[CONTRIBUTION FROM THE DIVISION OF STEROID RESEARCH, THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE, UNIVERSITY OF PENNSYLVANIA]

INVESTIGATIONS ON STEROIDS. XVII. DEHYDRATION STUDIES IN THE SERIES OF 3β,5,19-TRIHYDROXYETIOCHOLANIC ACID*[1]

PABLO TH. HERZIG AND MAXIMILIAN EHRENSTEIN

Received December 20, 1951

As has been stated previously (2, 3) it is intended to synthesize certain analogs of steroid hormones where the angular carbon atom between rings A and B is either missing (19-nor compounds) or is present in an oxygenated form, *i.e.* as a primary alcohol, aldehyde, or carboxyl group. In particular, compounds structurally derived from progesterone, 11-desoxycorticosterone, and 17-hydroxy-11-desoxycorticosterone (Reichstein's substance S) are of interest. A promising starting material appeared to be ethyl 3β,5,19-trihydroxyetiocholanate (I) which in turn can be obtained by partial synthesis from strophanthidin (1, 2). The purpose of the present investigation was the conversion of I into further suitable intermediates towards this general aim.

In an earlier publication (3) it was shown that, by means of N-bromoacetamide, I can be selectively oxidized to ethyl 3-keto-5,19-dihydroxyetiocholanate which under the influence of Girard's Reagent T is smoothly dehydrated to ethyl 3-keto-19-hydroxy-Δ^4-etiocholenate (IX). It has been found that the free 3β,5,19-trihydroxyetiocholanic acid (III), as obtained by saponification of I, can be subjected to the same reaction. Thus, when III was oxidized with N-bromoacetamide, followed by suitable treatment of the reaction product with Girard's Reagent T, a satisfactory yield of 3-keto-19-hydroxy-Δ^4-etiocholenic acid (X) was obtained. The methyl ester (XI) and the known ethyl ester (IX) (3) were prepared from X with diazomethane and diazoethane respectively. Treatment of X with acetic anhydride in pyridine gave the crystalline 3-keto-19-acetoxy-Δ^4-etiocholenic acid (Xa).

In another series of experiments it was examined whether 3β,19-diacetoxy-Δ^4 (or Δ^5)-etiocholenic acid was accessible because such a product would offer certain advantages in the projected syntheses. It was found earlier (1) that ethyl 3β,19-diacetoxy-5-hydroxyetiocholanate (II) is resistant to dehydration with phosphorus oxychloride in pyridine at room temperature. Enforcing the reaction at an elevated temperature was considered inadvisable in view of observations in the periplogenin series (4) indicating that under these conditions, at least in part, a $\Delta^{3,5}$-dienic system is formed. The Darzens dehydration (thionyl chloride in pyridine) has been applied to a number of 3β-acetoxy-5α-hydroxy steroids. It seems to lead preferentially to Δ^4-anhydro compounds (*cf.* 5, 6, 7, 15). The present example (II) represents the case of a 3β-acetoxy-5β-hydroxy steroid.

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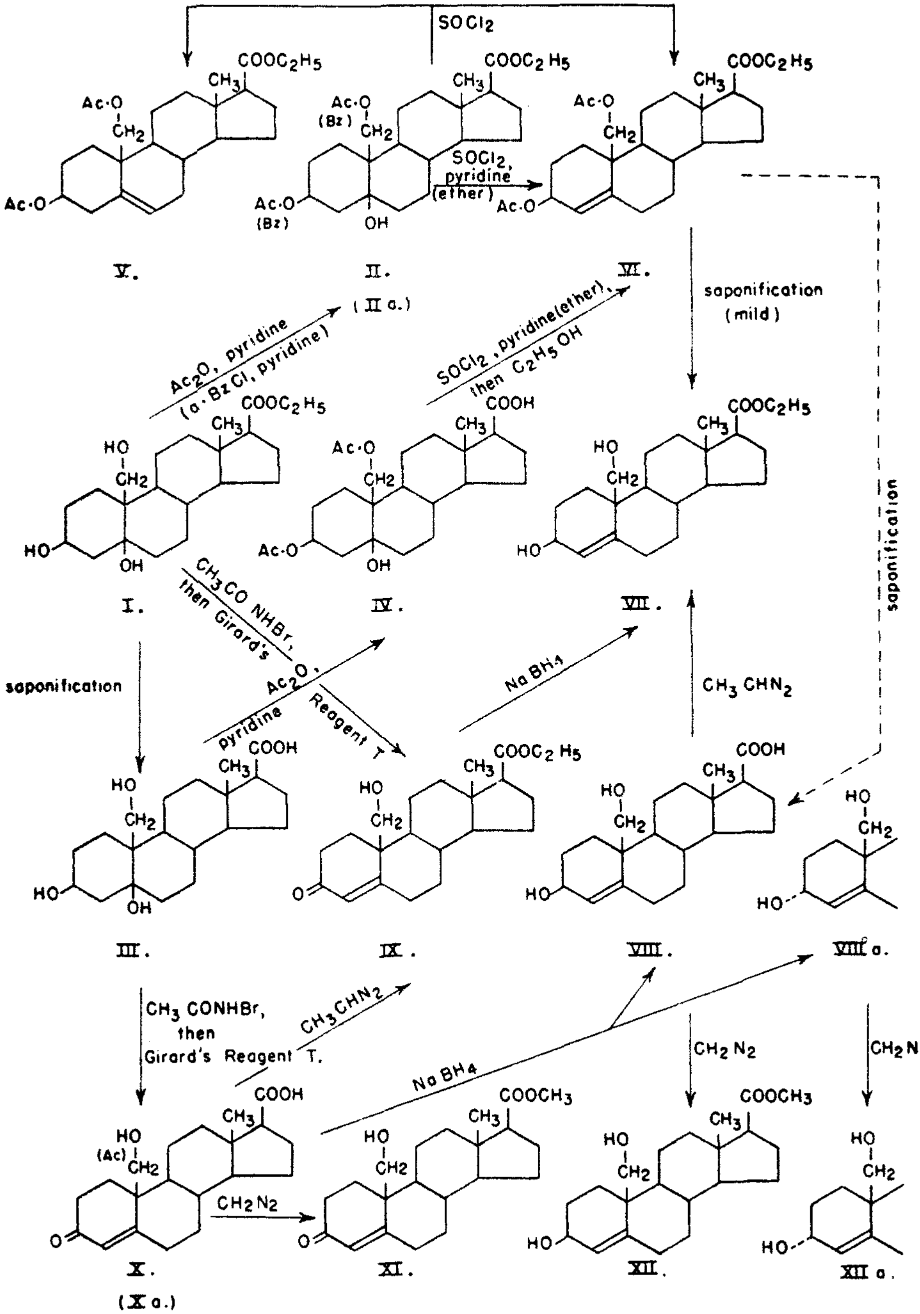
* This paper is dedicated to my teacher Prof. Dr. Adolf Windaus in Göttingen on the occasion of his 75th birthday (December 25, 1951). M. E.

[1] This investigation was supported by research grants from: (a) the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council; (b) the Rockefeller Foundation; (c) the National Cancer Institute of the National Institutes of Health, Public Health Service.



It was found that the course of the dehydration of II with thionyl chloride depends mainly on the reaction medium. On treating II in a solution in ether with thionyl chloride in the presence of pyridine, ethyl 3β,19-diacetoxy-Δ^4-etiocholenate (VI) (m.p. 70–72°; $[\alpha]_D$ +54.7°) resulted as the main product. In addition to VI only a small amount of starting material (II) was isolated.[2] A comparable yield of VI was likewise obtained, when 3β,19-diacetoxy-5-hydroxyetiocholanic acid (IV)[3] was treated in ether with thionyl chloride in the presence of pyridine, followed by treatment of the crude reaction product with ethanol. This result proves that the immediate reaction product was 3β,19-diacetoxy-Δ^4-etiocholenic acid chloride.

The important role of the solvent in this elimination reaction was illustrated by the finding that thionyl chloride and II in the absence of solvent yielded, after chromatographic separation, in addition to a small amount of starting material (II), about equal quantities of VI and ethyl 3β,19-diacetoxy-Δ^5-etiocholenate (V) (m.p. 135–137°; $[\alpha]_D$ $-54.2°$). Assignment of the structures V and VI for the dehydration products of II was made on the basis of the optical rotations of these compounds.

Saponification of VI under mild conditions yielded ethyl 3β,19-dihydroxy-Δ^4-etiocholenate (VII). This compound was also obtained by reducing IX with sodium borohydride. It is to be noted that VII, *i.e.* the 3β-hydroxy compound, was the only crystalline substance isolated from the reaction products. The reduction of IX to VII demonstrates the location of the double bond at the 4,5-position in the original dehydration product VI.

Analogous to the reduction of IX with sodium borohydride, X with the same reagent gave a product consisting essentially of 3β,19-dihydroxy-Δ^4-etiocholenic acid (VIII) accompanied by a small amount of the 3α-isomer (VIIIa). This mixture (VIII, VIIIa) appears to be rather unstable, even in the absence of mineral acid, a behaviour which is probably due to the tendency to undergo dehydration to a 3,5-diene.[4] The mixture consisting mainly of VIII gave with diazoethane a satisfactory yield of the ethyl ester VII, identical with the product obtained by hydrolysis of VI under mild conditions. It should be stated that saponification of VI under more vigorous conditions gave an acid product which apparently contained only small amounts of VIII. Thus, on treating this acid material with diazoethane, only a very small yield of the ethyl ester VII was obtained. The crude product (VIII, VIIIa), prepared by reduction of X with sodium borohydride, gave with diazomethane predominantly methyl 3β,19-dihydroxy-Δ^4 etiocholenate (XII), characterized as the diacetate XIII. On rechromatographing the mother liquors of XII, a small amount of the more easily eluted methyl 3α,19-dihydroxy-Δ^4-etiocholenate (XIIa) was isolated besides additional XII.[5]

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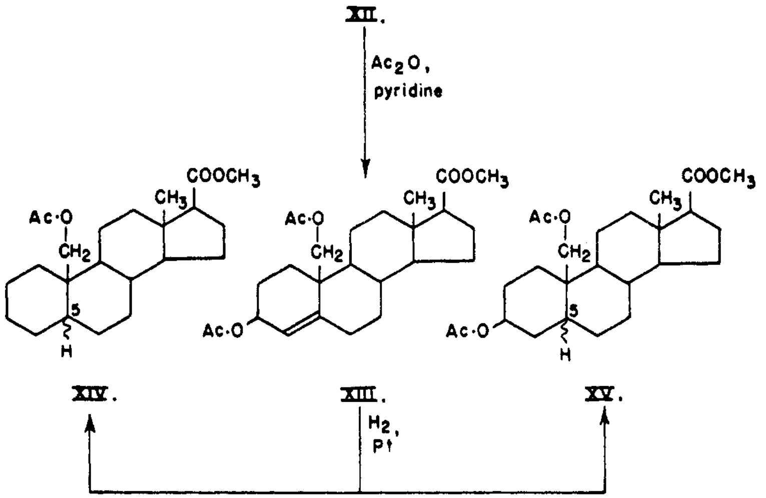
[2] On treating ethyl 3β,19-dibenzoxy-5-hydroxyetiocholanate (IIa) in ether with thionyl chloride in the presence of pyridine, about one-half of the invested material was recovered unchanged after chromatography. The remainder, which may have represented dehydrated material, resisted attempts at crystallization.

[3] This acid, previously (2) reported to be amorphous, has now been obtained in a crystalline form (*vide* expt'l part).

[4] For details see experimental part. The facile dehydration of Δ^4-3β-ols and Δ^4-3α-ols under the influence of dilute alcoholic hydrochloric acid is generally known (lit. *cf.* 8).

[5] Analogous cases regarding the order of chromatographic elution from aluminum oxide are: cholestane-3α-ol followed by cholestane-3β-ol (9) and coprostane-3α-ol closely followed by coprostane-3β-ol (10).

By catalytic hydrogenation of methyl 3β,19-diacetoxy-Δ^4-etiocholenate (XIII) with platinum in glacial acetic acid at room temperature two reduction products were obtained. Facile separation was achieved by chromatography. The compound eluted first (m.p. 106–107°) was obviously methyl 19-acetoxyetio(allo?)-cholanate (XIV), resulting from hydrogenolysis of the allylic ester, whereas the substance eluted later (m.p. 121–122°) was found to be the expected methyl 3β,19-diacetoxyetio(allo?)cholanate (XV). Because of the unknown influence of the C_{19}-acetoxy group upon the stereochemical course of the hydrogenation, no definite configurations at carbon atom 5 have been assigned to XIV and XV.[6] The infrared absorption spectra of XIV and XV were determined through the courtesy of Drs. Konrad Dobriner and Estella R. Katzenellenbogen of the Sloan-Kettering Institute for Cancer Research in New York City. The y wi be published elsewhere.



Among the various reactions described in the foregoing discussions, the fact that III can be obtained in excellent yield by saponification of I, is important. In turn, X and VIII are accessible in adequate yields from III. Thus, considering the ultimate aim of this investigation (*vide supra*), compound Xa as well as the diacetate of compound VIII may be considered as starting materials for future transformations.

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 Dr. E. W. D. Huffman, Denver 2, Colorado, on samples which were dried *in vacuo* over phosphorus pentoxide at 80–90°.

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[6] Analogous hydrogenation of $3\beta,6\beta$-diacetoxy-Δ^4-unsaturated steroids leads to the allo-configuration (6).

Ethyl 3β,19-dibenzoxy-5-hydroxyetiocholanate (IIa). To 100 mg. of ethyl 3β,5,19-trihydroxyetiocholanate (I) in 2 cc. of pyridine[7] was added at −10° a total of 0.5 cc. of redistilled benzoyl chloride. After keeping the reaction mixture at room temperature overnight, chopped ice was added, the benzoate was taken up in ether and the solvent was washed with *N* hydrochloric acid, with *N* sodium carbonate, and with water. After drying over sodium sulfate and evaporation of the ether, a viscous product resulted which was dissolved in a small amount of ligroin[8] and chromatographed over 8 g. of aluminum oxide [activity III (11); diam. of column, 10 mm.]. Elution with petroleum ether-benzene combinations gave a total of 152.4 mg. of resinous material which yielded by repeated crystallization from methanol-water 132.6 mg. (86%) of colorless plates; m.p. 166–167°. $[\alpha]_D^{31}$ +30° ±1° (13.31 mg. in 2.0 cc. of chloroform; l, 2 dm., α +0.40°).

Anal. Calc'd for $C_{36}H_{44}O_7$ (588.71): C, 73.44; H, 7.53.

Found: C, 73.62; H, 7.61.

Crystalline 3β,19-diacetoxy-5-hydroxyetiocholanic acid (IV). The amorphous modification of the acid was prepared as described previously (2, p. 275). Approximately 35 mg. of this product was dissolved in 100 cc. of benzene and chromatographed over 20 g. of silica gel.[9] After eluting with various solvent combinations (details in example, *vide infra*) a total of 31.7 mg. of crystalline material resulted which was recrystallized from ethanol-water yielding 23.3 mg. of needles; m.p. 156–158°. $[\alpha]_D^{31.5}$ +56° ±1° (11.92 mg. in 2.0 cc. of chloroform; l, 2 dm., α +0.67°).

Anal. Calc'd for $C_{24}H_{36}O_7$ (436.53): C, 66.03; H, 8.31.

Found:[10] C, 66.01; H, 8.31.

With this crystalline product available for seeding, part of the acid crystallized in subsequent experiments without chromatographic purification. A typical experiment follows: A solution of 4.28 g. of the amorphous acid in 20 cc. of ether was seeded with crystalline material and was kept in the refrigerator until the crystallization was complete; yield: 2.749 g. of coarse needles; m.p. 154–156°. After bringing the mother liquor to dryness, a solution of the residue (1.53 g.) in benzene was chromatographed over 100 g. of silica gel;[9] volume of each eluate: 200 cc. Nothing was eluted with benzene. Elution with benzene-chloroform[11] combinations (3:1, 1:1, 1:3) gave a total of 86.3 mg. of resinous material which resisted attempts at crystallization. Practically nothing was eluted with chloroform and certain chloroform-ether combinations (99:1, 49:1, 24:1). By elution with other chloroform-ether combinations (23:2, 17:3, 3:1, 1:1) there resulted a total of 869.4 mg. of resinous material which was recrystallized three times from ethanol-water yielding 231.4 mg. of m.p. 155–157°. Total yield of crystalline acid: 2.98 g.

Ethyl 3β,19-diacetoxy-Δ^4-etiocholenate (VI) (*a*) *from ethyl 3β,19-diacetoxy-5-hydroxyetiocholanate* (II). To 100 mg. of II (m.p. 104–105°) in 5 cc. of absolute ether, cooled to −15°, were successively added one droplet of pyridine[7] and 1 cc. of pure thionyl chloride (13, p. 381). The mixture was kept at room temperature for 4 hours, and was then brought to dryness *in vacuo* (room temp.). The ethereal solution of the residue was washed with *N* sodium bicarbonate, *N* hydrochloric acid, and water. After drying over sodium sulfate it was evaporated to dryness, and the resinous residue (101.8 mg.) was dissolved in a small amount of ligroin,[8] chromatographed over 10 g. of aluminum oxide [activity II–III (11); diam. of column, 10 mm.; time of elution of each 100-cc. fraction, 30 minutes]. The first two fractions (consisting of petroleum ether and petroleum ether-benzene, 9:1) yielded only traces of residues. Fractions 3 through 8 (petroleum ether-benzene [4:1, 7:3, 3:2, 1:1, 2:3] combinations) gave a total of 79.2 mg. of resins which, on separate crystallization

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[7] Distilled from barium oxide.

[8] Purified by shaking with conc'd sulfuric acid.

[9] Davison Silica Gel, lot TS-55-485, The Davison Chemical Corporation, Baltimore 3, Md.

[10] Special drying (12) was essential.

[11] The chloroform used for eluting was distilled over calcium chloride.

from methanol-water yielded a total of 64.1 mg. of identical material; needles; m.p. 70–72°. A solution in chloroform gave a slightly yellow color with tetranitromethane. $[\alpha]_D^{20.5}$ +54.7° ±2° (10.68 mg. in 2.0 cc. of chloroform; l, 2 dm., α +0.59°). Molecular weight determination (microcryoscopic, exaltone): 404; 419.

Anal. Calc'd for $C_{26}H_{38}O_6$ (446.56): C, 69.92; H, 8.57.

Found:[12, 13] C, 69.94, 70.11; H, 8.61, 8.54.

From one of the later eluates (benzene-ether, 3:2) 11.5 mg. of a resin was obtained which on crystallization from acetone-petroleum ether gave needles, m.p. 98–100°, consisting of starting material (II) identified by mixture m.p.

(*b*) *From 3β,19-diacetoxy-5-hydroxyetiocholanic acid* (IV). To 89.4 mg. of IV (m.p. 153–155°) in 5 cc. of absolute ether there were slowly added at −15° three drops of pyridine[7] and 1 cc. of pure thionyl chloride (13, p. 381). The mixture was kept at room temperature for one hour and the solvents were then evaporated *in vacuo* (room temp.). The residue was dissolved in dry benzene and the solution was brought to dryness. This procedure was repeated once and the residue was then treated for 10 minutes at room temperature with a mixture of 3 cc. of absolute alcohol and two drops of pyridine.[7] After removing the solvent *in vacuo*, the resinous residue was taken up in petroleum ether and the solution was washed with 10% sulfuric acid, *N* sodium carbonate, and water. After drying over sodium sulfate and evaporating the solvent, 82.8 mg. of resinous neutral material resulted. From the carbonate phase there was isolated in the usual fashion 10.1 mg. of resinous acid material. The neutral fraction was chromatographed as described under (*a*). The eluates consisting of petroleum ether-benzene combinations (7:3, 3:2, 1:1) gave a total of 48.1 mg. of resinous material. Repeated crystallization from ethanol-water yielded 39.5 mg. of needles; m.p. 70–72°; no depression of m.p. when mixed with an authentic sample of VI (*vide supra*). From the subsequent eluates (total of residues: 39.2 mg.) there resulted only traces of crystalline material which could not be identified.

Ethyl 3β,19-diacetoxy-Δ^4-etiocholenate (VI) *and ethyl 3β,19-diacetoxy-Δ^5-etiocholenate* (V) *from ethyl 3β,19-diacetoxy-5-hydroxyetiocholanate* (II). Compound II (100 mg.) (m.p. 104–105°) was dissolved in 1.5 cc. of pure thionyl chloride (13, p. 381) at −20° and the mixture kept at 0° overnight. After removing the excess reagent *in vacuo* (room temp.), the residue was taken up in 1.5 cc. of dry benzene and the resulting solution brought to dryness. This treatment was repeated once and the resinous residue was dissolved in ether. After washing the solvent with *N* sodium bicarbonate, drying over sodium sulfate, and evaporating, 102.3 mg. of resinous material resulted which was dissolved in a small amount of ligroin[8] and chromatographed over 10 g. of aluminum oxide [activity II–III (11), diam. of column, 10 mm.; time of elution of each 100-cc. fraction, 35 minutes]. The first two fractions (consisting of petroleum ether and petroleum ether-benzene, 9:1) yielded only traces of residues. The third fraction (petroleum ether-benzene, 4:1) gave 19.3 mg. of resinous material which on repeated crystallization from methanol-water yielded 14.8 mg. of needles; m.p. 69–71°; identified as VI by mixture m.p. The fourth fraction (petroleum ether-benzene, 7:3) gave 11.1 mg. of a resin which was not investigated. Fractions 5 and 6 (petroleum ether-benzene, 3:2 and 1:1) yielded 24.3 mg. of resinous material which on repeated crystallization from methanol-water gave 15.8 mg. of needles of constant m.p. 135–137°, recognized as ethyl 3β,19-diacetoxy-Δ^5-etiocholenate (V). A solution in chloroform gave a slightly yellow color with tetranitromethane. $[\alpha]_D^{20.5}$ −54.2° ±3° (9.23 mg. in 2.0 cc. of chloroform; l, 2 dm., α −0.50°). Molecular weight determination (microcryoscopic, exaltone): 410, 399.

Anal. Calc'd for $C_{26}H_{38}O_6$ (446.56): C, 69.92; H, 8.57.

Found:[12] C, 70.15, 70.23; H, 8.72, 8.61.

In one of the later eluates (benzene-ether, 3:2), wt. 31.7 mg., the presence of starting material (II) was established by determination of the mixture m.p.

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[12] As a precautionary measure special drying (12) was performed though it proved unnecessary.

[13] Dried at 50°.

Ethyl 3β,19-dihydroxy-Δ⁴-etiocholenate (VII). *A. By saponification of ethyl 3β,19-diacetoxy-Δ⁴-etiocholenate* (VI). To 26.4 mg. of VI (m.p. 70–71°; obtained by dehydration of II; *vide supra*) in 3 cc. of 96% alcohol was added a solution of 7 mg. of sodium hydroxide (3 equiv.) in 3.5 cc. of 96% alcohol. The mixture was kept at room temperature overnight and then brought to dryness *in vacuo* (25°). After dissolving the residue in ether the solvent was washed with *N* hydrochloric acid and water, dried over sodium sulfate, and evaporated, yielding 23.7 mg. of resinous material. Crystallization from ligroin[8] gave 12.5 mg. of fine needles of m.p. 136–137°. A solution in chloroform gave a slightly yellow color with tetranitromethane. $[\alpha]_D^{31}$ +80° ±2° (6.25 mg. in 2.0 cc. of chloroform; l, 2 dm., α +0.50°).

Anal. Calc'd for $C_{22}H_{34}O_4$ (362.49): C, 72.89; H, 9.45.

Found: C, 73.12; H, 9.42.

B. By reduction of ethyl 3-keto-19-hydroxy-Δ⁴-etiocholenate (IX). The ethyl ester IX was prepared by treatment of 3-keto-19-hydroxy-Δ⁴-etiocholenic acid (X) (m.p. 228–230°; *vide infra*) with diazoethane.[14] The product had m.p. 180–181° which was not depressed upon admixture with an authentic sample of IX (3). To 15 mg. of IX in 1 cc. of ethanol was added a solution of 10 mg. of sodium borohydride[15] in 1 cc. of ethanol-water (1:1) and the mixture was kept at 50° for 30 minutes. The excess reagent was then destroyed by slowly adding *N* hydrochloric acid and the mixture was kept at room temperature for 30 minutes. After extracting with ether, the ethereal solution was washed with water, dried over sodium sulfate, and evaporated to dryness. Yield: 18.8 mg. of resinous material which was chromatographed over 2 g. of aluminum oxide [activity III–IV (11); diam. of column: 6 mm.; 40-cc. eluates]. A total of 3.3 mg. of resinous material was eluted with petroleum ether and petroleum ether-benzene combinations (12 fractions). A total of 5.9 mg. of partly crystalline material was obtained with benzene and benzene-ether, 3:1; 1:1 (8 fractions; not investigated). Elution with benzene-ether, 1:3 and ether gave no material (4 fractions), whereas ether-methanol, 9:1 (1 fraction) yielded 6.0 mg. of a crystalline residue, m.p. 130–131°. Recrystallization from ethanol-water gave 4.7 mg. of plates, m.p. 138–140°. No depression of m.p. when mixed with sample obtained by procedure *A* (*vide supra*).

C. By esterification of 3β,19-dihydroxy-Δ⁴-etiocholenic acid (VIII). To 2.5 mg. of VIII (m.p. 200–202°) in ethanol was added an excess of ethereal diazoethane.[14] On evaporating the solvents, 2.4 mg. of a resinous residue was obtained which crystallized upon adding petroleum ether; m.p. 128–130°. By recrystallizing twice from acetone-petroleum ether 1.6 mg. of needles, m.p. 137–139°, was obtained. There was no depression of the m.p. when this product was mixed with the substance of m.p. 136–137°, obtained by procedure *A* (*vide supra*).

3-Keto-19-hydroxy-Δ⁴-etiocholenic acid (X) *from 3β,5,19-trihydroxyetiocholanic acid* (III). To 178 mg. of III in 5 cc. of *tert*-butyl alcohol was added 121 mg. of N-bromoacetamide[16] (1.6 moles) and 1 cc. of water. The mixture was kept at room temperature overnight and was then diluted with 50 cc. of water causing a precipitate which was filtered after some standing; yield: 172.5 mg. of needles; m.p. 172–175° (decomp.); representing probably impure 3-keto-5,19-dihydroxyetiocholanic acid. This material was dissolved in 4 cc. of ethanol and, after the addition of 250 mg. of Girard's Reagent T and 0.15 cc. of glacial acetic acid, the mixture was refluxed for 1 hour. After reaching room temperature it was poured into ice-cold water and the aqueous solution was extracted twice with ethyl acetate, followed by washing of the solvent with water, drying over sodium sulfate and evaporation. Yield: 29 mg. of non-ketonic material which, upon recrystallizing twice from ethanol-water, gave 17.3 mg. of m.p. 262–265°. No depression of m.p. when mixed with starting material (III). The combined aqueous phases were acidified with *N* hydrochloric acid and, after standing for ½ hour at room temperature, were extracted with ethyl acetate which was

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[14] Prepared from ethylnitrosourea according to the Organic Syntheses directions for diazomethane (14).

[15] Metal Hydrides, Inc., Beverly, Mass.

[16] Arapahoe Chemicals, Inc., Boulder, Colo.

dried over sodium sulfate and evaporated. Yield: 124.8 mg. of crystalline material which by recrystallizing once from acetone-petroleum ether gave 100 mg. of m.p. 216–218°. To verify the purity, this material was chromatographed over 15 g. of silica gel[9] (diam. of column: 15 mm.; 50-cc. eluates). Practically nothing was eluted with benzene, benzene-chloroform[11] combinations, chloroform, and chloroform-ether combinations up to an ether content of 25%. The major part of crystalline material was eluted with chloroform-ether, 1:1, and some additional crystalline fractions were obtained with chloroform-ether, 1:3, and with ether. All of this material was found to be identical (total: 90.9 mg.) and was recrystallized from ethanol-water, yielding 79 mg. of needles of constant m.p. 228–230°. (λ_{max}^{alc} 242 mμ; ϵ, 15,000). $[\alpha]_D^{23}$ +107° ±2° (9.44 mg. in 2.0 cc. of chloroform containing one drop of ethanol; l, 2 dm., α +1.01°).

Anal. Calc'd for $C_{20}H_{28}O_4$ (332.42): C, 72.26; H, 8.49.

Found:[10] C, 72.40, 72.36; H, 8.44, 8.58.

In the foregoing experiment the ketonic material was chromatographed in order to demonstrate its purity. It was found that reasonably pure material can be secured also without this manipulation. Even under seemingly identical conditions the yield of ketonic material fluctuated. In any case the non-ketonic fraction consisted of starting material.

3-Keto-19-acetoxy-Δ^4-etiocholenic acid (Xa). To 70 mg. of X in 2 cc. of pyridine was added 0.5 cc. of acetic anhydride. The mixture was kept at room temperature overnight followed by diluting it with water, extracting with ether, and washing the solvent with *N* hydrochloric acid and *N* sodium carbonate. Only 5 mg. of resinous neutral material resulted from the ether. After acidifying the carbonate phase with *N* hydrochloric acid, extracting with ether, washing the solvent with water, and drying over sodium sulfate, evaporation to dryness gave 72 mg. of crystalline acid material; m.p. 185–187°. Recrystallizing twice from methanol-water gave 60 mg. of fine needles; m.p. 193–194°. $[\alpha]_D^{27}$ +193° ±3° (17.68 mg. in 2.0 cc. of chloroform; l, 2 dm., α +3.41°). $[\alpha]_D^{27}$ +200° ±3° (20.50 mg. in 2.0 cc. of chloroform; l, 2 dm., α +4.10°).

Anal. Calc'd for $C_{22}H_{30}O_5$ (374.46): C, 70.57; H, 8.07.

Found:[12] C, 70.66, 70.53; H, 8.14, 8.14.

Methyl 3-keto-19-hydroxy-Δ^4-etiocholenate (XI). To 59.7 mg. of X (m.p. 228–230°) in 3 cc. of methanol was added a slight excess of a 1.8% ethereal solution of diazomethane. Evaporation to dryness and repeated recrystallization of the residue from ethanol-water gave 51.2 mg. of needles of constant m.p. 176–177°. (λ_{max}^{alc} 242 mμ; ϵ, 14,000). $[\alpha]_D^{23}$ +111° ±2° (8.02 mg. in 2.0 cc. of chloroform; 1 = l, 2 dm., α +0.90°).

Anal. Calc'd for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73.

Found:[12] C, 72.56; H, 8.60.

3β,19-Dihydroxy-Δ^4-etiocholenic acid (VIII) [*containing a small amount of 3α,19-dihydroxy-Δ^4-etiocholenic acid* (VIIIa)] *by reduction of 3-keto-19-hydroxy-Δ^4-etiocholenic acid* (X). To 75 mg. of X in 3 cc. of 0.1 *N* sodium hydroxide was added a solution of 25 mg. of sodium borohydride[15] in 1.5 cc. of water and the mixture was kept at room temperature for one hour. The excess reagent was destroyed by slowly adding 10% acetic acid and the precipitate was filtered, washed, and dried, yielding 73.2 mg. of m.p. 192–195°. Recrystallization by dissolving in ethanol, gradually adding benzene and evaporating until crystallization started; needles of m.p. 205–207°. The product appeared to be rather unstable. Thus on repeated recrystallization from aqueous alcohol, the melting point was gradually lowered. Simultaneously the material, which originally was transparent in the ultraviolet, gave an absorption indicating the presence of conjugated double bonds. An attempt at further purifying the product of m.p. 205–207° by chromatography over silica gel likewise resulted in a decomposition of the major part of the compound.

An attempt was made to obtain VIII also by refluxing VI with a large excess of ethanolic potassium hydroxide. This gave a rather unsatisfactory yield (8.0 mg. from 34.0 mg. of VI) of an acid product; plates from ethanol-water; m.p. 158–160°. This was apparently not uniform, because on treating it with diazoethane,[14] only an inferior yield of a crystalline substance, m.p. 135–137°, was obtained after chromatography; identified by mixture m.p. as VII.

Methyl 3β,19-dihydroxy-Δ⁴-etiocholenate (XII) *and methyl 3α,19-dihydroxy-Δ⁴-etiocholenate* (XIIa). To 73.2 mg. of a mixture containing essentially VIII and some VIIIa (m.p. 192–195°) in methanol was added an excess of ethereal diazomethane. Evaporation of the solvents yielded 73.2 mg. of a crystalline residue; m.p. 145–155°. This was chromatographed over 10 g. of aluminum oxide [activity V (11); diam. of column: 14 mm.; 50-cc. eluates]. Elution with petroleum ether, petroleum ether-benzene combinations, and benzene yielded no material. Benzene-ether combinations gave only traces of resinous residues and by elution with pure ether no material was secured. By elution with ether-methanol, 20:1, four successive fractions were isolated: (a) 19.1 mg., partly crystalline; (b) 23.5 mg., crystalline, m.p. 172–175°; (c) 10.4 mg., crystalline, m.p. 158–160°; (d) 8.3 mg., crystalline, m.p. 175–177°. A total of 8.3 mg. of resinous products was eluted with ether-methanol. Separate recrystallization of the crystalline fractions from acetone-ligroin[8] gave stout plates: (a1) 8.0 mg., m.p. 150–155°; (b1) 19.4 mg., m.p. 186–187°; (c1) 9.2 mg., m.p. 188–189°; (d1) 6.9 mg., m.p. 188–189°. The latter three fractions represented identical material (mixture melting points) and were combined (35.5 mg.). Renewed crystallization for analysis gave 31.3 mg. of m.p. 187–189°; representing methyl 3β,19-dihydroxy-Δ⁴-etiocholenate (XII). $[\alpha]_D^{27}$ +88° ±2° (13.16 mg. in 2.0 cc. of chloroform; l, 2 dm., α +1.16°).

Anal. Calc'd for $C_{21}H_{32}O_4$ (348.47): C, 72.38; H, 9.26.

Found:[12] C, 72.05, 71.92; H, 8.91, 8.89.

The mother liquors resulting from the isolation of XII in the above experiment and in repetitions of it were pooled and the material (51.9 mg.) was rechromatographed over 7 g. of aluminum oxide [activity V (11); diam. of column: 14 mm.; 50-cc. eluates]. The chromatogram showed two peaks resulting from exhaustive elution with (a) ether-methanol, 50:1 and (b) ether-methanol, 20:1 respectively. Weight of pooled residues: (a) 20.5 mg.; (b) 19.2 mg. Recrystallization of (b) from acetone-ligroin[8] gave 15.9 mg. of plates, m.p. 185–187° identical with XII; mixture m.p. Repeated recrystallization of (a) from methanol-water gave 10.2 mg. of needles; constant m.p. 133–135°; representing methyl 3α,19-dihydroxy-Δ⁴-etiocholenate (XIIa).

Anal. Calc'd for $C_{21}H_{32}O_4$ (348.47): C, 72.38; H, 9.26.

Found:[10] C, 72.26, H, 9.41.

Methyl 3β,19-diacetoxy-Δ⁴-etiocholenate (XIII). To 22.3 mg. of XII (m.p. 186–188°) in 0.5 cc. of pyridine[7] was added 0.25 cc. of acetic anhydride. After keeping the mixture at room temperature overnight, subsequently cooling with ice and diluting with water, the reaction product was extracted with ether and the solvent was washed with *N* hydrochloric acid, *N* sodium carbonate, and with water. After drying over sodium sulfate, evaporation of the ether yielded 24.9 mg. of resinous material which was dissolved in petroleum ether and chromatographed over 10 g. of aluminum oxide [activity V (11); diam. of column: 14 mm.]. After removal of impurities by exhaustively washing with petroleum ether, the diacetate was eluted with petroleum ether-benzene, 2:3. This gave 22.1 mg. of resinous material which was crystallized from methanol-water. Yield: 20.3 mg. of fine needles; m.p. 82–83°, resolidification at 86–88°, remelting at 101–102°. The product was used in a hydrogenation experiment without subjecting it to analysis.

Methyl 19-acetoxyetio(allo?)cholanate (XIV) *and methyl 3β,19-diacetoxyetio(allo?)cholanate* (XV) *by hydrogenation of methyl 3β,19-diacetoxy-Δ⁴-etiocholenate* (XIII). A solution of 19 mg. of XIII (*vide supra*) in 1 cc. of glacial acetic acid and 2 cc. of the same solvent for rinsing were added to a suspension of 11.5 mg. of previously reduced platinum oxide in 1.5 cc. of glacial acetic acid. Hydrogenation proceeded rapidly during the first five minutes and came to a standstill after about ½ hour. Total absorption: 1.2 cc. (28°; 759 mm.); calc'd for one mole H_2: 1.07 cc. The solvent was removed *in vacuo*, yielding 19.5 mg. of resinous material which was chromatographed over 10 g. of aluminum oxide [activity V (11); diam. of column: 14 mm.; 50-cc. eluates]. Eluates 1 through 3 (petroleum ether) gave 1.8 mg. of resinous residue. Eluates 4 through 6 (petroleum ether-benzene, 9:1) yielded a total of 10.4 mg. of crystalline material, melting points between 98 and 106° (= product A). Eluates 7 through 8 (petroleum ether-benzene, 9:1), 9 through 10 (petroleum ether-benzene, 3:2), 11 through 12 (petroleum ether-benzene, 1:1), and 13 through 14 (petroleum ether-benzene, 2:3) gave a total of only 0.5 mg. of resinous material. Eluates 15 through 16 (benzene) and 17 (benzene-ether, 4:1) yielded a total of 8.0 mg. of crystalline material; melting points between 121 and 123° (= product B). Total recovery: 20.7 mg.

Methyl 19-acetoxyetio(allo?)cholanate (XIV): The pooled product A (10.4 mg.) was recrystallized from methanol-water, yielding 8.2 mg. of needles, m.p. 106–107°. Mixture m.p. with starting material (XIII): 75–78°. A solution of the substance in chloroform gave no color with tetranitromethane. $[\alpha]_D^{25}$ $+45° \pm 1°$. (8.1 mg. in 2.0 cc. of chloroform; l, 2 dm., α $+0.37°$). For infrared spectrum see theoretical part.

Anal. Calc'd for $C_{23}H_{36}O_4$ (376.52): C, 73.36; H, 9.64.

Found:[12, 17] C, 73.41; H, 9.70.

Methyl 3β,19-diacetoxyetio(allo?)cholanate (XV): On recrystallizing the pooled product B (8.0 mg.) from methanol-water, 7.7 mg. of plates was obtained; m.p. 121–122°. Mixture m.p. with starting material (XIII): 83–86°. A solution of the compound in chloroform gave no color with tetranitromethane. $[\alpha]_D^{25}$ $+31° \pm 1°$. (7.65 mg. in 2.0 cc. of chloroform; l, 2 dm., α $+0.24°$). For infrared spectrum see theoretical part.

Anal. Calc'd for $C_{25}H_{38}O_6$ (434.55): C, 69.09; H, 8.82.

Found:[12, 18] C, 69.13; H, 8.74.

The mixture melting point of XIV and XV was 87–89°.

SUMMARY

1. 3β,5,19-Trihydroxyetiocholanic acid (III) and some of its derivatives were subjected to dehydrations under varying conditions. Several new compounds have been correlated by additional chemical reactions.

2. In the presence of pyridine, treatment with thionyl chloride of ethyl 3β,19-diacetoxy-5-hydroxyetiocholanate (II) yielded ethyl 3β,19-diacetoxy-Δ^4-etiocholenate (VI). In the absence of pyridine, a mixture of VI and ethyl 3β,19-diacetoxy-Δ^5-etiocholenate (V) resulted. VI was also obtained from 3β,19-diacetoxy-5-hydroxyetiocholanic acid (IV) by treating with thionyl chloride in pyridine and dissolving the resulting acid chloride in ethanol.

3. Mild hydrolysis of VI gave ethyl 3β,19-dihydroxy-Δ^4-etiocholenate (VII). A second route for the preparation of VII was the reduction with sodium borohydride of ethyl 3-keto-19-hydroxy-Δ^4-etiocholenate (IX).

4. Oxidation of III with N-bromoacetamide, followed by treatment with Girard's Reagent T, gave 3-keto-19-hydroxy-Δ^4-etiocholenic acid (X) which was characterized by the acetate (Xa), the methyl ester (XI), and the known ethyl ester (IX).

5. Reduction with sodium borohydride of X gave mainly 3β,19-dihydroxy-Δ^4-etiocholenate (VIII) and, to a minor extent, the 3α-isomer VIIIa, characterized by the methyl esters XII and XIIa respectively. Treatment with diazoethane of the mixture of VIII and VIIIa represented a third route for the preparation of VII.

6. Acetylation of XII gave methyl 3β,19-diacetoxy-Δ^4-etiocholenate (XIII) which by catalytic hydrogenation (Pt, acetic acid) gave a mixture of methyl 19-acetoxyetio(allo?)cholanate (XIV) and methyl 3β,19-diacetoxyetio(allo?)-cholanate (XV). No definite configurations at carbon atom 5 have been assigned to XIV and XV.

PHILADELPHIA 4, PA.

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[17] Dried at 65°.

[18] Dried at 75°.

REFERENCES

(1) Ehrenstein and Johnson, *J. Org. Chem.*, **11,** 823 (1946).
(2) Ehrenstein, Johnson, Olmsted, Vivian, and Wagner, *J. Org. Chem.*, **15,** 264 (1950).
(3) Ehrenstein, Barber, and Gordon, *J. Org. Chem.*, **16,** 349 (1951).
(4) Speiser and Reichstein, *Helv. Chim. Acta*, **30,** 2143 (1947).
(5) Petrow, Rosenheim, and Starling, *J. Chem. Soc.*, 677 (1938).
(6) Mancera, Rosenkranz, and Djerassi, *J. Org. Chem.*, **16,** 192 (1951).
(7) Fieser and Rigaudy, *J. Am. Chem. Soc.*, **73,** 4660 (1951).
(8) Dauben and Eastham, *J. Am. Chem. Soc.*, **72,** 2305 (1950).
(9) Shoppee, *J. Chem. Soc.*, 1147 (1946).
(10) Shoppee, private communication.
(11) Brockmann and Schodder, *Ber.*, **74,** 73 (1941).
(12) Milner and Sherman, *Ind. Eng. Chem.*, *Anal. Ed.*, **8,** 427 (1936).
(13) Fieser, *Experiments in Organic Chemistry*, 2nd. ed., D. C. Heath and Company, New York, 1941.
(14) Blatt, *Org. Syntheses*, Coll. Vol. II, 165, 461 (1943).
(15) Prelog and Tagmann, *Helv. Chim. Acta*, **27,** 1867 (1944).