[CONTRIBUTION FROM THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE AND THE GEORGE S. COX MEDICAL RESEARCH INSTITUTE, UNIVERSITY OF PENNSYLVANIA]

INVESTIGATIONS ON STEROIDS. XII. NEW STEROIDS DERIVED FROM STROPHANTHIDOL: ETHYL 3β,5-DIHYDROXY-8,19-OXIDOETIOCHOLANATE AND TRANSFORMATION PRODUCTS[1]

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Received July 25, 1950

In a previous publication (1) it was shown that on treating ethyl 3β,5,19-trihydroxyetiocholanate (X) with Raney nickel in the presence of cyclohexanone, a substantial part of the material underwent aromatization leading to ethyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate (XI). In view of this fact it was decided to subject to the same reaction a compound to which had been tentatively assigned the structure of ethyl 3β,5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate (V) (2). The question arose whether under the experimental conditions, after the aromatization of ring A of the steroid nucleus (VIII), the isolated double bond in the 8,14-position would migrate into ring B and thus facilitate the more extensive aromatization to a compound of the equilenin type (IX). The experiment showed that apparently no aromatization took place. The characterization and investigation of the reaction products threw new light on the constitution of the starting material. It can be considered proven that it does not possess the structure of ethyl 3β,5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate (V); probably it does possess the structure of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI).

It had been shown in an earlier paper (2) that by degrading strophanthidol (I), 3β,5,14,19-tetrahydroxy-14-isoetiocholanic acid (II) can be obtained and that this compound undergoes facile dehydration with 0.1 *N* absolute alcoholic hydrogen chloride. About 85 per cent of the reaction products consists of neutral material and obviously represents essentially a mixture of ethyl esters. The main reaction product is ethyl 3β,5,19-trihydroxy-Δ^{14}-etiocholenate (IV) which can easily be hydrogenated to ethyl 3β,5,19-trihydroxyetiocholanate (X). By-products of the neutral fraction were a compound $C_{20}H_{28}O_4$, tentatively formulated as a lactone (1, 2), and in particular the compound previously (2) interpreted to be ethyl 3β,5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate (V), but now considered to possess the structure of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI). The dehydration reaction has been carried out numerous times and in each instance the bulk of the main product, *i.e.* IV and also of the so-called lactone $C_{20}H_{28}O_4$ was removed from the neutral fraction by direct crystallization. Thus a large amount of neutral mother liquors had accumulated. With a view to obtaining substantial quantities of the compound now considered to be ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI), they were subjected to

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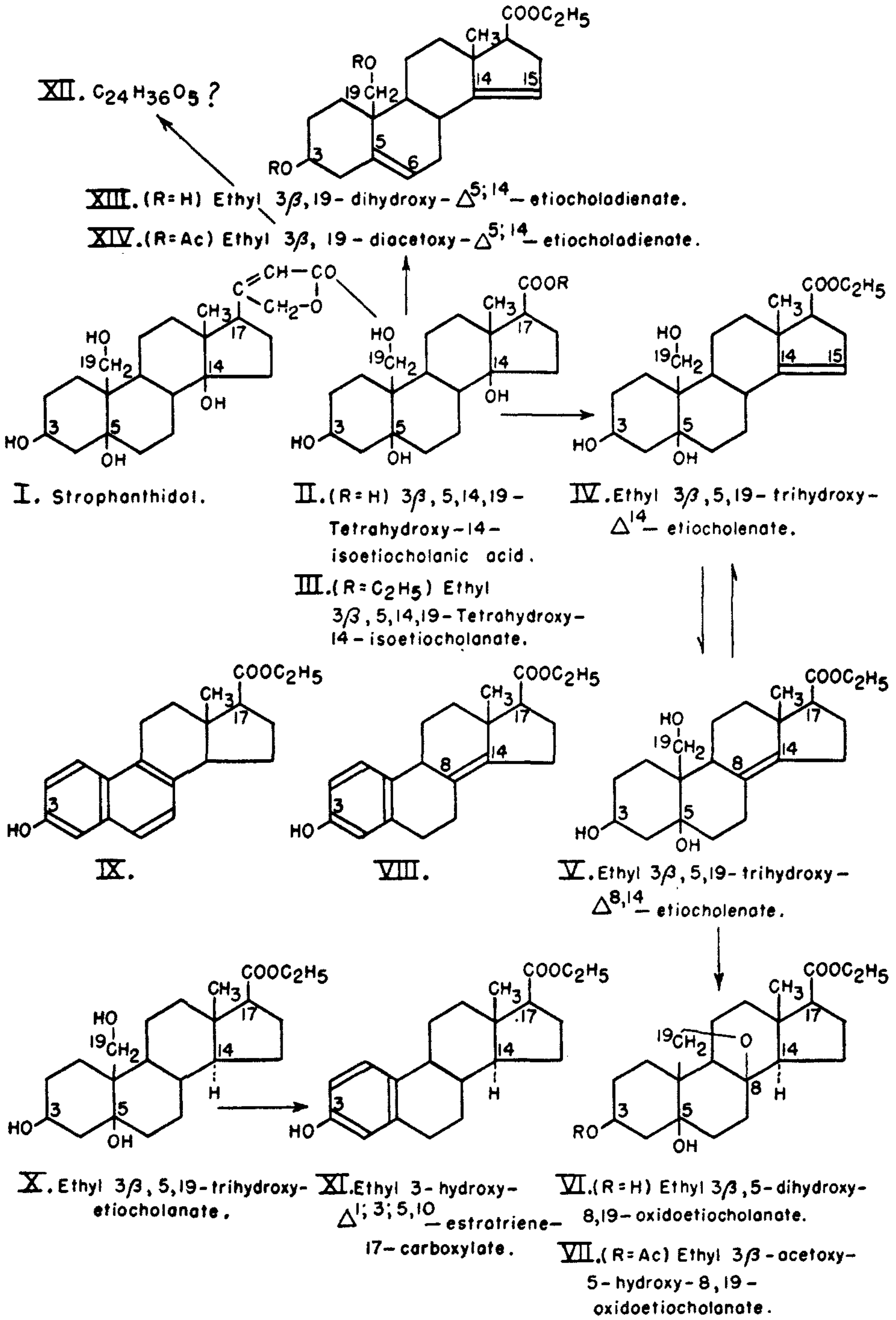
[1] This investigation was supported by research grants from Sharp and Dohme, Inc. in Philadelphia and from the National Cancer Institute of the National Institutes of Health, Public Health Service. Presented before the Division of Medicinal Chemistry at the 118th Meeting of the American Chemical Society in Chicago, September 6, 1950. [*cf.* Ehrenstein and Neumann, Abstracts of Papers, 118th Meeting of the American Chemical Society, p. 12M (1950)].



XII. $C_{24}H_{36}O_5$?

XIII. (R=H) Ethyl 3β,19-dihydroxy-$\Delta^{5;14}$-etiocholadienate.

XIV. (R=Ac) Ethyl 3β,19-diacetoxy-$\Delta^{5;14}$-etiocholadienate.

I. Strophanthidol.

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

III. (R=C_2H_5) Ethyl 3β,5,14,19-Tetrahydroxy-14-isoetiocholanate.

IV. Ethyl 3β,5,19-trihydroxy-Δ^{14}-etiocholenate.

IX.

VIII.

V. Ethyl 3β,5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate.

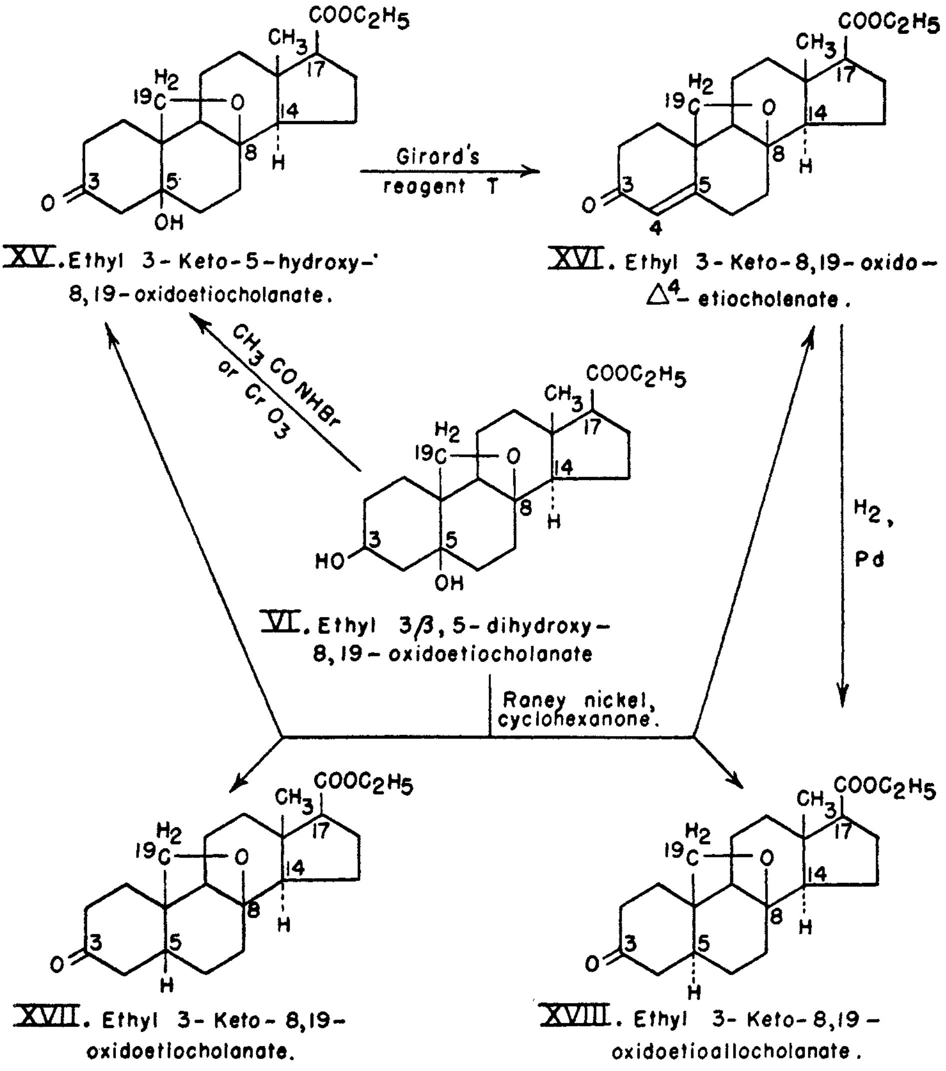
X. Ethyl 3β,5,19-trihydroxy-etiocholanate.

XI. Ethyl 3-hydroxy-$\Delta^{1;3;5,10}$-estratriene-17-carboxylate.

VI. (R=H) Ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate.

VII. (R=Ac) Ethyl 3β-acetoxy-5-hydroxy-8,19-oxidoetiocholanate.

a separation by chromatography. This resulted in the isolation of appreciable amounts of this compound. Furthermore, additional quantities of IV and also two new compounds were obtained from this chromatographic separation.



XV. Ethyl 3-Keto-5-hydroxy-8,19-oxidoetiocholanate.

XVI. Ethyl 3-Keto-8,19-oxido-Δ^4-etiocholenate.

VI. Ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate

XVII. Ethyl 3-Keto-8,19-oxidoetiocholanate.

XVIII. Ethyl 3-Keto-8,19-oxidoetioallocholanate.

In the order of polarity there was eluted first a new, apparently unsaturated compound of the possible formula $C_{24}H_{36}O_5$ (XII) which resisted attempts at acetylation. The amounts were too small to warrant further investigation. Next was the compound now assigned the structure of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI). The average yield of this substance in the dehydration reaction is 4.4%. In accordance with the assigned structure, acetylation yielded a monoacetate, *i.e.*, ethyl 3β-acetoxy-5-hydroxy-8,19-oxidoetiocholanate (VII).

From the more polar eluates of the chromatogram there resulted additional (3.1%) quantities of IV so that the total yield of this compound from the dehydration experiments is now 36 to 38%.

In another chromatographic separation of the neutral reaction products obtained from the dehydration of II a crystalline unsaturated compound $C_{22}H_{32}O_4$ resulted to which probably has to be assigned the structure of ethyl 3β,19-dihydroxy-$\Delta^{5;14}$-etiocholadienate (XIII). It was characterized by the crystalline diacetate, *i.e.*, ethyl 3β,19-diacetoxy-$\Delta^{5;14}$-etiocholadienate (XIV).

The dehydration of etio acids having a β-hydroxyl group at carbon atom 14 usually leads to a mixture of the Δ^{14}- and $\Delta^{8,14}$-isomers in which the former component seems to predominate [*cf. e.g.* (3), p. 533]. Accordingly, when the dehydration was applied to II in a solution of absolute alcohol in the presence of hydrogen chloride, the main reaction product was interpreted to be ethyl 3β,5,19-trihydroxy-Δ^{14}-etiocholenate (IV) and the isomeric minor product ethyl 3β,5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate (V) (2). As has been stated, the chemical behaviour of the latter compound is not in agreement with the assumed structure. In particular, the substance gives a monoacetate whereas structure V would be expected to yield a diacetate, unless the rather unlikely assumption were made that the primary alcohol group at carbon atom 19 is sterically hindered. With the presence of a hydroxymethyl group at carbon atom 10 instead of a methyl group, the double bond in the 8,14-position is apparently unstable and hence an oxygen bridge is formed. One might consider such a bridge to extend between carbon atoms 8 and 19 or between carbon atoms 14 and 19. According to models the latter possibility is most unlikely and hence the structural formula is probably that of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI). The structure of the corresponding free etio acid [*cf.* (2), p. 827] will have to be changed accordingly. This is a tentative formulation, in particular also with regard to the configuration at carbon atom 14. These conclusions are analogous to the interpretation by Fieser and Fieser [(3), pp. 523, 524] of the structures of certain compounds originally described by Jacobs. Also in the present instance the cyclized compound, *i.e.*, ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI; $[\alpha]_{\mathrm{D}}^{27}$ +67.4°) is slightly more dextrorotatory than the uncyclized precursor, ethyl 3β,5,14,19-tetrahydroxy-14-isoetiocholanate (II; $[\alpha]_{\mathrm{D}}^{23.5}$ +45.7°) (2) just as in the case of the Jacobs' compounds [*cf.* (3), p. 524].

As has been stated, the treatment of VI with Raney nickel in the presence of cyclohexanone apparently did not lead to aromatization. Four crystalline reaction products were isolated and their structures (XV, XVI, XVII, XVIII) established. These structures are closely interrelated and hence the probable reaction mechanism is indicated. It seems that VI is first dehydrogenated to ethyl 3-keto-5-hydroxy-8,19-oxidoetiocholanate (XV). This compound in turn is dehydrated to ethyl 3-keto-8,19-oxido-Δ^4-etiocholenate (XVI). The structure is supported by the ultraviolet absorption curve (Figure 1). Subsequently, the double bond is saturated by hydrogen originating probably from the cyclohexanol present in the reaction mixture. This leads to two different stereochemical configurations at carbon atom 5. To the lower-melting compound (m.p. 112–113°;

$[\alpha]_D^{17.5} + 56.9°$), which is eluted from the chromatographic column somewhat more easily than its stereoisomer, was assigned the "*cis*" configuration, *i.e.*, the structure of ethyl 3-keto-8,19-oxidoetiocholanate (XVII). To the slightly more difficultly eluted, higher-melting compound (m.p. 136–137°; $[\alpha]_D^{17.5} + 25.7°$) was assigned the "*trans*" configuration, *i.e.*, the formula of ethyl 3-keto-8,19-oxidoetioallocholanate (XVIII).[3] This substance appeared to be the main product resulting from the Raney nickel reaction. It was characterized by its crystalline oxime.

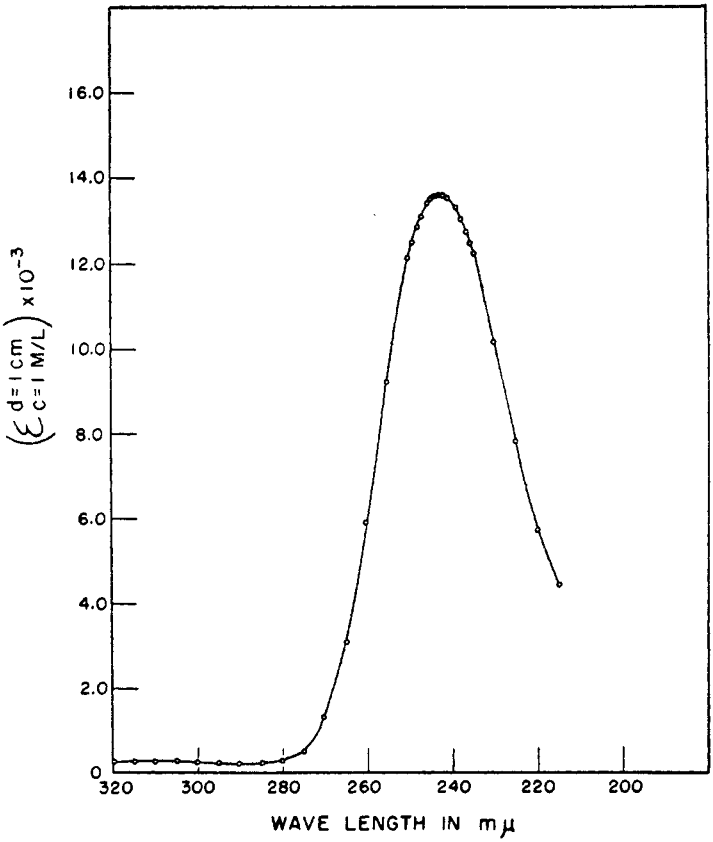


FIGURE 1.[2] ETHYL 3-KETO-8,19-OXIDO-Δ^4-ETIOCHOLENATE (XVI); mol. wt. 358.46; m.p. 121–123°; concentration of solution measured was 0.1646 mg. per 10 cc. of absolute alcohol. λ max. 242.5 mμ (ϵ = 13,611).

In another series of experiments VI was oxidized to XV with N-bromoacetamide or preferably with chromic acid. On treating XV with Girard's reagent T in the usual fashion, dehydration occurred leading to XVI. When this com-

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[2] The ultraviolet absorption curve was determined by Mr. K. B. Streeter of the Sharp and Dohme Research Laboratories.

[3] For an analogous case of assignment of stereochemical configurations *cf.* (4).

pound was hydrogenated catalytically (Pd in ethyl acetate), XVIII resulted. Identity with the compound obtained by the reaction with Raney nickel-cyclohexanone (*vide supra*) was established by the determination of the mixed melting points and infrared spectra. The latter were taken under identical conditions in chloroform. There was identity particularly regarding the position and the intensity of the absorption bands in the region 1100–850 cm^{-1} (Figure 2). Furthermore, in agreement with the assigned structure, there was no indication of the presence of a hydroxyl group.

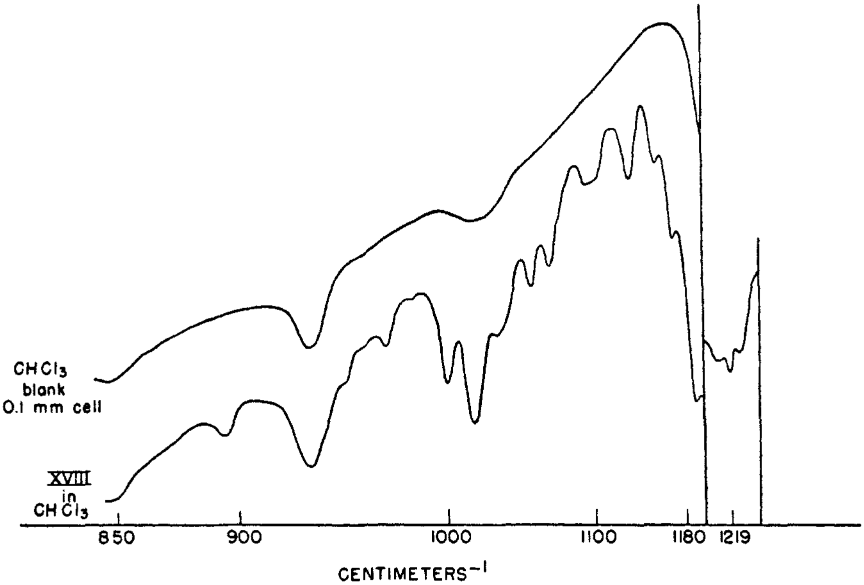

FIGURE 2.[4] ETHYL 3-KETO-8,19-OXIDOETIOALLOCHOLANATE (XVIII). Infrared absorption spectrum in the region 1100–850 $\mathrm{cm.}^{-1}$.

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. All microanalyses were carried out by Dr. E. W. D. Huffman, Denver 2, Colo.; unless otherwise stated, all analytical samples were dried *in vacuo* over phosphorus pentoxide at 80°.

Dehydration of 3β,5,14,19-tetrahydroxy-14-isoetiocholanic acid (II): Isolation of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI) and other compounds. The dehydration of crystalline II, samples melting above 200°, by means of 0.1 *N* absolute alcoholic hydrogen chloride was carried out as described earlier [(1), p. 273]. From the neutral part of the reaction product there was obtained by direct crystallization from acetone a yield of 33–35% of IV. The remaining resinous neutral material (12.926 g.) resulting from a total of 25.585 g. of crystalline II (9 experiments) was subjected to further purification by chromatography. For this purpose it was dissolved in a mixture of 700 cc. of benzene and 300 cc.

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[4] Obtained through the courtesy of Dr. Konrad Dobriner and Mrs. Phyllis Humphries of the Sloan-Kettering Institute for Cancer Research in New York.

of petroleum ether which was passed through a column (diam. 42 mm.) of 400 g. of alkali-free aluminum oxide[5] within a period of three hours. The eluting was done at the rate of approximately one hour for each fraction. The eluates 8, 19, and 24 were interrupted overnight.

Chromatographic Fractionation

NO. OF FRACTION	SOLVENT	WEIGHT OF RESIDUE, MG.	APPEARANCE OF RESIDUE
1	1,000 cc. of benzene-petr. ether (7:3) (original solution)	81.6	Greasy liquid
2–4	500 cc. each of benzene	31.3	Yellowish resin
5–7	500 cc. each of benzene-ether (1:1), ether, ether-chloroform (7:3)	75.9	Yellowish resin
8	500 cc. of ether-chloroform (3:2)	164.7	Resin and brittle foam
9–10	500 cc. each of ether-chloroform (1:1)	564.9	Resin and brittle foam
11–12	500 cc. each of ether-chloroform (2:3)	387.5	Resin and brittle foam
13	500 cc. of ether-chloroform (3:7)	170.6	Crystalline
14–15	500 cc. each of ether-chloroform (1:4)	395.3	Cryst. and resin
16–17	500 cc. each of chloroform	305.0	Cryst. and resin
18	500 cc. of chloroform + 0.5 cc. of methanol	130.0	Cryst. and resin
19	500 cc. of chloroform + 1 cc. of methanol	215.2	Cryst. and resin
20	500 cc. of chloroform + 3 cc. of methanol	474.0	Cryst. and resin
21	500 cc. of chloroform + 5 cc. of methanol	1,946.4	Cryst. and resin
22	500 cc. of chloroform + 5 cc. of methanol	2,473.8	Brittle foam
23	500 cc. of chloroform + 10 cc. of methanol	1,218.7	Brittle foam
24–26	500 cc. each of chloroform-methanol (19:1), (19:1), (9:1)	2,870.5	Brittle foam
27	500 cc. of methanol	698.7	Brownish brittle foam
Total		12,204.1	

Attempts were made to crystallize the residues of fractions 8 through 27. Fraction 8 crystallized from acetone-ether. Fractions 9 and 10 resisted attempts at crystallization. Fractions 11 to 23 furnished crystalline material from acetone alone.

Compound $C_{24}H_{36}O_5$ (XII)?. Fraction 8 yielded from acetone-ether several crops of rectangular crystals melting between 178 and 181°; total, 20.1 mg. Repeated recrystallization from ether-petroleum ether and finally from ether alone raised the melting point to 186–187°. A solution of the substance in chloroform gave a light yellow color with tetranitromethane. An attempt at acetylating with acetic anhydride in a solution of pyridine at room temperature resulted in the isolation of unchanged starting material (mixed m.p.). $[\alpha]_D^{20}$ +70.6° (15.3 mg. in 2.0 cc. of chloroform, l, 1.51 dm., α +0.82°).

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[5] One part of Adsorption Alumina (80–200 MM) of the Fisher Scientific Co. 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 200° for four hours.

Anal. Calc'd for $C_{24}H_{36}O_5$ (404.52): C, 71.25; H, 8.96.
Found: C, 70.98; H, 8.91.

Molecular Weight (cryoscopic): Wt. of solvent (camphor): 6.30 mg., wt. of sample: 0.571 mg., K = 40°; Δt = 0.9°; Mol. wt. 403.
Wt. of solvent (camphor): 4.12 mg.; wt. of sample: 0.337 mg., K = 40°; Δt = 8.2°; Mol. wt. 399.

Ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI) [*Previously (2) designated: Ethyl 3β,5,19-trihydroxy-*$\Delta^{8,14}$*-etiocholenate* (V)]. By gradually concentrating the acetone solutions of fractions 11 to 21, seventeen crops of crystals were obtained; melting points: between 205 and 212°; total: 1.1657 g., *i.e.*, 4.4% of the theoretical yield. Renewed recrystallization of the combined material from acetone gave small prismatic or rectangular crystals, m.p. 213–214.5°; $[\alpha]_D^{27}$ +67.4° (21.8 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +1.11°). The value reported previously [(2), p. 828] was obviously obtained with somewhat less pure material. A sample in chloroform gave no color with tetranitromethane.

Ethyl 3β,5,19-trihydroxy-Δ^{14}*-etiocholenate* (IV). By concentrating the acetone solutions of fractions 22 and 23, two crops of crystals resulted; melting points, 175–180° and 183–185° respectively; no depression with an authentic sample; total: 0.821 g., *i.e.*, 3.1% yield. With this additional material the previously reported yield (*vide supra*) was raised to a total of 36–38%. Recrystallization from acetone furnished rosettes of small rods, m.p. 188–189°. There is an indication that the combined mother liquors of the crystals obtained from fractions 22 and 23 contain additional VI.

Ethyl 3β-acetoxy-5-hydroxy-8,19-oxidoetiocholanate (VII). To a solution of 32 mg. of VI in 1 cc. of pyridine was added 1 cc. of acetic anhydride and the mixture allowed to stand at room temperature overnight. It was subsequently poured into 10 cc. of water causing the immediate separation of crystalline material which was washed with *N* hydrochloric acid and with water. Weight of the crystalline material after drying, 31.2 mg.; m.p. 201–203°. By extracting the filtrates with ether, washing the ethereal phase with *N* hydrochloric acid, *N* sodium carbonate, and water, 3.4 mg. of additional material was obtained. The combined acetate was recrystallized from acetone and then acetone-ether which yielded needles; 1st crop: wt. 15.3 mg., m.p. 206–207°; 2nd crop: wt. 13.1 mg., m.p. 205–206°. Mixed m.p. with starting material, 175–180°; $[\alpha]_D^{26}$ +82.2° (19.1 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +1.19°).

Anal. Calc'd for $C_{24}H_{36}O_6$ (420.52): C, 68.54; H, 8.63.
Found: C, 68.70, 68.57; H, 8.66, 8.59 (Dried at 90°).

Further investigations on dehydration products. In another experiment the resinous part of the material which had resulted from the saponification of 3β,19-diacetoxy-5,14-dihydroxy-14-isoetiocholanic acid [(1), p. 273] was combined with a fraction analogous to the mixture which had served as starting material for the above described chromatographic separation. The pooled material (3.7 g.) was subjected to treatment with 0.1 *N* absolute alcoholic hydrogen chloride. By recrystallizing from acetone, the resulting neutral fraction of the dehydration product yielded 237 mg. of IV, m.p. 185–187°. The material contained in the mother liquors was subjected to three successive chromatographic separations yielding 312 mg. of additional IV and 133 mg. of VI.

Ethyl 3β,19-dihydroxy-$\Delta^{5,14}$*-etiocholadienate* (XIII)?. This compound was isolated from chromatographic fractions located between those from which the last two mentioned substances were obtained. Treatment with acetone gave several crystalline crops (total 68.8 mg.) of material melting between 150 and 162°. Recrystallization from acetone gave a fluffy arrangement of thin, long needles, m.p. 168.5–169.5°. The substance gave a yellow color with tetranitromethane in a solution of chloroform; $[\alpha]_D^{27}$ −28.9° (13.1 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α −0.29°). There was no depression of the melting point when this substance was mixed with a sample of a compound described earlier [$C_{22}H_{34}O_5$ or $C_{22}H_{32}O_5$; m.p. 167–168°; *cf.* (1), p. 274], though the analytical findings were different. The product was dried as usual *in vacuo* for analysis. The dried material appeared hygroscopic. In view of this, the carbon-hydrogen determination was performed with certain modifications.

Anal. Calc'd for $C_{22}H_{32}O_4$ (360.47): C, 73.29; H, 8.95.
Found: C, 72.83; H, 8.97.

Molecular Weight (cryoscopic): Wt. of solvent (camphor): 2.03 mg., wt. of sample: 0.200 mg.; K = 40°; Δt = 10.7°; Mol. wt. 368.
Wt. of solvent (camphor): 3.52 mg., wt. of sample: 0.316 mg.; K = 40° Δt = 9.9°; Mol. wt. 363.

Ethyl 3β,19-diacetoxy-$\Delta^{5;14}$-etiocholadienate (XIV)?. To a solution of 12.9 mg. of the preceding substance (XIII) in 1 cc. of pyridine was added 1 cc. of acetic anhydride. After standing at room temperature for a period of two days the mixture was poured into water. After standing for one hour, the precipitate was extracted with ether and the combined ethereal phases washed neutral with *N* hydrochloric acid, *N* sodium carbonate, and water. After drying over sodium sulfate the ether solution was brought to dryness yielding 16.1 mg. of a partially crystalline residue. Recrystallization from ether gave several crops of long, slender prismatic rods, m.p. 152–153°. $[\alpha]^{27}_{D}$ −52.4° (9.5 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α −0.38°).

Anal. Calc'd for $C_{26}H_{36}O_6$ (444.5): C, 70.24; H, 8.16.
Found: C, 70.20; H, 8.05.

Treatment of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI) with Raney nickel in the presence of cyclohexanone. Fresh active Raney nickel was prepared from 3 g. of nickel-aluminum alloy (5, 6), giving an estimated yield of 1.2 g. of catalyst. A solution of 500 mg. of VI in 10 cc. of redistilled cyclohexanone (88.5%) was added to a suspension of the catalyst in 20 cc. of dry toluene. The mixture was stirred and refluxed at a bath temperature of 125–135° for a period of 24 hours, followed by filtration of the catalyst and washing with acetone. The acetone was evaporated at the water pump, and the toluene, excess cyclohexanone, and cyclohexanol were removed by warming under an oil-pump vacuum. The residue, which possessed a phenolic odor, was taken up in 200 cc. of ether and the solution washed with three 10-cc. portions of *N* sodium hydroxide and six 5-cc. portions of water. The phenolic phase (307 mg.) which was recovered from the alkaline extract and aqueous washings, obviously consisted essentially of phenol, because on bringing it to dryness in an oil-pump vacuum (4 mm.), only 1 mg. of a residue was obtained. After drying with sodium sulfate, the neutral ether extract yielded a limpid residue (717 mg.), probably still containing cyclohexanol, cyclohexanone, and difficultly removable high-boiling condensation products of cyclohexanone. By heating *in vacuo* (4 mm.) at 50° for a period of two hours and subsequently at 100° for 2½ hours, the weight of the oily neutral residue was reduced to 603 mg. Obviously it still contained about 100 mg. of non-steroid material.

The residue was dissolved in a mixture of 10 cc. of benzene and 45 cc. of petroleum ether which was chromatographed over a column of 25 grams of alkali-free aluminum oxide[5] (diam. 17 mm.). The original solution and the eluates were passed through, each within about 15 minutes.

The residues of fractions 10 to 20 were separately recrystallized from a mixture of ether and petroleum ether. Rosettes of needles; in each case the first crop of crystals melted at about 130°. These crystals proved to be identical material and were therefore combined (99.5 mg.).

The subsequent crops of crystals obtained from fractions 10 to 20 had lower melting points (about 100°). They were combined (57.1 mg.) and subjected to another chromatographic separation. The eluting (25 fractions) was done essentially with benzene-petroleum ether combinations (petroleum ether content gradually decreasing). From the earlier fractions there resulted by recrystallization from ether-petroleum ether nine crops of crystals (total, 16.6 mg.) melting at 110° and somewhat lower. They were identical and were combined. Similar recrystallization of the later fractions yielded several crystalline crops (total, 2.5 mg.) melting about 130°. They were identical and were combined with material of the same melting point (99.5 mg.) which had resulted from the original chromatogram (*vide supra*).

The resinous material which had resulted from fractions 10 to 20 of the original chromatogram, after the collection of crystals, was combined with residue 9 of the same chromatogram. To this was added all of the resinous material remaining from the second chromatogram after the separation of the crystalline fractions. The total (dry wt., 122.3 mg.) was subjected to another chromatographic separation. Also in this third chromatogram the eluting was done essentially with benzene-petroleum ether combinations (petroleum ether content gradually decreasing). By recrystallization from ether-petroleum ether, the residues of the early eluates furnished ten crops of crystals (total, 47.3 mg.), m.p. between 105–113°. They were combined with 16.6 mg. of identical material secured from the second chromatogram, so that the final yield was 63.9 mg. This combined material was subjected to further purification [*vide infra:* Ethyl 3-keto-8,19-oxidoetiocholanate (XVII)]. By recrystallization from ether-petroleum ether, the residues of the later eluates furnished six crops of crystals; m.p. about 130°, total wt., 11.8 mg. Combined with identical material obtained from the first (99.5 mg.) and second (2.5 mg.) chromatogram the total yield was 112.9 mg. This was subjected to further purification [*vide infra:* Ethyl 3-keto-8,19-oxidoetioallocholanate (XVIII)].

Chromatographic Fractionation

NO. OF FRACTION	SOLVENT	WEIGHT OF RESIDUE, MG.	APPEARANCE OF RESIDUE
1	55 cc. of benzene-petr. ether (2:9) (original solution)	10.7	Limpid oil
2–3	55 cc. each of benzene-petr. ether (2:9)	19.7	Yellowish oil
4–5	50 cc. each of benzene-petr. ether (1:4)	13.5	Yellowish oil
6–8	50 cc. each of benzene-petr. ether (3:7), (3:7), (2:3)	8.6	Colorless resin
9	50 cc. of benzene-petr. ether (11:14)	9.0	Colorless resin
10	50 cc. of benzene-petr. ether (1:1)	42.4	Colorless resin
11–13	50 cc. each of benzene-petr. ether (1:1)	91.8	Partly resin, pt. cryst.
14–16	50 cc. each of benzene-petr. ether (3:2)	59.1	Partly resin, pt. cryst.
17–18	50 cc. each of benzene-petr. ether (7:3)	30.3	Partly resin, pt. cryst.
19–20	50 cc. each of benzene-petr. ether (4:1)	28.1	Partly resin, pt. cryst.
21	50 cc. of benzene-petr. ether (9:1)	14.2	Colorless resin
22–25	50 cc. each of benzene	48.7	Largely cryst.
26	50 cc. of benzene-ether (49:1)	7.0	Partly resin, pt. cryst.
27–32	50 cc. each of benzene-ether (9:1), (9:1), (4:1), (4:1), (7:3), (3:2)	36.8	Colorless resin
33	50 cc. of benzene-ether (3:2)	11.0	Colorless resin
34–36	50 cc. each of benzene-ether (1:1), (2:3), (1:4)	28.8	Colorless resin
37	50 cc. of ether	8.4	Colorless resin
38–39	50 cc. of ether-methanol (49:1), (9:1)	68.1	Partly cryst.; yellowish resin
40	50 cc. of ether-methanol (1:1)	3.8	Yellowish film
41	50 cc. of methanol	0.9	Yellowish film
Total		540.9	

Recrystallization of the residues of fractions 21 to 26 of the original chromatogram from a mixture of ether and petroleum ether gave twelve crops of microcrystalline material (total wt., 31.7 mg.); melting points between 108 and 128°. They apparently were identical and were combined [*vide infra:* Ethyl 3-keto-8,19-oxido-Δ^4-etiocholenate (XVI)].

No crystalline material could be secured from fractions 27 to 32 of the original chromatogram. Fraction 33 furnished from ether-petroleum ether beautiful needles, m.p. 151–153°. The amount (1.5 mg.) was too small to permit further characterization. No crystals resulted from fractions 34 to 37.

Fractions 38 and 39 gave from a mixture of acetone and ether a total of 23.0 mg. of crystalline material, m.p. 195–201°; there was a depression of 15–25° when mixed with VI. For further identification [*vide infra:* Ethyl 3-keto-5-hydroxy-8,19-oxidoetiocholanate (XV)].

Ethyl 3-keto-8,19-oxidoetiocholanate (XVII). The crude crystalline material melting between 105 and 113° (63.9 mg., *vide supra*) was recrystallized from a mixture of ether and petroleum ether; granules of thick prisms. A chloroform solution gave no yellow color with tetranitromethane. The melting point of the purest sample was 112–113°. $[\alpha]^{17.5}_{D}$ +56.9° (19.9 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +0.86°).

Anal. Calc'd for $C_{22}H_{32}O_4$ (360.47): C, 73.29; H, 8.95.
Found: C, 73.38; H, 9.02.

Molecular Weight (cryoscopic): Wt. of solvent (exaltone): 3.64 mg., wt. of sample: 0.359 mg.; K = 21°; Δt = 5.9°; Mol. wt. 351.
Wt. of solvent (exaltone): 2.18 mg., wt. of sample: 0.223 mg.; K = 21°; Δt = 6.0°; Mol. wt. 358.

The substance gave a 2,4-dinitrophenylhydrazone which crystallized from ethyl acetate in yellow-orange needles, m.p. 214–216°. Treatment of XVII with hydroxylamine hydrochloride gave an oily oxime which resisted attempts at crystallization.

Ethyl 3-keto-8,19-oxidoetioallocholanate (XVIII). The crude compound melting between 131 and 134° (112.9 mg., *vide supra*) was recrystallized by dissolving it in ether and concentrating. On cooling, bunches of stout prismatic needles separated. In chloroform it gave no yellow color with tetranitromethane. The melting point of the purest material was 136–137°. $[\alpha]^{17.5}_{D}$ +25.7° (20.2 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +0.39°).

Anal. Calc'd for $C_{22}H_{32}O_4$ (360.47): C, 73.29; H, 8.95.
Found: C, 73.38; H, 9.06.

Molecular weight (cryoscopic): Wt. of solvent (exaltone): 2.93 mg.; wt. of sample: 0.280 mg.; K = 21°; Δt = 5.3°; Mol. wt. 378.
Wt. of solvent (exaltone): 2.33 mg.; wt. of sample: 0.226 mg.; K = 21°; Δt = 5.5°; Mol. wt. 370.

For infrared spectrum see theoretical part. Treatment of this compound with pyridine and acetic anhydride at room temperature yielded unchanged starting material. The substance gave a 2,4-dinitrophenylhydrazone which crystallized from ethyl acetate in yellow-orange needles, m.p. 203–206°. There was only a very slight depression of the melting point when mixed with the 2,4-dinitrophenylhydrazone of XVII (*vide supra*).

Oxime. To 10.8 mg. of XVIII dissolved in 2.0 cc. of redistilled alcohol was added a mixture of 20 mg. of hydroxylamine hydrochloride and 30 mg. of anhydrous sodium acetate in 0.15 cc. of water. After refluxing for three hours and subsequent standing at room temperature overnight, the mixture was brought to dryness *in vacuo*. The residue was transferred to a separatory-funnel with water and ether. The ether extract was washed with water, dried with sodium sulfate, and then gradually concentrated. Three crops of crystals (total, 7.6 mg.) with melting points between 188.5 and 190° resulted; silky long needles. Renewed crystallization from a mixture of ether and petroleum ether (1:1) yielded 5.3 mg., m.p. 190–191°.

Anal. Calc'd for (monoöxime) $C_{22}H_{33}NO_4$ (375.49): N, 3.73. Found: N, 3.77.

Ethyl 3-keto-8,19-oxido-Δ^4-etiocholenate (XVI). The substance which had resulted from fractions 21 to 26 of the original chromatogram, m.p. between 108 and 128° (31.7 mg., *vide supra*), was difficult to purify. When dissolved in either acetone or ether, the concentration of such solutions was accompanied by the separation of very small amounts of a gelatinous precipitate which proved insoluble in the respective solvent. Because of this difficulty the clear solution was brought quickly to dryness *in vacuo* and purified by leaching repeatedly with small amounts of ether. Thereby some yellowish material went into solution leaving some small, white, feathery needles; 6.2 mg., m.p. 121–123°. The filtrate was again brought to dryness *in vacuo* and the residue leached with ether. This gave another crop of white crystals: 7.8 mg., m.p. 119–122°. On evaporating the filtrate to dryness a yellowish crystalline residue was obtained, m.p. 103–106°. In chloroform the substance gave no yellow color with tetranitromethane. The analytical data were secured with the purest material available.

Anal. Calc'd for $C_{22}H_{30}O_4$ (358.46): C, 73.71: H, 8.43.
Found: C, 73.11; H, 8.84.

Molecular weight (cryoscopic): Wt. of solvent (exaltone): 2.72 mg.; wt. of sample: 0.265 mg.; K = 21°; Δt = 5.6°; Mol. wt. 365.
Wt. of solvent (exaltone): 3.11 mg.; wt. of sample: 0.308 mg.; K = 21°; Δt = 5.8°; Mol. wt. 359.

The ultraviolet absorption curve ($\lambda_{\max}^{\text{alc}}$ 242.5 mμ; ε, 13,611) is presented in the theoretical part. The compound yielded a red 2,4-dinitrophenylhydrazone.

Ethyl 3-keto-5-hydroxy-8,19-oxidoetiocholanate (XV). The material which had resulted from fractions 38 and 39 of the original chromatogram (23.0 mg., m.p. 195–201°) was recrystallized from acetone. This yielded several crops of needles and leaflets (total, 15.7 mg.), m.p. 199–201.5°. In a solution of chloroform the substance gave no yellow color with tetranitromethane.

Anal. Calc'd for $C_{22}H_{32}O_5$ (376.47): C, 70.18; H, 8.57.
Found: C, 70.02; H, 8.67 (Dried at 90°).

Molecular weight (cryoscopic): Wt. of solvent (camphor): 2.595 mg.; wt. of sample: 0.250 mg.; K = 40°; Δt = 10.0°; Mol. wt. 385.
Wt. of solvent (camphor): 3.18 mg.; wt. of sample; 0.311 mg.; K = 40°; Δt = 10.5°; Mol. wt. 373.

The 2,4-dinitrophenylhydrazone of this substance was obtained as a yellow precipitate which gradually turned reddish on standing in solution.

Ethyl 3-keto-5-hydroxy-8,19-oxidoetiocholanate (XV) by oxidation of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI): (a) With N-bromoacetamide. In preliminary experiments it was shown that the application of 2 moles of the reagent furnished only about 25% of ketonic material, whereas somewhat higher amounts increased the yield appreciably. To a solution of 100 mg. of VI in 4.0 cc. of *tert*-butyl alcohol and 1.5 cc. of water was added 120 mg. of 85% N-bromoacetamide (approx. 3 moles). After standing at room temperature overnight, a concentrated aqueous solution of 200 mg. of sodium sulfite was added and the reaction mixture extracted with ether. The combined ether phase was successively washed with a solution of sodium carbonate and with water. After drying with sodium sulfate, 97.3 mg. of a crystalline residue was obtained which was subjected to purification by chromatography (7 g. of alkali-free aluminum oxide;[5] diam. of column: 11 mm.). The material was dissolved in a mixture of 20 cc. of benzene and 10 cc. of petroleum ether and the eluting was performed with benzene-petroleum ether, benzene, benzene-ether, ether, ether-chloroform, chloroform, and chloroform-methanol. No appreciable residues resulted from the benzene-petroleum ether and benzene eluates. The benzene-ether eluates (total residue, 12.3 mg.) apparently contained XVI. The ether and the major part of the ether-chloroform fractions contained identical material which, after separate crystallization from acetone-ether, was pooled (46 mg.) and once more recrystallized from acetone only; short stout rods; m.p. 185–192° (decomp.). No depression of melting point with sample of XV obtained in the Raney nickel reaction (*vide supra*). $[\alpha]_D^{25}$ +60.3° (20.5 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm; α +0.93°). Treatment with pyridine and acetic anhydride at room temperature yielded unchanged starting material.

Anal. Calc'd for $C_{22}H_{32}O_5$ (376.47): C, 70.18; H, 8.57.
Found: C, 70.02; H, 8.54.

(*b*) *With chromic acid.* To a solution of 37.8 mg. (0.1 millimole) of VI in 3.0 cc. of 95% acetic acid there was added over a period of one hour at room temperature 7.34 mg. of chromium trioxide (the equivalent of 1.1 atoms of oxygen) dissolved in 2.0 cc. of 95% acetic acid. After standing overnight at room temperature, 1.0 cc. of 95% alcohol was added and the mixture brought to dryness (4 mm.) at room temperature. The crystalline residue was taken up in water and ether. The aqueous phase was extracted twice more with 20-cc. portions of ether. The combined ether extracts were washed with two 5-cc. portions of *N* sodium carbonate. From the combined carbonate phase only traces of acidic material could be isolated. The ether extract was washed with 10 cc. of water and dried with sodium sulfate; wt. of neutral residue, 38.2 mg. Recrystallization from acetone gave rosettes of long needles; 1st crop: wt., 12.6 mg., m.p. 201–202.5°; 2nd crop: wt, 10.8 mg., m.p. 199–200°. There was no rapid decomposition on melting as in the case of the identical substance obtained under (*a*). It seems possible that the rapid decomposition is facilitated by the presence of small amounts of impurities. The substance as obtained in this experiment gave no depression of the melting point when mixed with a sample of XV secured by the Raney nickel reaction (*vide supra*). $[\alpha]_D^{27}$ +60.1° (22.2 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm.; α +1.01°).

Ethyl 3-keto-8,19-oxido-Δ⁴-etiocholenate (XVI) by dehydrating XV. When the crude reaction product resulting from the oxidation of VI with N-bromoacetamide or chromic acid was treated with Girard's reagent T, the separation of the ketonic matter was connected with simultaneous complete dehydration leading to XVI. At the same time any unchanged VI was recovered from the non-ketonic fraction. As an example, the Girard separation is presented in an instance where VI had been oxidized with an insufficient amount of N-bromoacetamide.

To a solution of approximately 300 mg. of the reaction product in 7.5 cc. of absolute alcohol was added 0.7 cc. of glacial acetic acid and 360 mg. of Girard's reagent T. After refluxing for one hour, a small piece of ice was added and the reaction mixture transferred to a separatory-funnel by means of a little cold ether and water in a cold-room. After the addition of an ice-cold solution of 602 mg. of sodium carbonate in 5 cc. of water, the mixture was quickly extracted with two 50-cc. portions of cold ether and the combined ether phase washed with two 5-cc. portions of water. After drying, the ether phase yielded 71.2 mg. of non-ketonic material, identified as unchanged VI. The combined carbonate phase and aqueous washings were made acid to Congo Red by the addition of 10% sulfuric acid and, after allowing the mixture to stand at room temperature for about one hour, it was extracted with four 25-cc. portions of ether. The combined ether phases were washed neutral with *N* sodium carbonate and water. After drying with sodium sulfate, 206.0 mg. of ketonic material resulted. In recrystallizing this substance from ether, three crops of microcrystalline material (total, 146.2 mg.) were obtained; m.p. 120–121° or slightly below. There was no depression of the melting point when the substance was mixed with a sample of the ethyl 3-keto-8,19-oxido-Δ⁴-etiocholenate (m.p. 121–123°) obtained in the Raney nickel reaction (*vide supra*). Also in this instance the recrystallization was accompanied by the separation of small amounts of an insoluble fluffy precipitate. Renewed crystallization gave m.p. 122–123°; $[\alpha]_D^{22.5}$ −46.8° (17.8 mg. in 2.0 cc. of chloroform; *l*, 1.51 dm., α −0.63°).

Anal. Calc'd for $C_{22}H_{30}O_4$ (358.46): C, 73.71; H, 8.43.

Found: C, 73.46; H, 8.41.

Treatment of this compound with acetic anhydride or benzoyl chloride in pyridine at room temperature yielded unchanged starting material. The substance gave a low-melting oxime (from aqueous alcohol, m.p. 90–92°) which was difficult to recrystallize.

Ethyl 3-keto-8,19-oxidoetioallocholanate (XVIII) by catalytic hydrogenation of XVI. A suspension of 22 mg. of 5% palladium on carbon [(7), p. 78, method D] in 10 cc. of redistilled ethyl acetate was saturated with hydrogen. After the addition of a solution of 51.5 mg. of XVI (see preceding experiment) in a few ml. of ethyl acetate hydrogenation was performed at room temperature (27°) and atmospheric pressure. The hydrogen uptake stopped after about 40 minutes of shaking. Total absorption: 4.2 cc. (27°). Calc'd for 1 mole of H_2: 4.1 cc. (27°). After filtering from the catalyst, the solution was brought to dryness yielding 56 mg. (not quite dry) of a colorless resinous residue which was separated into ketonic and non-ketonic material.

To a solution of the residue in 2.5 cc. of absolute alcohol was added 0.25 cc. of glacial acetic acid and 60 mg. of Girard's reagent T. After refluxing the mixture for one hour, the separation was carried out as described before (see preceding experiment) after neutralizing with 215 mg. of sodium carbonate. Yield: ketonic, 32.2 mg.; non-ketonic, 21.4 mg.; both residues were resinous. Recrystallization of the ketonic fraction from ether-petroleum ether gave a total of 8.5 mg. of small crystals, m.p. 128–131°. The ketonic material was again pooled and subjected to a chromatographic separation (7 g. of alkali-free aluminum oxide;[5] diam. of column: 10 mm.). The desired substance was contained in the benzene and in some of the benzene-ether (9:1; 8:2) eluates. Total of the residues of these eluates: 25.8 mg. By recrystalization from ether-petroleum ether, these residues yielded a total of 9.3 mg. of material melting between 130 and 135°. The melting point of the purest fraction (4.0 mg.) was 133–134°; it gave no depression of the m.p. when mixed with a sample of XVIII obtained by the Raney nickel reaction (*vide supra*). The infrared spectra of the two samples likewise proved their identity (see also theoretical part).

SUMMARY

1. On the basis of new evidence the compound formerly interpreted to be ethyl 3β,5,19-trihydroxy-$\Delta^{8,14}$-etiocholenate (V) (2) has been assigned the structure of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI) and characterized by the monoacetyl derivative (VII). Correspondingly, the free acid, previously named 3β,5,19-trihydroxy-$\Delta^{8,14}$-etiocholenic acid (2) will henceforth be called 3β,5-dihydroxy-8,19-oxidoetiocholanic acid.

2. A method for the isolation of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI) from the mixture of the dehydration products of 3β,5,14,19-tetrahydroxy-14-isoetiocholanic acid (II) has been described. A new compound obtained from this mixture probably possesses the structure of ethyl 3β,19-dihydroxy-$\Delta^{5;14}$-etiocholadienate (XIII). It was characterized by the diacetyl derivative (XIV).

3. Treatment of ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI) with Raney nickel in the presence of cyclohexanone (1), led to the isolation of four reaction products (XV, XVI, XVII, XVIII). A reaction mechanism is postulated according to which VI is first dehydrogenated to ethyl 3-keto-5-hydroxy-8,19-oxidoetiocholanate (XV) which in turn is dehydrated to ethyl 3-keto-8,19-oxido-Δ^{4}-etiocholenate (XVI). The latter compound is reduced to ethyl 3-keto-8,19-oxidoetiocholanate (XVII) and ethyl 3-keto-8,19-oxidoetioallocholanate (XVIII) respectively. The main product of the reaction is XVIII.

4. Ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (VI) was oxidized to ethyl 3-keto-5-hydroxy-8,19-oxidoetiocholanate (XV) with either N-bromoacetamide or chromic acid. On treating XV with Girard's reagent T, dehydration occurred leading to ethyl 3-keto-8,19-oxido-Δ^{4}-etiocholenate (XVI). Catalytic hydrogenation of XVI (Pd, ethyl acetate) yielded ethyl 3-keto-8,19-oxidoetioallocholanate (XVIII).

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