

A reoxidation of the neutral fraction (14.1 mg.) under analogous conditions yielded 8.0 mg. of neutral and 2.8 mg. of acid material. Both residues refused to crystallize.

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[19] In an additional experiment hydrolysis (20 hours) of 499.4 mg. of XXIX gave 269.5 mg. (85.0%) of pure XXX; m.p. 154–156° (decomp.)

SUMMARY

1. The process of making ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) from strophanthidin (I) has been revised and simplified. A detailed description of the preparation of the crystalline intermediate ethyl 3(β),5,19-trihydroxy-Δ^{14}-etiocholenate (VI) and its diacetyl derivative (VII) is given.

2. Ethyl 3(β),19-diacetoxy-5-hydroxyetiocholanate (XI) can be distilled in a high-vacuum without decomposition. On subjecting the corresponding free acid, 3(β),19-diacetoxy-5-hydroxyetiocholanic acid (X), to the same treatment, a mixture of substances resulted in which probably 3(β),19-diacetoxy-Δ^5-etiocholenic acid (XIV) is present. It was characterized by the crystalline methyl ester XV. Part of the material (X) is probably aromatized to 3-acetoxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylic acid (XVI). This was characterized by transforming it into the methyl ester XVII which was hydrolyzed to methyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate (XVIII). The latter was compared with an authentic sample (6).

3 A partial oxidation of ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) with chromic acid in a solution of acetic acid led to a mixture of substances from which two compounds have been isolated in a pure crystalline form. One of them is probably ethyl 3-keto-5,19-dihydroxyetiocholanate (XIX).[20]

4. Contrary to expectations ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) could not be made to undergo the Oppenauer reaction.

5. On treating ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) with Raney nickel and cyclohexanone under mild conditions, a mixture of substances resulted from which three different compounds were isolated in a pure crystalline form. One of them was found to be ethyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate (XXII). It was saponified to 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylic acid (XXIV). The latter was compared with a sample of the same substance as prepared from cholesterol (6). A correlation has been established between the cardiac aglycones and the estrogenic hormones. Both, XXII and XXIV possess no significant estrogenic activity.

6. Attempts to subject ethyl 3(β),19-diacetoxy-5-hydroxyetiocholanate (XI) to a partial deacetylation failed.

7. A partial succinylation of ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) yielded the monohemisuccinate XXV which was characterized by the crystalline methyl ester XXVI.

8. By tritylation, ethyl 3(β),5,19-trihydroxyetiocholanate (VIII) was converted into ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (XXVII) as main product and ethyl 3(β),19-ditritoxy-5-hydroxyetiocholanate (XXVIII) as by-product. In an acid medium both of these trityl ethers are easily hydrolyzed to VIII. Acetylation of ethyl 19-tritoxy-3(β),5-dihydroxyetiocholanate (XXVII) yielded the resinous ethyl 3(β)-acetoxy-19-tritoxy-5-hydroxyetiocholanate (XX-

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[20] (Addition to proof, February 10, 1950). Recent experiments by Mary A. Wagner indicate that the compound to which was assigned the structure of ethyl 3-keto-5,19-dihydroxyetiocholanate (XIX) is actually ethyl 19-oxo-3(β),5-dihydroxyetiocholanate. Details will be published shortly.

IX) which by alkaline solvolysis was reconverted into XXVII. Acid hydrolysis of XXIX gave ethyl 3(β)-acetoxy-5,19-dihydroxyetiocholanate (XXX). The latter was oxidized with chromic acid to 3(β)-acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (XXXI).[8]

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