[CONTRIBUTION FROM THE GEORGE S. COX MEDICAL RESEARCH INSTITUTE, UNIVERSITY OF PENNSYLVANIA]

INVESTIGATIONS ON STEROIDS. IV. NEW DEGRADATION PRODUCTS OF CHOLIC ACID AND STUDIES ON THE SYNTHESIS OF 7,12-DIHYDROXYPROGESTERONE[1]

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Received October 1, 1940

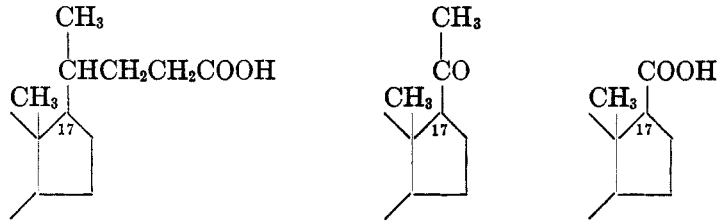
In a previous paper (1) it was pointed out that it appears desirable to obtain for physiological examination compounds which are derived from progesterone or desoxycorticosterone by the attachment of hydroxyl groups to various carbon atoms of the sterol nucleus.

Four such *hydroxylated progesterones* are known at the present time. A 12-hydroxyprogesterone was synthesized from desoxycholic acid (2), a 17-hydroxyprogesterone was isolated from beef adrenal glands (3), and recently an 11-hydroxyprogesterone was prepared from corticosterone by Reichstein (4). These three compounds possess no noticeable progestational activity; definite data are not available concerning the adrenal cortical activity. Ehrenstein and Stevens (1) prepared from pregnenolone the acetate of 6(α)-hydroxyprogesterone, which manifests a distinct progestational effect, and possibly also slight adrenal cortical activity. All known *hydroxylated desoxycorticosterones* have been found in the adrenal cortex. In all of these, hydroxyl groups are attached to carbon atoms 11 or 17 of the sterol nucleus, or to both. These compounds counteract the manifestations of adrenal insufficiency; some of them are known to possess also slight progestational activity.

Certain bile acids appear to afford a suitable starting material for the preparation of compounds of the above mentioned types. They carry hydroxyl groups at carbon atoms of the sterol nucleus where they cannot be easily introduced by the available chemical procedures. By systematic degradation of bile acids according to the method of Wieland (5) one can obtain a 17-methyl ketone and a 17-carboxylic acid (etio acid). Such a 17-methyl ketone can serve as starting material for the preparation of a compound belonging to the progesterone series, whereas the 17-carboxylic acid (etio acid) may be transformed into a substance derived from desoxycorticosterone.

[1] Aided by a grant from the Smith, Kline, and French Laboratories in Philadelphia.

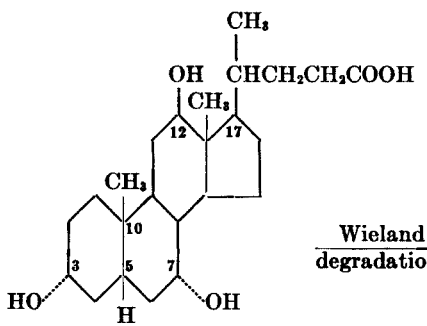
Reported on October 28, 1940 at the Autumn Meeting of the National Academy of Sciences at the University of Pennsylvania.



Bile acid type | 17-Methyl ketone | 17-Carboxylic acid (etio acid)

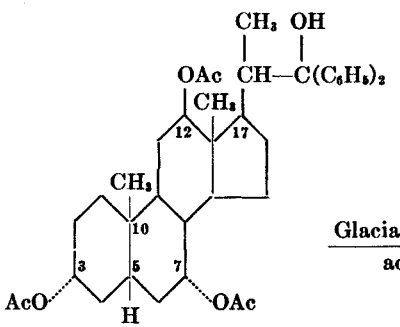
Dalmer (6) and his associates found that the Wieland degradation (5) is applicable not only to cholanic acid, but also to hydroxylated cholanic acids, provided that the hydroxyl groups are protected by acetylation. A number of bile acids were subsequently subjected to such a degradation. Sawlewicz and Reichstein (7) degraded lithocholic acid [3(α)-hydroxycholanic acid] to etiolithocholic acid [3(α)-hydroxyetiocholanic acid] which in turn was transformed into desoxycorticosterone (8). Recently Hoehn and Mason (9) repeated this degradation and isolated etiolithocholic acid along with 3(α)-hydroxypregnane-20-one. Desoxycholic acid [3(α),12-dihydroxycholanic acid] was degraded by Hoehn and Mason (10) as well as Reichstein and von Arx (11) to etiodesoxycholic acid [3(α),12-dihydroxyetiocholanic acid] and 3(α),12-dihydroxypregnane-20-one. The latter substance was transformed into 12-hydroxyprogesterone (2). Chenodesoxycholic acid [3(α),7-dihydroxycholanic acid] was subjected to a Wieland degradation by Ishihara (12); thus etiochenodesoxycholic acid [3(α),7-dihydroxyetiocholanic acid] and 3(α),7-dihydroxypregnane-20-one were obtained. Kimura and Sugiyama (13) subjected hyodesoxycholic acid [3(α),6-dihydroxycholanic acid] to a similar series of reactions, the end-product of which was 3(α),6-dihydroxypregnane-20-one; the corresponding etio acid was not described. The most readily available bile acid is cholic acid [3(α),7,12-trihydroxycholanic acid] (I). Morsman, Steiger, and Reichstein (14) subjected it to a Wieland degradation. The end-product was 3(α),7,12-trihydroxypregnane-20-one (V); the corresponding etio acid (VIII) was not described.

We decided to repeat the degradation of cholic acid as worked out by Reichstein and his associates (14). It was our intention to transform the 3(α),7,12-triacetoxypregnane-20-one (IV) into 7,12-dihydroxyprogesterone or its diacetate (XV). Another object of our investigation was the preparation of the still unknown etiocholic acid [3(α),7,12-trihydroxyetiocholanic acid] (VIII), which we intend later to transform into 7,12-dihydroxy-11-desoxycorticosterone (7,12,21-trihydroxy-4-pregnene-3,20-dione). We found the procedure described by Reichstein (14) easily reproducible. Diphenyl-[3(α),7,12-triacetoxy-ter-norcholyl]



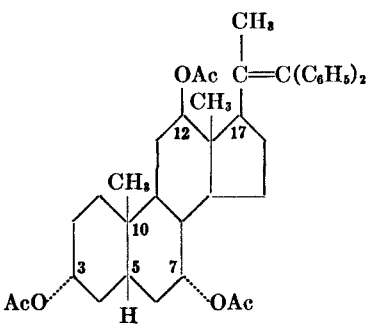
I. Cholic acid.

Wieland degradation.



II. Diphenyl-[3(α),7,12-triacetoxy-ter-norcholyl] carbinol.

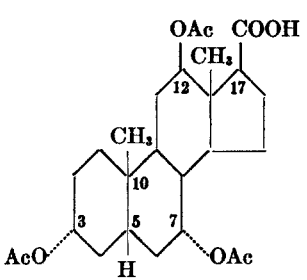
Glacial acetic acid.



III. 1,1-Diphenylmethyl-[3(α),7,12-triacetoxyetiocholyl]ethylene.

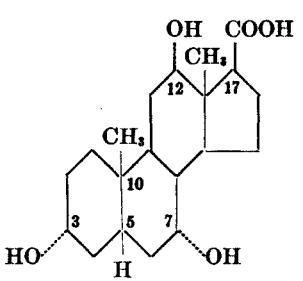
CrO_3

Ozone.

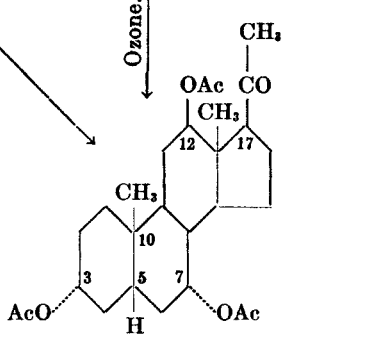


VII. 3(α),7,12-Triacetoxyetiocholanic acid.

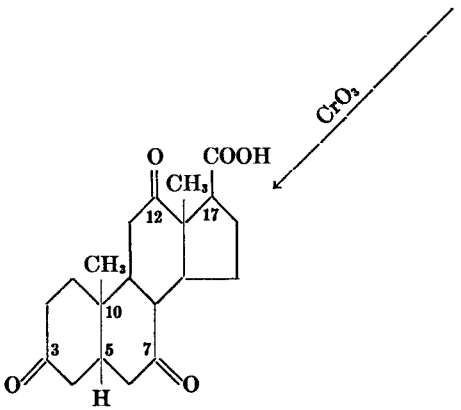
sapon.



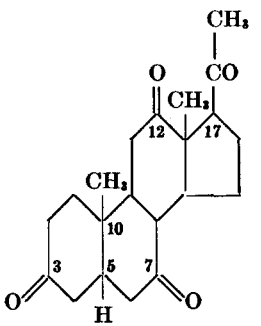
VIII. Etiocholic acid.



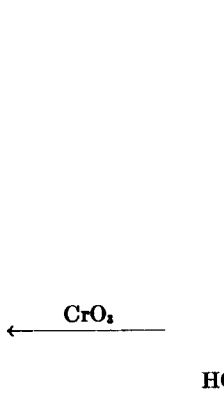
IV. 3(α),7,12-Triacetoxypregnane-20-one.

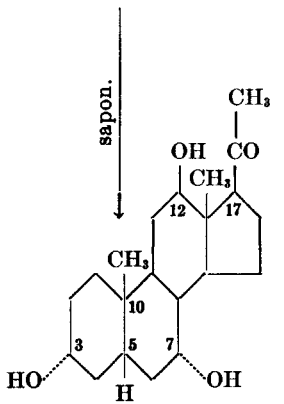


IX. Dehydroetiocholic acid.

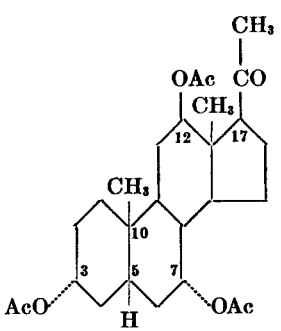


VI. Pregnane-3,7,12,20-tetraone.

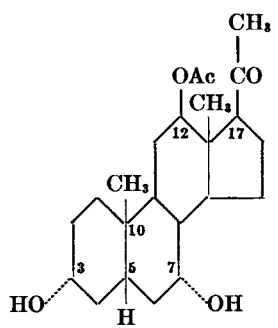




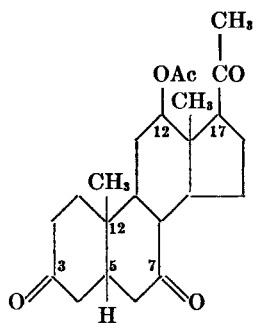
V. 3(α),7,12-Trihydroxypregnane-20-one.



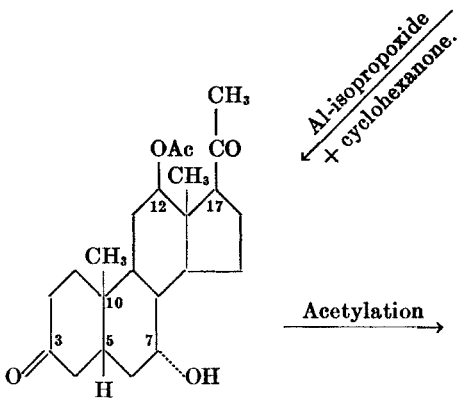
IV. 3(α),7,12-Triacetoxypregnane-20-one.



X. 12-Acetoxypregnane-3(α),7-diol-20-one.



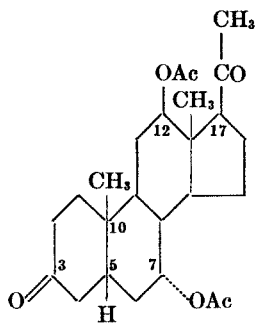
XI. 12-Acetoxypregnane-3,7,20-trione.



XII. 12-Acetoxypregnane-7-ol-3,20-dione.

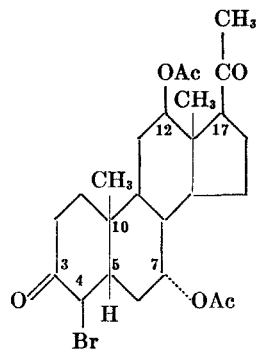
Al-isopropoxide + cyclohexanone.

Acetylation



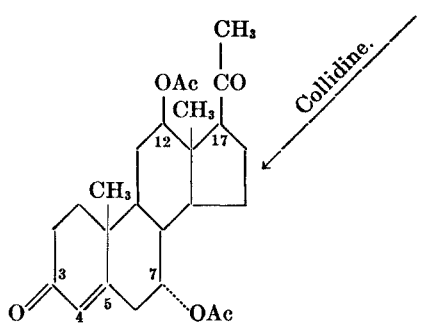
XIII. 7,12-Diacetoxypregnane-3,20-dione.

1 mole Br_2



XIV. 4-Bromo-7,12-diacetoxypregnane-3,20-dione.

Collidine.



XV. 7,12-Diacetoxy-4-pregnene-3,20-dione. [7,12-Diacetoxyprogesterone; 7,12-dihydroxyprogesterone diacetate]

carbinol (II) was subjected to oxidation with chromic acid. Attempts to isolate from the acid fraction of this oxidation the 3(α),7,12-triacetoxyetiocholanic acid (VII) in a crystalline form failed. After saponification of the acid fraction the crystalline etiocholic acid (VIII) was obtained. Attempts to secure the crystalline 3(α),7,12-triacetoxyetiocholanic acid (VII) by re-acetylating the pure etiocholic acid (VIII) were of no avail. From the neutral fraction of the oxidation with chromic acid we were able to isolate a small amount of 3(α),7,12-triacetoxypregnane-20-one (IV). We had already finished this part of our investigation when Hoehn and Mason (10) described a new procedure for the preparation of etio acids (17-carboxylic acids) by way of the 17-methyl ketones. The latter method was slightly modified by Reichstein and von Arx (11) and generally utilized by Marker and Wittle (15). It is possible that the new procedure applied to 3(α),7,12-trihydroxypregnane-20-one (V) will furnish better yields of the etiocholic acid (VIII) than the above described method. When etiocholic acid (VIII) was oxidized with chromic acid, a compound was obtained which we consider to be dehydroetiocholic acid (3,7,12-triketoetiocholanic acid) (IX), although the analytical figures are in better agreement with a monohydroxydiketoetiocholanic acid.

The 3(α),7,12-trihydroxypregnane-20-one (V) was secured according to the procedure given by Reichstein and his associates (14). Oxidation of this compound with chromic acid yielded the hitherto unknown pregnane-3,7,12,20-tetraone (VI). We attempted the partial hydrolysis of 3(α),7,12-triacetoxypregnane-20-one (IV) with the original intention of hydrolyzing the acetyl group at carbon atom 3 only. The observations of Wieland and Kapitel (16) as well as Miyazi and Isaka (17) on acylated bile acids indicate that distinct differences exist between the rates of hydrolysis at carbon atoms 3, 7, and 12 respectively. Treatment of 3(α),7,12-triacetoxypregnane-20-one (IV) with potassium carbonate did not yield the monohydroxy compound desired, but crystalline material whose analysis indicated that the hydrolysis had resulted in a mixture which consisted predominantly of a dihydroxy compound. We therefore tried to saponify IV with exactly one mole of potassium hydroxide. Also under these experimental conditions the dihydroxy rather than the monohydroxy compound was the main product of the reaction; purification by chromatographic adsorption yielded almost the theoretical amount of 12-acetoxypregnane-3(α),7-diol-20-one (X). Hence the conclusion may be drawn that in this case the rates of hydrolysis at carbon atoms 3 and 7 are of about the same order. Therefore it was decided to subject IV to a saponification with two moles of potassium hydroxide in the expectation that this would furnish a satisfactory

yield of 12-acetoxypregnane-3(α),7-diol-20-one (X). In this case a mixture was obtained, which on repeated chromatographic treatments and recrystallizations gave about 78% of the theoretical yield of the dihydroxy compound (X). When X was oxidized with chromic acid, 12-acetoxypregnane-3,7,20-trione (XI) was obtained.

Recently Gallagher (18) reported briefly that Oppenauer's method (19) for the dehydrogenation of secondary alcohols can be utilized in the bile acid series for the selective dehydrogenation of a hydroxyl group at carbon atom 3. When we treated 12-acetoxypregnane-3(α),7-diol-20-one (X) according to Oppenauer's method with aluminum isopropoxide and cyclohexanone we observed also a selective dehydrogenation of the hydroxyl group at carbon atom 3. The reaction-product (12-acetoxypregnane-7-ol-3,20-dione, XII) was not isolated in a pure form but immediately acetylated to 7,12-diacetoxypregnane-3,20-dione (XIII). We consider it probable that the same substance (XIII) can also be obtained by treatment of 3(α),7,12-trihydroxypregnane-20-one (V) with aluminum isopropoxide and cyclohexanone and subsequent acetylation.

Butenandt and his associates (20) found that in such steroids of the allo series (H at carbon atom 5 trans to CH_3 at carbon atom 10) in which carbon atom 3 forms a keto group, bromination takes place at carbon atom 2. The corresponding compounds of the coprostane series (H at carbon atom 5 cis to CH_3 at carbon atom 10) brominate at carbon atom 4. Treatment of 7,12-diacetoxypregnane-3,20-dione (XIII) with one mole of bromine furnished a crystalline monobromide which could not be obtained in a completely pure form. Since cholic acid and hence also compound XIII possess the coprostane configuration the monobromo compound must be assigned the structure of 4-bromo-7,12-diacetoxypregnane-3,20-dione (XIV).

When 4-bromo compounds of this type are refluxed with pyridine they suffer a splitting of hydrogen bromide from the molecule to form α,β-unsaturated ketones (4:5). Compound XIV was subjected to a debromination by refluxing it with collidine (2,4,6-trimethylpyridine). This was recently introduced as a debrominating agent by Butenandt and his associates (21); its action was plausibly interpreted by Inhoffen and his co-workers (22). We obtained a bromine-free compound to which must be assigned the structure of 7,12-diacetoxy-4-pregnene-3,20-dione (7,12-diacetoxyprogesterone; 7,12-dihydroxyprogesterone diacetate, XV). It was obviously not pure. The ultraviolet absorption spectrum of a sample of this substance (Figure I) was determined in the Department of Physics of the Massachusetts Institute of Technology (Professor George R. Harrison). The maximum is at the expected wave-length (about

240 mμ). The unusual shape of the absorption curve is probably due to the impurity.

EXPERIMENTAL

All melting points were determined with the Fisher-Johns melting point apparatus of the Fisher Scientific Company (Pittsburgh, Pa.). The readings are sufficiently near the true melting points so that no corrections have been made. All microanalyses, unless otherwise stated, were carried out by Mr. William Saschek, Columbia University, New York. Valuable assistance was rendered by Mrs. Marguerite Twaddell Decker in the preparation of the starting material for this investigation.

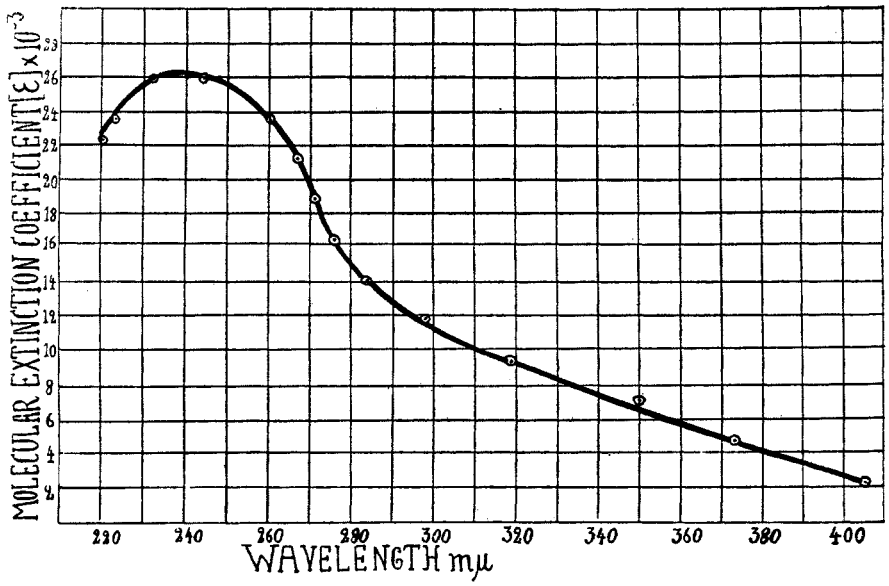


FIG. I. ABSORPTION CURVE OF A SAMPLE OF 7, 12-DIACETOXYPROGESTERONE (1.1 MG. IN 60 CC. OF ABSOLUTE ALCOHOL)

Etiocholic acid [3(α),7,12-trihydroxyetiocholanic acid] (VIII) by oxidation of diphenyl-[3(α),7,12-triacetoxy-ter-norcholyl] carbinol (II) with chromic acid. To a solution of 2.6 g. of carbinol (II) in 115 cc. of glacial acetic acid was added on a water-bath over a period of 25 minutes 2.3 g. of chromium trioxide dissolved in a mixture of 2.3 cc. of water and 60 cc. of glacial acetic acid. The heating was continued for 5½ hours; stirring was applied during the whole procedure. The major part of the acetic acid was removed *in vacuo* (55-60°). Water was added to the sticky residue and the whole was extracted three times with ether. After the washing of the combined ether phases with dilute sulfuric acid and with water, the acid products of the oxidation were removed by treatment with dilute sodium carbonate. The ether phase, containing the neutral products of the oxidation, was dried with sodium sulfate and brought to dryness; weight of the almost colorless viscous residue 1.23 g. (neutral). The sodium carbonate solution was acidified (Congo red) by the

addition of hydrochloric acid, which caused a precipitate to appear. The acid products of the oxidation were obtained by extracting the acidified phase three times with ether, drying with sodium sulfate, and evaporating to dryness; weight of the almost colorless brittle residue: 1.04 g. (acid).

Etiocholic acid [3(α),7,12-trihydroxyetiocholanic acid] (VIII) About 910 mg. of the acid residue was distilled in a high vacuum. The main part distilled between 230° and 240° at a pressure of approximately 5×10^{-5} mm.; slightly yellow glass, weight 657 mg. The forerun and the afterrun were not investigated. When the main fraction was treated with several solvents and combinations of solvents it did not show any tendency to crystallize.

Saponification: To a solution of 0.38 g. of the above described main fraction in 2.0 cc. of methanol was added a solution of 0.30 g. of potassium hydroxide (calc'd 0.18 g.) in 0.6 cc. of water. This mixture was refluxed on a water-bath for two hours. Water was added and the methanol removed *in vacuo*. The aqueous solution was acidified to Congo red and extracted several times with ethyl acetate. After short drying (about 1 hour) with sodium sulfate, the ethyl acetate extracts were concentrated to a low volume *in vacuo*, which caused crystals to separate; wt. 0.090 g., m.p. 232–237°. From the mother liquor (solid content 0.033 g.) another 0.014 g. of crystals was obtained. Repeated crystallization from acetone yielded 0.074 g. of beautiful white needles of constant m.p. 254–258°; $[\alpha]_D^{27.5}$ +65.2° (50 mg. in 2.0 cc. of absol. alcohol).

Anal. Calc'd for $C_{20}H_{32}O_5$: C, 68.13; H, 9.16.
Found: C, 67.85; H, 8.98.

3(α),7,12-triacetoxypregnane-20-one (IV) The neutral fraction of the oxidation (1.23 g.) was subjected to treatment with Girard's reagent T (23) which furnished 0.15 g. of ketonic (almost colorless resin) and 1.02 g. of non-ketonic material. Crystals were obtained from the ketonic fraction by dissolving it in ether and gradually adding petroleum ether; clusters of prisms grew slowly; wt. 0.036 g., m.p. 149–151°. More prisms were obtained from the mother liquor.

Anal. Calc'd for $C_{27}H_{40}O_7$: C, 68.02; H, 8.46.
Found: C, 68.13, 68.24; H, 8.26, 8.36.

Dehydroetiocholic acid (3,7,12-triketoetiocholanic acid) (IX) Twenty-nine milligrams of etiocholic acid (VIII) of m.p. 254–256° was dissolved in 1.0 cc. of glacial acetic acid and 17.3 mg. of chromium trioxide (= 3.15 atoms O) in 1.0 cc. of 90% acetic acid was added. The mixture was allowed to stand at room temperature for 17 hours, and after the addition of ten drops of methanol, the solution was brought almost to dryness *in vacuo*. Some water was added to the residue; after a few minutes standing the separation of beautiful crystals began. These were filtered the next day, washed with water, and dried; wt. 16.7 mg., m.p. 240.5–243.5°. After recrystallizing from a mixture of acetone and ether the melting point was raised to 245–246°.

Anal. Calc'd for $C_{20}H_{26}O_5$: C, 69.32; H, 7.57.
(for $C_{20}H_{28}O_5$: C, 68.92; H, 8.10.)
Found: C, 68.85; H, 7.92.

3(α),7,12-trihydroxypregnane-20-one (V) 3(α),7,12-triacetoxypregnane-20-one (IV) was prepared according to Reichstein's procedure (14) by ozonizing 1,1-diphenylmethyl-[3(α),7,12-triacetoxyetiocholyl]ethylene (III). The average yield was 61.8%; the yield obtained by Reichstein was 57.3%. The melting point of the substance was 150–152°; Reichstein recorded 134–135°. The difference of the melting points may be due to the existence of two polymorphic forms.

To a solution of 1.3 g. of IV in 5.2 cc. of methanol was added a solution of 0.68 g.

of potassium hydroxide in 1.6 cc. of water. The mixture was refluxed on a water-bath for two hours. After the addition of some water the methanol was removed *in vacuo*. The whole was extracted three times with ether and three times with ethyl acetate. The extracts were washed with water and dried with sodium sulfate. From the ether extract was secured 0.3 g. of crystalline material melting slightly above 120°. The ethyl acetate extract furnished several crops of crystalline material totalling 0.41 g. and melting between 120° and 125°. The saponification was carried out several times; the average yield was 72.4%, the yield computed from Reichstein's figures is 34.0%.

Pregnane-3,7,12,20-tetraone (VI) To a solution of 160 mg. of 3(α),7,12-trihydroxypregnane-20-one (V) in 40 cc. of glacial acetic acid was added 130 mg. of chromium trioxide (calc'd for 3 atoms O: 91.4 mg. of CrO_3) dissolved in 10 cc. of 90% acetic acid. The mixture was allowed to stand at room temperature for one hour. After the addition of about 10 drops of methanol the solution was concentrated to a very low volume *in vacuo*. Water was added, which caused a white precipitate to appear. Because this was rather difficultly soluble in ether, the suspension was extracted 5 times with ample quantities of ether and thereafter 3 times with ethyl acetate. The ether and ethyl acetate extracts were washed with *N* hydrochloric acid, *N* sodium carbonate, and water. After drying over sodium sulfate, the ether was brought to a small volume, which caused the separation of 102 mg. of long, irregularly-shaped plates; m.p. 238–241°. The ethyl acetate was brought completely to dryness and the residue treated with some ether. By this means about 12 mg. of crystals melting at 239–241° was secured. The total yield of almost pure crystalline material was 114 mg. The optical rotation and analysis refer to a sample of m.p. 238–242°; $[\alpha]_D^{26}$ +76.3° (20 mg. in 2.0 cc. of acetone).

Anal.[2] Calc'd for $C_{21}H_{28}O_4$: C, 73.21; H, 8.20.
Found: C, 73.30, 73.51; H, 8.15, 8.08.

12-Acetoxypregnane-3(α),7-diol-20-one (X) To a solution of 0.486 g. of 3(α),7,12-triacetoxypregnane-20-one (IV) in 35 cc. of absol. alcohol was added over a period of 48 hours in 0.75-cc. quantities, 19.5 cc. of a solution of 0.1 *N* potassium hydroxide in absol. alcohol (about 2 moles of KOH). After standing at room temperature for two more days the solution was made neutral to litmus by the addition of 50% acetic acid. It was then concentrated to a low volume *in vacuo*. After the addition of some water, a white crystalline precipitate (designated A; dry wt. 0.170 g.) appeared, which was filtered and washed with water. Further concentration furnished 0.110 g. of additional crystalline material (designated B). A third concentration *in vacuo* furnished a sticky precipitate which was taken up in chloroform. The aqueous phase was re-extracted three times with chloroform. All chloroform extracts were combined, washed with water, dried with sodium sulfate, and evaporated. The dry residue (designated C; wt. 0.120 g.) was a yellow resin. The crystalline precipitates A and B were separately and repeatedly recrystallized from a mixture of acetone and ether. A total of 0.260 g. of material was secured, which melted between 220° and 230°. To these combined fractions was added 0.015 g. of similar material from a previous experiment. The whole (0.275 g.) was subjected to a further purification by means of chromatographic adsorption. It was dissolved in a mixture of 25 cc. of chloroform and 30 cc. of benzene to which 100 cc. of petroleum ether was added. This solution was allowed to run slowly through a properly prepared column of 13.75 g. of aluminum oxide (aluminum oxide anhydrous; standardized for chromato-

[2] Microanalysis by Dr. Ing. A. Schoeller, Berlin-Schmargendorf.

graphic adsorption acc. to Brockmann, E. Merck, Darmstadt). Long needles separated from this solution before the filtration through the column was finished; they were poured with the solution onto the aluminum oxide. The adsorbed material was eluted first with 100 cc. of chloroform, thereafter with a mixture of 25 cc. of chloroform and 25 cc. of methanol, and eventually with 50 cc. of methanol. The original filtrate and the last eluate contained only a little material and were hence discarded. The residue of the first eluate (*a*) weighed 0.115 g.; m.p. 225–232°. The weight of the residue of the second eluate (*b*) was 0.145 g.; m.p. 222–230°.

By recrystallization of residue (*a*) from mixtures of chloroform, benzene, and petroleum ether, as well as acetone and ether, a total of 0.106 g. melting at 228–232° was obtained. No satisfactory purification could be achieved when residue (*b*) was subjected to recrystallization. It was therefore decided to combine residue (*b*) with the filtrates from the recrystallization of residue (*a*) and to subject the total to chromatographic adsorption. For this purpose the material was dissolved in a mixture of benzene and chloroform to which petroleum ether was added. The eluting was performed with chloroform and thereafter with four mixtures of chloroform and methanol (methanol content gradually increasing). After working up the several fractions, a total of 0.130 g. melting at 228–232° was secured.

The resinous residue C (0.120 g.) was combined with low-melting fractions obtained during the purification procedures described above; also low-melting material from previous experiments was added. The total (0.207 g.) was subjected to chromatographic adsorption. The material was dissolved in a mixture of 40 cc. of benzene and 16 cc. of petroleum ether. This solution was allowed to drip through a column of 10 g. of aluminum oxide. The eluting was begun with a mixture of 20 cc. of benzene and 5 cc. of petroleum ether, and continued with 25 cc. of benzene, a mixture of 12.5 cc. of benzene and 12.5 cc. of ether, a mixture of 12.5 cc. of ether and 12.5 cc. of chloroform, and finally with several mixtures of chloroform and methanol (methanol content gradually increasing). A crystalline residue (85.6 mg.) was obtained only from the first chloroform-methanol eluate (24.5 cc. of chloroform + 0.5 cc. of methanol). Recrystallization of this material furnished 64 mg. of m.p. 228–232°.

The analyzed sample had the melting point 230–233°; $[\alpha]_D^{27}$ +81.6° (20 mg. in 2.0 cc. of acetone).

Anal. Calc'd for $C_{23}H_{36}O_5$: C, 70.36; H, 9.25.
Found: C, 70.40; H, 9.23.

12-Acetoxypregnane-3,7,20-trione (*XI*) To a solution of 49 mg. of 12-acetoxypregnane-3(α),7-diol-20-one (X) in 11 cc. of glacial acetic acid was added 1.8 cc. (the equivalent of about 2.2 atoms of O) of a solution of 1 g. of chromium trioxide in 100 cc. of 90% acetic acid. The mixture was allowed to stand at room temperature for about 16 hours. After the addition of 20 drops of methanol it was brought to dryness *in vacuo*. Water was added to the residue and it was extracted three times with redistilled ether. The combined ether phases were washed with *N* hydrochloric acid, *N* sodium carbonate, and water, and were finally dried with sodium sulfate. On concentrating this ether solution to a small volume, stout needles separated slowly, wt. 33.4 mg., m.p. 158–161°. The constant melting point was 160.5–163.5°; $[\alpha]_D^{28}$ +125.9° (14 mg. in 2.0 cc. of acetone).

Anal. Calc'd for $C_{23}H_{32}O_6$: C, 71.09; H, 8.31.
Found: C, 71.04; H, 8.24.

7,12-Diacetoxypregnane-3,20-dione (*XIII*) To 88 mg. of 12-acetoxypregnane-3(α),7-diol-20-one (X) was added 15 cc. of toluene (dried over calcium chloride),

3 cc. of cyclohexanone, and 300 mg. of aluminum isopropoxide (Eastman Kodak). This mixture was refluxed for two hours and then brought to dryness *in vacuo* at a temperature of about 70°. After the addition of water and *N* sulfuric acid the whole was extracted four times with redistilled ether. The combined ether extracts were washed with water, with a solution of sodium carbonate, and again with water. After drying over sodium sulfate, the ethereal solution was brought to a small volume. While standing overnight crystals separated out, which were filtered and dried; wt. 41.0 mg., m.p. 210–226°. No appreciable quantity of crystals could be secured from the filtrate or its acetylation product.

The crystalline material was dissolved in a mixture of 1 cc. of pyridine and 1 cc. of acetic anhydride. This solution was heated on the water-bath for about 4 hours and then brought to dryness *in vacuo*. The addition of some water caused an apparently crystalline residue to appear; wt. about 32 mg. A little more crystalline material could be secured by extracting the filtrate with chloroform. Because the crystals were fairly insoluble in acetone they were recrystallized by dissolving in chloroform and adding acetone. Repeated recrystallizations yielded 22.8 mg. of m.p. 256–262°; $[\alpha]_D^{24}$ +113.7° (20 mg. in 2.0 cc. of chloroform).

Anal. Calc'd for $C_{25}H_{36}O_6$: C, 69.40; H, 8.39.
Found: C, 69.17; H, 8.26.

4-Bromo-7,12-diacetoxypregnane-3,20-dione (*XIV*) To a solution of 76.1 mg. of 7,12-diacetoxypregnane-3,20-dione (XIII) in 5.25 cc. of glacial acetic acid was added one drop of 40% hydrogen bromide and thereafter over a period of 20 minutes 28.7 mg. of bromine (1 mole Br_2 = 28.1 mg.) dissolved in 0.95 cc. of glacial acetic acid. After standing for 20 minutes, water was added to the decolorized solution, which caused very fine, short white crystals to appear. The crystallization was completed by allowing the solution to stand in a refrigerator overnight. Weight of the first crop 77.8 mg.; m.p. 202–216°. A second crop was secured by concentrating the filtrate to a very low volume *in vacuo*; wt. 6.0 mg.; m.p. 202–215°. It was decided to purify the first fraction (77.8 mg.) by chromatographic adsorption, for which purpose it was dissolved in a mixture of 24 cc. of benzene and 15 cc. of petroleum ether. This solution was allowed to run through a suitably prepared column of 3.5 g. of aluminum oxide (acc. to Brockmann). The adsorbed material was successively eluted with a mixture of 24 cc. of benzene and 6 cc. of petroleum ether, with 30 cc. of benzene, with a mixture of 24 cc. of benzene and 6 cc. of chloroform, with a mixture of 15 cc. of benzene and 15 cc. of chloroform, and finally with 30 cc. of chloroform. Thereafter the aluminum oxide was thoroughly extracted with methanol. The total recovered material weighed 74.6 mg.; the crystalline fractions totalled only 43 mg. It appears possible that the chromatographic treatment caused destruction of part of the crystalline material. The crystalline fractions were repeatedly recrystallized from mixtures of chloroform and 95% alcohol. The melting point was finally 210–218° (decomp.). Analyses were performed with the last substance, as well as with material of m.p. 211–217° obtained from a preliminary experiment (no chromatographic treatment). The melting points and the analytical figures indicate that the substances were not quite pure.

Anal. Calc'd for $C_{25}H_{35}BrO_6$: C, 58.69; H, 6.90.
Found: C, 59.69, 59.80; H, 7.33, 7.24.

7,12-Diacetoxy-4-pregnene-3,20-dione (*7,12-diacetoxyprogesterone*) (*XV*) A solution of 36.5 mg. of 4-bromo-7,12-diacetoxypregnane-3,20-dione (XIV) in 1.0 cc. of collidine was refluxed (metal-bath, temperature about 190°) for four hours, causing it to turn dark brown. The collidine (Eastman-Kodak) had been freshly distilled

(b.p. 165°) and dried overnight with potassium hydroxide. After the addition of *N* hydrochloric acid the solution was extracted four times with ether. The combined ether extracts were washed with *N* hydrochloric acid, *N* sodium carbonate, and water. The ether solution was dried over sodium sulfate and concentrated to a small volume, which caused the separation of crystals (5.6 mg.; m.p. 215–232°). The filtrate of these crystals was brought to dryness; weight of the residue 20.2 mg. It was decided to combine the crystals and the residue again and to subject this material (25.8 mg.) to chromatographic adsorption. For this purpose it was dissolved in a mixture of 15 cc. of benzene and 15 cc. of petroleum ether and was allowed to drip through a column of 1.8 g. of aluminum oxide (acc. to Brockmann). The elution was carried out with 50-cc. quantities of the following solvents: benzene-petroleum ether 3:2; benzene-petroleum ether 4:1; benzene only; benzene-ether 1:1; ether only; ether-chloroform 1:1; chloroform only; and finally with three mixtures of chloroform and increasing amounts of methanol. Only the second and third eluate yielded crystalline residues (8.3 + 4.1 mg.; clusters of long white needles). These (total: 12.4 mg.) were washed with ether and repeatedly recrystallized from mixtures of chloroform and ether. Eventually material (4.9 mg.) melting between 240° and 249° was secured; it was combined with a fraction (0.8 mg.) of similar melting point obtained in a preliminary experiment. Renewed recrystallization yielded 5.1 mg. of m.p. 249.5–252° (without decomp.).

Anal. Calc'd for $C_{25}H_{34}O_6$: C, 69.72; H, 7.96.
Found: C, 68.15; H, 8.21.

SUMMARY

1. Cholic acid [3(α),7,12-trihydroxycholanic acid] (I) was degraded to etiocholic acid [3(α),7,12-trihydroxyetiocholanic acid] (VIII) and 3(α),7,12-trihydroxypregnane-20-one (V).

2. Etiocholic acid [3(α),7,12-trihydroxyetiocholanic acid] (VIII) was oxidized to a substance considered to be dehydroetiocholic acid (3,7,12-triketoetiocholanic acid) (IX).

3. 3(α),7,12-Trihydroxypregnane-20-one (V) was oxidized to pregnane-3,7,12,20-tetraone (VI).

4. 3(α),7,12-Triacetoxypregnane-20-one (IV) was partially saponified to 12-acetoxypregnane-3(α),7-diol-20-one (X). The latter compound was oxidized to 12-acetoxypregnane-3,7,20-trione (XI).

5. 12-Acetoxypregnane-3(α),7-diol-20-one (X) was selectively dehydrogenated with aluminum isopropoxide and cyclohexanone to 12-acetoxypregnane-7-ol-3,20-dione (XII). This substance was acetylated to 7,12-diacetoxypregnane-3,20-dione (XIII) and the latter converted by bromination to 4-bromo-7,12-diacetoxypregnane-3,20-dione (XIV). The bromo compound (XIV) was debrominated to 7,12-diacetoxy-4-pregnene-3,20-dione (7,12-diacetoxyprogesterone; 7,12-dihydroxyprogesterone diacetate, XV). The last two substances were obviously not pure.

PHILADELPHIA, PA.

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