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

INVESTIGATIONS ON STEROIDS. XIII. TRANSFORMATION OF STROPHANTHIDIN INTO COMPOUNDS STRUCTURALLY RELATED TO STEROID HORMONES[1]

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As has been stated in an earlier publication (1), it is intended to prepare certain analogs of steroid hormones. In particular the synthesis of compounds appears desirable in which 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 a steroid model the proximity of carbon atoms 11 and 19 appears obvious. Spacially a hydroxyl group attached to carbon atom 19 is very close to the location of a β-hydroxyl group at carbon atom 11, whereas a hydroxyl group in position 12 instead of 19 is farther away. Hence as part of the program the preparation of such isomers of adrenal cortical hormones is planned in which the one of the oxygen atoms is in position 19 rather than 11. The question arises whether compounds of such a type possess physiological activity.

I

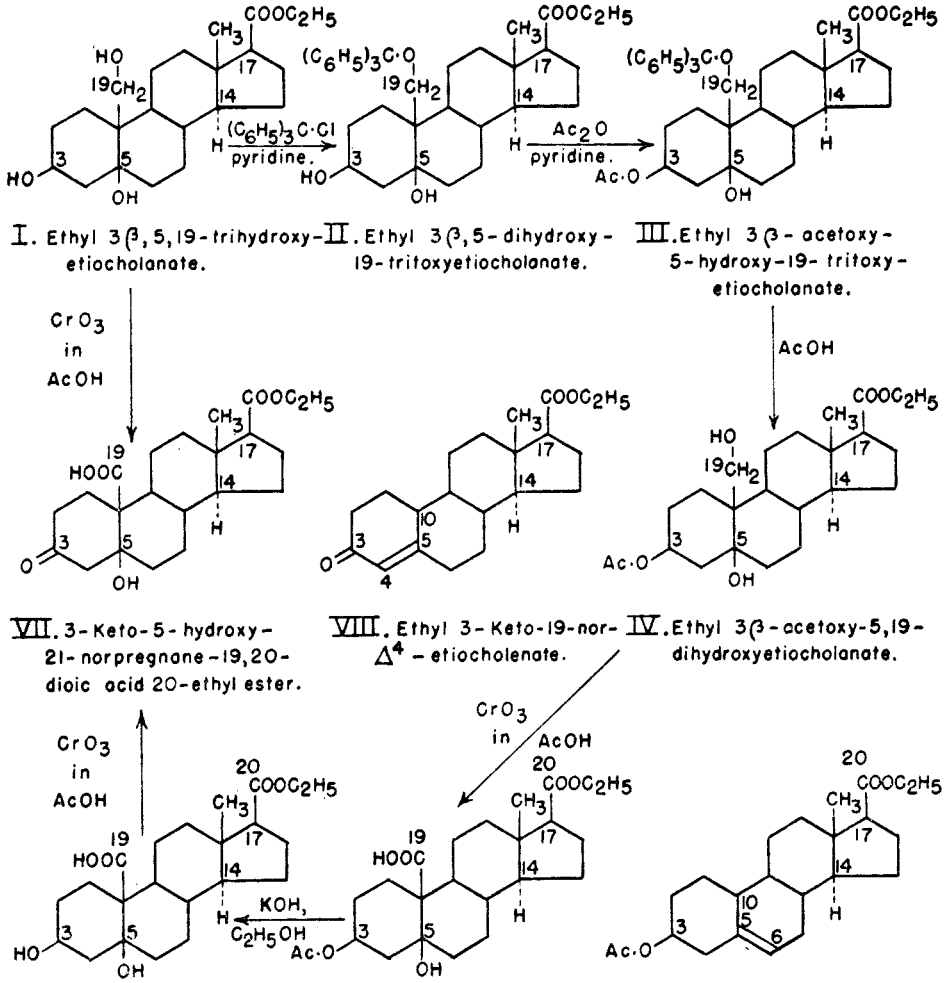
The conversion of strophanthidin into ethyl 3β,5,19-trihydroxyetiocholanate (I) has been described previously (1, 2). The latter compound is a key intermediate in the proposed work. Before subjecting this substance to any transformations at carbon 17, it appears desirable to explore the conditions under which the intended structural changes can be brought about in ring A and at carbon atom 19.

As has been described (1), I can be selectively tritylated to ethyl 3β,5-dihydroxy-19-tritoxyetiocholanate (II) which by acetylation can be transformed into

[1] This investigation was supported by research grants from: (a) Sharp and Dohme, Inc. in Philadelphia; (b) the National Cancer Institute of the National Institutes of Health, Public Health Service; (c) the Damon Runyon Memorial Fund for Cancer Research and the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council. Part I of this paper was presented before the American Society of Biological Chemists at the 34th annual meeting of the Federation of American Societies for Experimental Biology in Atlantic City, April 18, 1950 [*cf.* Ehrenstein and Wagner, *Federation Proceedings*, **9**, 167 (1950)]. Part II was presented before the Biological Chemistry Section at the Miniature Meeting of the Philadelphia Section of the American Chemical Society, January 18, 1951. (cf. Barber and Ehrenstein, Abstracts of Papers, Fourth Meeting-in-Miniature, Philadelphia Section of the American Chemical Society, p. 16 [1951]),

[2] Mrs. Mary Wagner Gordon was in charge of the experiments dealing with the transformation products of ethyl 3β,5,19-trihydroxyetiocholanate (Section I); Dr. George W. Barber performed the investigations on related compounds in the 14-iso-17-iso series (Section II).

ethyl 3β-acetoxy-5-hydroxy-19-tritoxyetiocholanate (III). Hydrolysis of III in slightly dilute acetic acid yields ethyl 3β-acetoxy-5,19-dihydroxyetiocholanate (IV) which may be oxidized with chromic acid to 3β-acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester[3] (V). An improved method of pre-



paring V is presented in the experimental section. The appreciable neutral fraction resulting from this oxidation, possibly containing IV and the aldehyde XV, awaits further investigation.

In continuation of this work, ethanolysis under mild conditions of V provided

[3] This nomenclature is based on recent proposals by Reichstein (3). A different nomenclature has been suggested by Fieser (4, page 522; footnote 15).

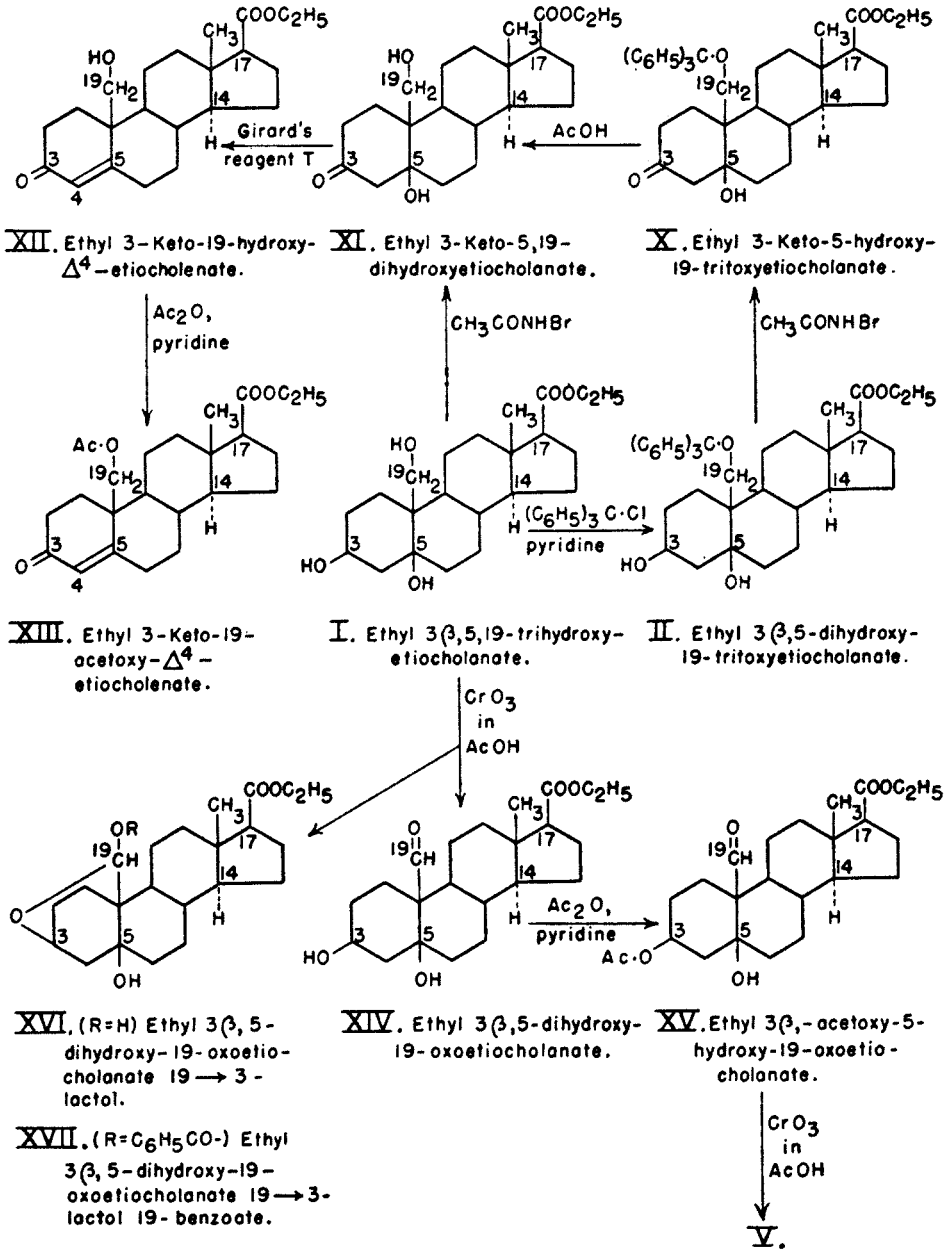
a good yield of 3β,5-dihydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester[3] (VI). Subsequent oxidation with chromic acid of VI gave 3-keto-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester[3] (VII). An attempt was made to obtain VII by direct oxidation with chromic acid of I. In this case the purification of the reaction product was difficult and the yield unsatisfactory. Investigations are under way to transform VII into ethyl 3-keto-19-nor-Δ^4-etiocholenate (VIII) by simultaneous dehydration and decarboxylation.

In a preliminary experiment V was subjected to distillation in a high vacuum. In analogy with earlier observations (5), it was anticipated that this would result in simultaneous dehydration and decarboxylation leading to ethyl 3β-acetoxy-19-nor-Δ^5-etiocholenate (IX) or an isomer having the double bond in the 4,5 or 5,10-position. Unexpectedly, only about 30% of the initial substance (V) was transformed into neutral material, which awaits further investigation. The major part of the distillation product consisted of unchanged V. Stereoisomers of compounds V, VI, VII, and VIII will be discussed in the second section of this paper (14-iso,17-iso series).

In another series of transformations the conditions were studied under which in I ring A can be transformed into the arrangement of an α,β-unsaturated ketone with retention of the primary alcohol group at carbon atom 19. Oxidation of II with 2 moles of N-bromoacetamide (lit. cit. 1) yielded ethyl 3-keto-5-hydroxy-19-tritoxyetiocholanate (X). Cleavage of the ether linkage of X with slightly dilute acetic acid gave ethyl 3-keto-5,19-dihydroxyetiocholanate (XI). When prepared by this method, XI was difficult to purify, mainly on account of its tendency to undergo dehydration and, in addition, because of the presence of small amounts of non-ketonic material. It was subsequently found that the preparation of XI can be substantially simplified. The primary alcohol group at carbon atom 19 seems to be inert towards oxidation by N-bromoacetamide. Hence, when I was treated directly with 2 moles of N-bromoacetamide, a satisfactory yield of fairly pure XI resulted. Also in this instance the purified product seemed to contain a certain amount of non-ketonic material, probably consisting of unchanged I. An attempt to remove these impurities by treatment with Girard's Reagent T was accompanied by practically complete dehydration of XI. Hence ethyl 3-keto-19-hydroxy-Δ^4-etiocholenate (XII) resulted as the main product. By chromatography it could be separated from a small amount of XI. It appears, therefore, that the preparation of the α,β-unsaturated ketone (XII) can be appreciably simplified. Oxidation with N-bromoacetamide of I, followed by treatment of the crude reaction product XI with Girard's Reagent T leads directly to XII which was characterized by its crystalline oxime. By acetylation, XII was converted into ethyl 3-keto-19-acetoxy-Δ^4-etiocholenate (XIII). Both XII and XIII gave ultraviolet absorption curves characteristic of α,β-unsaturated ketones. They are in agreement with the assigned structures (Figure 1).

No attempt is made to explain the possible role of Girard's Reagent T in the transformation of XI into XII. The formation of the hydrazone derivative (*i.e.*, the Girard condensation product) is carried out in the presence of a small amount

of acetic acid which *per se* facilitates the dehydration. Hence it cannot be stated whether the reaction is in any way analogous to the dehydrobromination of



XII. Ethyl 3-Keto-19-hydroxy-Δ^4-etiocholenate.

XI. Ethyl 3-Keto-5,19-dihydroxyetiocholanate.

X. Ethyl 3-Keto-5-hydroxy-19-tritoxyetiocholanate.

XIII. Ethyl 3-Keto-19-acetoxy-Δ^4-etiocholenate.

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

II. Ethyl 3(β),5-dihydroxy-19-tritoxyetiocholanate.

XVI. (R=H) Ethyl 3(β),5-dihydroxy-19-oxoetiocholanate 19 ⟶ 3-lactol.

XVII. (R=C_6H_5CO-) Ethyl 3(β),5-dihydroxy-19-oxoetiocholanate 19 ⟶ 3-lactol 19-benzoate.

XIV. Ethyl 3(β),5-dihydroxy-19-oxoetiocholanate.

XV. Ethyl 3(β),-acetoxy-5-hydroxy-19-oxoetiocholanate.

V.

3-keto-4-bromo steroids *via* the 2,4-dinitrophenylhydrazones (6, *cf.* also 7, 8) or semicarbazones (8).

Previously (1) an attempt was made to subject I to selective oxidation either

in position 3 or 19. Oxidation at room temperature with one equivalent of chromic acid yielded 80% of neutral material. Crystallization from ether gave a substance $C_{22}H_{34}O_5$ (m.p. 199–203°; $[\alpha]_D$ +76.2°, oxime: m.p. 188–189°) to which had been tentatively assigned the structure of ethyl 3-keto-5,19-dihydroxy-

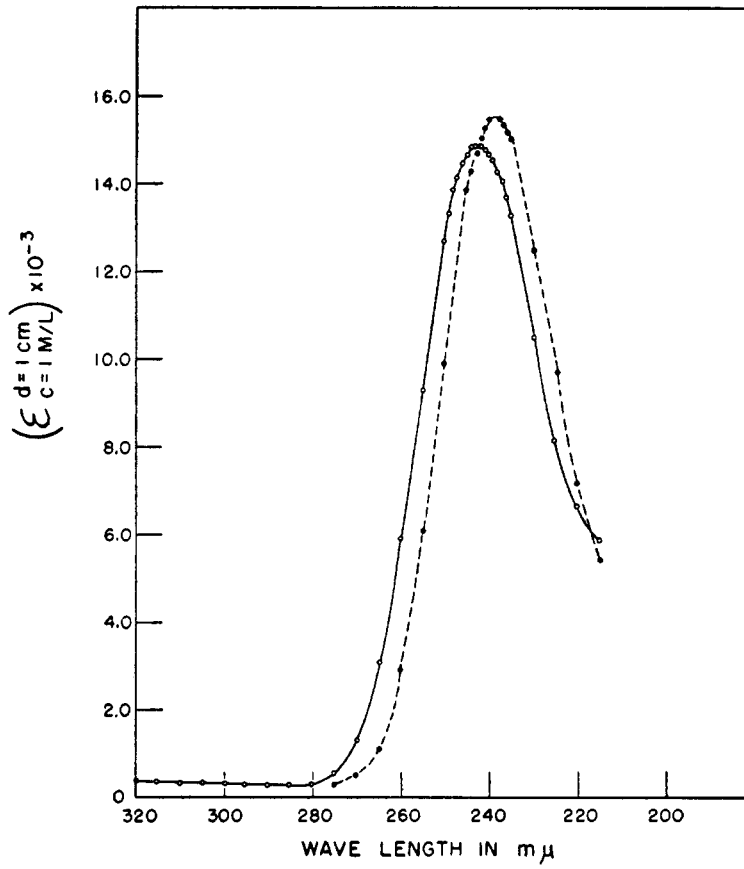


FIGURE 1.[4] SOLID CURVE, Ethyl 3-keto-19-hydroxy-Δ^4-etiocholenate (XII); mol. wt. 360.25; m.p. 182–185°; concentration of solution measured was 0.177 mg. per 10 cc. of absolute alcohol. λ_{max} 243 mμ (ϵ 14,858). BROKEN CURVE, Ethyl 3-keto-19-acetoxy-Δ^4-etiocholenate (XIII); mol. wt. 402.25; amorphous; concentration of solution measured was 0.2008 mg. per 10 cc. of absolute alcohol. λ_{max} 239 mμ (ϵ 15,465).

etiocholanate (XI). Chromatography of the neutral mother liquors yielded additional quantities of this compound and another, more polar, crystalline substance of the possible formulas $C_{24}H_{36}O_6$ or $C_{20}H_{30}O_5$ (m.p. 171–173°; $[\alpha]_D$ −11.2°. No oxime.)

The compound proven in the present paper to possess structure XI (*vide supra*) is different from the previously described (1) substance to which had

[4] The absorption curves were determined by Messrs. J. L. Ciminera, R. C. Shultz, and K. B. Streeter of the Sharp and Dohme Research Laboratories.

been tentatively assigned the same formula. Hence a reinvestigation of the latter substance was indicated. When it was subjected to purification by Girard's Reagent T in the usual fashion, only a small amount of nonketonic material resulted. After acidification and a few hours standing, the isolation of the ketonic fraction yielded only a trace of material. It was concluded that the substance in question had formed a very stable Girard condensation product and was therefore probably an aldehyde. It is stated in Fieser's monograph (4, pp. 308–309): "Aldehydes react readily to form hydrazone derivatives so stable to acid hydrolysis that a sharp separation of ketones from aldehydes can be made; no practicable method has been found for the regeneration of aldehydes". On the other hand, the recovery of aldehydes from Girard condensation products has recently been reported (9). After the failure to extract tangible amounts of

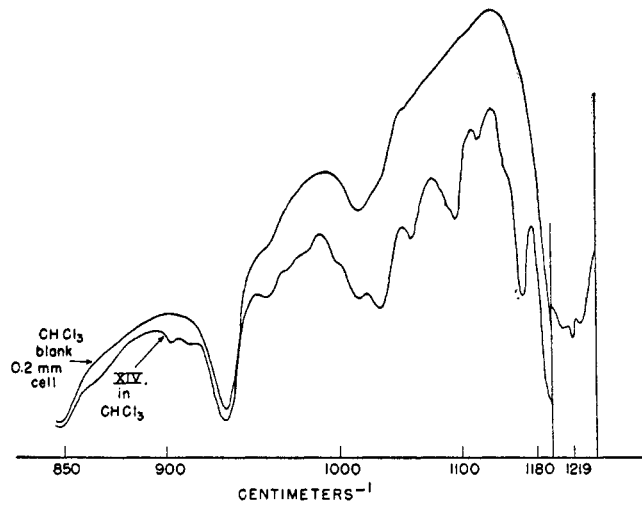


FIGURE 2.[5] ETHYL 3β,5-DIHYDROXY-19-OXOETIOCHOLANATE (XIV). Infrared absorption spectrum in the region 1100–850 cm^{-1}.

ketonic material in the present instance, the acidified aqueous solution was allowed to stand at room temperature for nine days. This resulted in the separation of crystals (m.p. 214–218°) which did not give a depression of the melting point when mixed with material (m.p. 199–203°) which had not been subjected to a Girard separation. Likewise the two samples gave identical infrared spectra (Figure 2). The properties and chemical behaviour of the substance therefore indicated that formula XI had to be ruled out and that the aldehydic structure of ethyl 3β,5-dihydroxy-19-oxoetiocholanate (XIV) was the only alternative. Further support was given by acetylating this compound and oxidizing the resulting acetate (XV) with chromic acid to the known and well characterized compound V.

The second, more polar, oxidation product of I which had failed to furnish

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

an oxime, was also subjected to treatment with Girard's Reagent T. It was recovered practically completely from the non-ketonic fraction. After recrystallization, the melting point was 172.5°. The structural formula of ethyl 3β,5-dihydroxy-19-oxoetiocholanate 19→ 3 lactol (XVI) is tentatively considered. It is known that compounds of such lactol type do not react with ketone reagents.[6] On benzoylation the substance yielded a monobenzoate (XVII?).

II

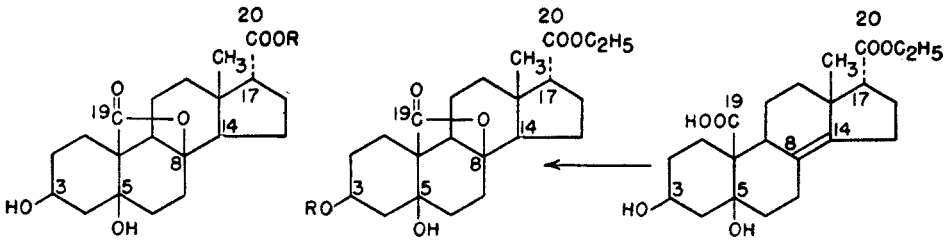
In a previous publication (5) the preparation of 19-norprogesterone[7] and 19-nor-11-desoxycorticosterone acetate[7] was described. The steroids of this series possess the iso-configurations at carbon atoms 14 and 17 (*cf.* 3, 12). Both end products and several intermediates in their syntheses were amorphous and probably represented mixtures of stereoisomers (*cf.* 13, p. 475). It has been decided to subject this matter to a reinvestigation with a view towards carrying out each chemical reaction with material whose chemical identity and stereochemical purity has been definitely established. The first steps in this direction are reported at this time, because some of the products obtained represent stereoisomers of compounds mentioned in the first section of this paper.

3β,5,14-Trihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid[3] (XVIII) was prepared from strophanthidin as described earlier (5) with only minor modifications. In previous experiments it had been shown (5, 14) that treatment of this compound with 0.1 *N* absolute alcoholic hydrogen chloride leads to 3β,5-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid[3] (XIX). It is known that a carboxyl group at carbon atom 17 is more reactive when it is in "*cis*" rather than in "*trans*" position to the angular methyl group at carbon atom 13. Hence esters of normal etio acids are hydrolyzed easier than those of the 17-isoetio acids (*cf. e.g.* 4, p. 362; 18). In turn it may be expected that esterification with an alcohol in the presence of mineral acid will proceed much easier with a normal etio acid than with a 17-isoetio acid. In 3β,5,14,19-tetrahydroxy-14-iso-etiocholanic acid the carboxyl group at carbon atom 17 is in the normal position. Consequently when this compound was treated with 0.1 *N* absolute alcoholic hydrogen chloride, not only dehydration took place, but the major part of the reaction product underwent simultaneous esterification (1, 2, 15). On the other hand, it had not appeared surprising that similar treatment of the 17-isocarboxylic acid (XVIII) had so far not resulted in the isolation of an esterified product. A reinvestigation showed, however, that XIX is not the only compound formed in the reaction. An appreciable part of the material is converted into the half ester, *i.e.* 3β,5-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid 20-ethyl ester[3] (XX). Due to its easier solubility it had previously escaped isolation.

In addition to these two main reaction products a very small amount of a neutral substance was isolated to which was tentatively assigned the structure of 3β,5,8-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→ 8-lactone

[6] *Cf. e.g.*, pseudostrophanthidin (4, pp. 523–524) (10, 11).

[7] The original designations were 10-norprogesterone and 10-nor-11-desoxycorticosterone acetate respectively. The new terminology is in better agreement with the established nomenclature (*cf.* also 4, p. 522).



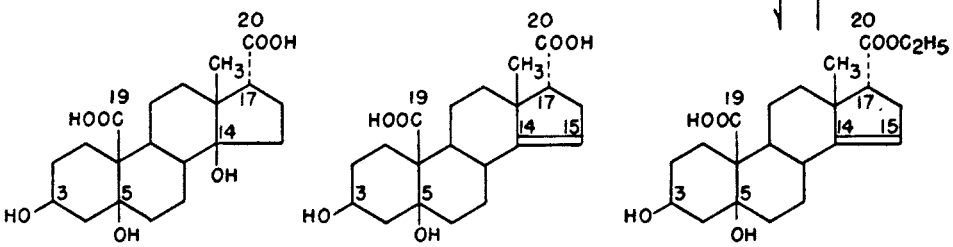
XXIV. (R=H) 3β,5,8-Trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone.

XXII. (R=H) 3β,5,8-Trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone 20-ethyl ester.

XXI. 3β,5-Dihydroxy-17-iso-21-nor-$\Delta^{8,14}$-pregnene-19,20-dioic acid 20-ethyl ester.

XXV. (R=CH_3) 3β,5,8-Trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone 20-methyl ester.

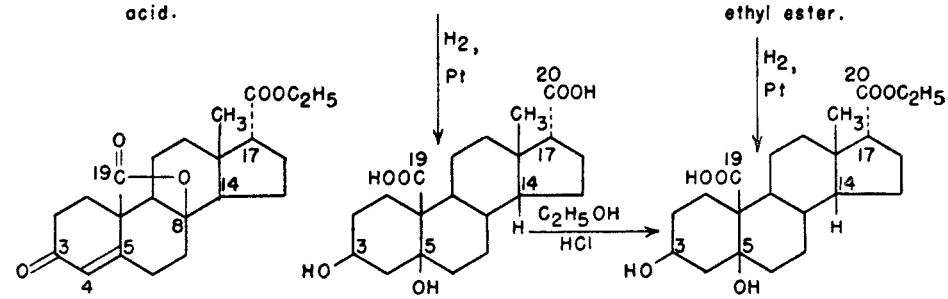
XXIII. (R=C_6H_5CO-) 3β-Benzoxy-5,8-dihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone 20-ethyl ester.



XVIII. 3β,5,14-Trihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid.

XIX. 3β,5-Dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid.

XX. 3β,5-Dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid 20-ethyl ester.



XXVIII. 3-Keto-8-hydroxy-17-iso-21-nor-Δ^{4}-pregnene-19,20-dioic acid 19→8-lactone 20-ethyl ester.

XXVI. 3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid.

XXVII. 3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester.

20-ethyl ester[3] (XXII). It was characterized by the benzoate (XXIII). As has been discussed elsewhere (15), an equilibrium may exist between Δ^{14}- and $\Delta^{8,14}$-unsaturated steroids, thus in the present instance between compounds XX and XXI. With a carboxyl group attached to carbon atom 10, the compound with

the double bond in the 8,14 position (XXI) seems to be unstable and hence cyclization occurs to compound XXII. The reasons why the bridge is formulated to extend between carbon atoms 8 and 19 have been discussed in an analogous case (15).

In a previous publication (5) a compound was mentioned which represents an isomer of XIX. It was obtained by treatment of XVIII with sulfuric acid in a solution of dioxane. At that time the structure was tentatively formulated as differing from that of XIX by the location of the double bond ($\Delta^{8,14}$- instead of Δ^{14}-position) (5, footnote on p. 446). When this compound was treated with diazomethane a neutral monomethyl ester resulted. In view of this fact and in consideration of the isolation of compound XXII in the present investigation, it is now considered very probable that the isomer of XIX, as obtained from XVIII by the action of sulfuric acid in dioxane, possesses structure XXIV and the corresponding methyl ester structure XXV. Compound XXIV has not been isolated in the present investigation. It is possible that small amounts of this substance are present in the mother liquors. The configuration at carbon atom 14 in formulas XXII, XXIII, XXIV, and XXV is not proven.

Catalytic hydrogenation of XIX yielded 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid[3] (XXVI). The stereochemical course of this hydrogenation has been discussed previously (13, p. 475). Because of the discrepancy of the optical rotations given in the literature (5, 14), the rotation of XXVI was redetermined and found in agreement with the figure reported earlier (5).

By catalytic hydrogenation (Pt;ethanol) XX was transformed into 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester[3] (XXVII).[8] The latter compound could also be obtained by esterification of XXVI with ethanol in the presence of hydrogen chloride. In accordance with expectations, *i.e.*, due to the iso position of the carboxyl group at carbon atom 17, the esterification proceeded only at a moderate rate. The ester XXVII is a stereoisomer of compound VI mentioned earlier in this paper.

In a previous investigation (1) it was shown that on treating I with Raney nickel in the presence of cyclohexanone, a fair amount of the material undergoes aromatization leading to ethyl 3-hydroxy-$\Delta^{1;3;5;10}$-estratriene-17-carboxylate. Hence the question arose whether the ester XXVII, when subjected to identical experimental conditions, might undergo decarboxylation followed by aromatization of ring A. The experiment showed that in this instance a substantial amount of the starting material (XXVII) is recovered. The only new substances which have been isolated in small amounts from the reaction mixture are an α,β-unsaturated ketone of the possible formula $C_{22}H_{28-32}O_5$ and ethyl 3-keto-14-iso-17-

[8] On acetylating XXVII under mild conditions (acetic anhydride, pyridine; room temperature), an amorphous reaction product resulted. The analytical figures (Found: C, 65.53, 65.47; H, 8.25, 8.29) are in better agreement with a diacetate (Calc'd for $C_{26}H_{38}O_8$: C, 65.25; H, 8.00) than with a 3-monoacetate (Calc'd for $C_{24}H_{36}O_7$: C, 66.03; H, 8.31). On the other hand, the formation of a 3,5-diacetate under these conditions appears unlikely. This will be further investigated. A 3-monoacetate should be hydrolyzable to the starting material under mild conditions.

iso-19-nor-Δ^4-etiocholenate (XXX) (*cf.* also *infra*). The former compound is different from the α,β-unsaturated ketone XXVIII obtained from XXII by

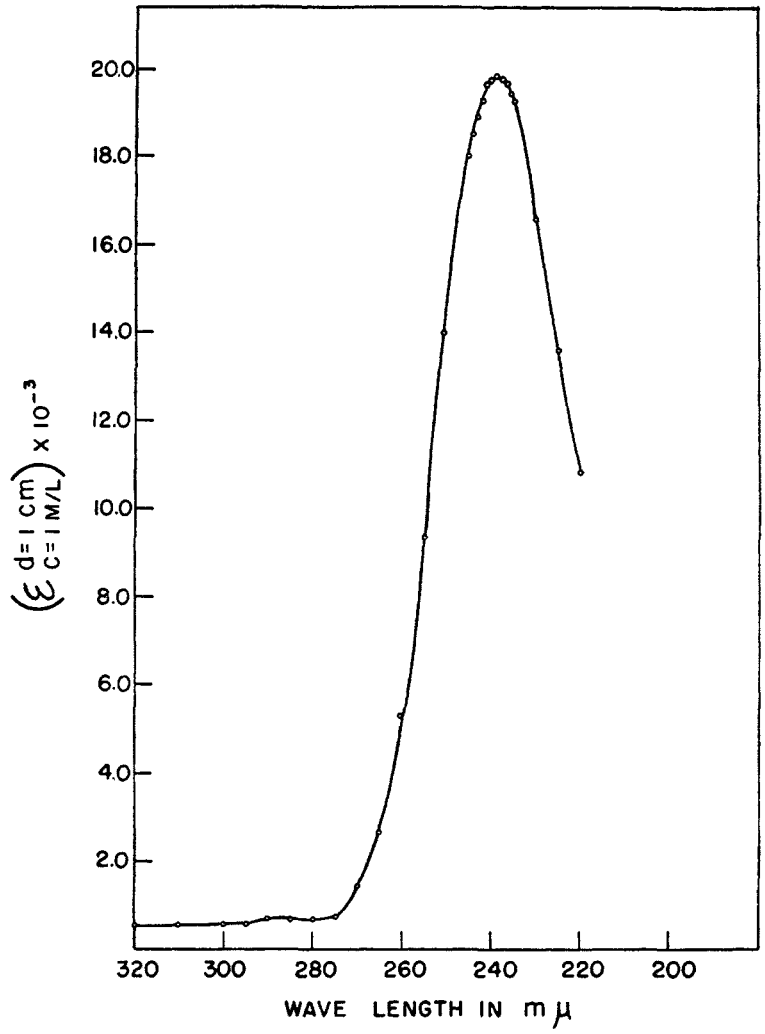


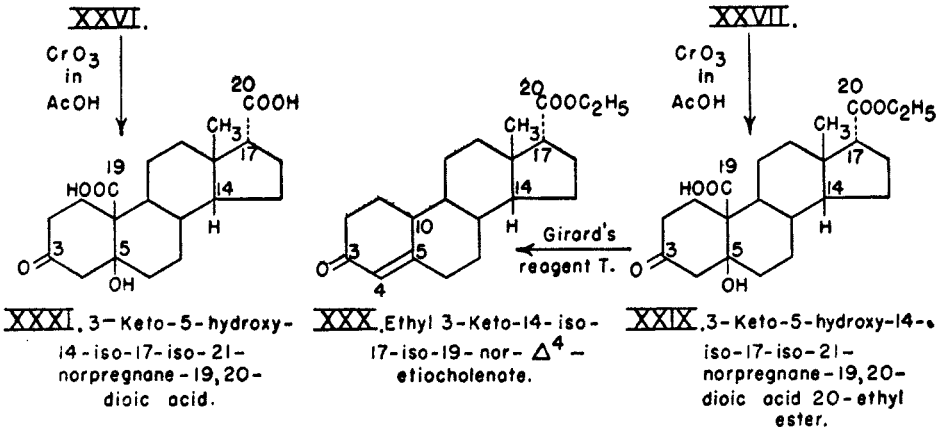
FIGURE 3.[10] ETHYL 3-KETO-14-ISO-17-ISO-19-NOR-Δ^4-ETIOCHOLENATE (XXX); mol. wt. 330.45; m.p. 88.5°; concentration of solution measured was 0.1125 mg. per 10 cc. of absolute alcohol. λ_{max} 239 mμ (ϵ 19,856).

oxidation and subsequent dehydration.[9] The transformation of XXVII into XXX by means of Raney nickel-cyclohexanone appears plausible on the basis of analogous observations made with structurally related compounds (15).

[9] To be published later.

[10] The absorption curve was determined by Messrs. Edward H. Unger and K. B. Streeter of the Sharp and Dohme Research Laboratories.

By treatment with chromic acid XXVII was converted into 3-keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester[3] (XXIX) which is a stereoisomer of compound VII mentioned in the first section of this paper. It was attempted in various ways to subject XXIX to simultaneous dehydration and decarboxylation. Such a reaction should lead to compound XXX. In agreement with expectations, refluxing XXIX with glacial acetic acid gave a neutral reaction product which apparently represented a mixture containing the desired product (XXX). Various attempts at purification have been unsuccessful. On the other hand, when XXIX was subjected to treatment with Girard's Reagent T, a neutral crystalline product was obtained which gave the ultraviolet absorption curve of an α,β-unsaturated ketone (Figure 3) and, therefore, represents ethyl 3-keto-14-iso-19-nor-Δ^4-etiocholenate (XXX). The configuration at carbon atom 10 remains uncertain. A very small amount of a by-product is probably identical with compound $C_{22}H_{28-32}O_5$ (Raney nickel experiment; *vide supra*).



XXXI. 3-Keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid.

XXX. Ethyl 3-Keto-14-iso-17-iso-19-nor-Δ^4-etiocholenate.

XXIX. 3-Keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester.

Oxidation with chromic acid of the dicarboxylic acid XXVI (14) gave an improved yield of 3-keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid[3] (XXXI).

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 (C, H, N, and mol. wt. determinations) 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°.

SECTION *I*

3β-Acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (V) *by oxidation with chromic acid of* IV. When the oxidation was carried out under the conditions described earlier (1), the yield of crystalline V was variable: (a) 23.9% (original expt.), (b) 29.5%, (c) 39.9% (d) 22.4%. (e). An improved and more uniform yield was obtained by observing the following revised procedure: To a solution of 393.2 mg. of IV in 10 cc. of glacial acetic acid, which was cooled in a ice-salt mixture, was added 136.6 mg. (the equiv. of 2.2 atoms of oxygen) of chromium trioxide dissolved in 13.66 cc. of 95% acetic acid. The reaction

mixture was gently shaken at room temperature until it became liquid. It was returned to the ice-salt bath and the entire system allowed to reach room temperature. The following day a large amount of water was added and the turbid solution extracted with three approx. 500-cc. portions of ether. The combined ether phases were washed with *N* sodium carbonate and water, dried with sodium sulfate, and evaporated to dryness; wt. of the resinous neutral residue: 188.1 mg. The combined carbonate phases and aqueous washings were cooled in an ice-salt bath and made acid to Congo Red with 10% sulfuric acid. The resulting gelatinous precipitate was taken up in 500 cc. of ether and the aqueous layer extracted twice with large amounts of ether. The combined acid ether extracts were washed five times with small amounts of water, dried with sodium sulfate, and evaporated to dryness; wt. of the crystalline acid residue: 209.0 mg. Recrystallization from a mixture of acetone and petroleum ether yielded: 1st crop, wt. 131.0 mg., m.p. 206–208° (decomp.); 2nd crop, wt. 39.1 mg., m.p. 206–208° (decomp.); 3rd crop, wt. 6.7 mg., m.p. 202–204° (decomp.). Total yield of crystalline acid: 176.8 mg. (43.5%). (f). Repetition of the experiment under analogous conditions (starting material: 161.3 mg.) gave a yield of 40.9% of crystalline acid.

In one instance (b) an attempt was made to purify the neutral fraction (61.2 mg.) by chromatography. The presence of several crystalline compounds was indicated, none of which could be conclusively identified as IV or XV.

3β,5-Dihydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (VI) *by ethanolysis of* V. To a solution of 68.0 mg. of V (m.p. 205–207°; decomp.) in 2 cc. of absolute ethanol was added 3.3 cc. of 0.1 *N* absolute alcoholic potassium hydroxide (approx. 2.2 equiv.). After standing at room temperature for about 20 hours, 2 cc. of water and enough 0.1 *N* hydrochloric acid to make the solution neutral to litmus were added. The ethanol was subsequently removed *in vacuo* and the resulting white suspension made acid to Congo Red with three drops of conc'd hydrochloric acid. The material was taken up in 300 cc. of ether, the solution washed a few times with small amounts of water, and then extracted with *N* sodium carbonate. After cooling with ice, the extract was made acid to Congo Red with conc'd hydrochloric acid and the resulting precipitate taken up in 300 cc. of ether. Drying with sodium sulfate and subsequent evaporation yielded 66.0 mg. of a brittle foam, which was crystallized from ether-petroleum ether as clusters of short, rectangular crystals. Yield of pure material: 51.9 mg., m.p. 165–167°, solidifies, then remelts at 183–184° (effervescence). Some additional crystalline material (2.3 mg.) with a lower m.p. could be secured. $[\alpha]_D^{31}$ +35.0° (23.9 mg. in 2.0 cc. of chloroform; *l*, 1.51 dm., α +0.63°).

Anal. Calc'd for $C_{22}H_{34}O_6$ (394.27): C, 66.93; H, 8.69.

Found: C, 66.69; H, 8.82.

3-Keto-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (VII). *A. By oxidation with chromic acid of* VI. To a solution of 51.5 mg. of VI in 2.93 cc. of redist. glacial acetic acid there was added at room temperature during 30 minutes, a solution of 10.44 mg. (1.2 equivalents) of chromium trioxide in 6.2 cc. of 95% acetic acid. After two days standing, some ethyl alcohol was added and the solution brought to dryness *in vacuo* (45°). In order to remove the acetic acid completely, the residue was repeatedly dissolved in small amounts of alcohol and taken to dryness. Finally 2 cc. of water was added, the white and gummy mass was taken up in 100 cc. of ethyl acetate, and this solution washed with 2 cc. of *N* sulfuric acid and several times with small amounts of water. The ethyl acetate phase was subsequently extracted with four 1-cc. portions of *N* sodium carbonate and then washed four times with small amounts of water. After the addition of ice, the combined carbonate extracts and aqueous washings were made acid to Congo Red with 2 cc. of 4 *N* sulfuric acid. The precipitated material was thoroughly extracted with ethyl acetate and the solution washed with water. After drying with sodium sulfate and evaporation of the solvent, 46.7 mg. of resinous acid material was obtained which was recrystallized from a mixture of ether and petroleum ether. Mushroom-like arrangements of small, rectangular crystals; weight of the first crop 24.0 mg; m.p. 153–154.5° (effervesc.). By recrystallization from

ether the melting point was raised to 158–160° (effervesc.). $[\alpha]_D^{30}$ +29.9° (24.4 mg. in 2.0 cc. of chloroform; *l*, 1.51 dm.; α +0.55°).

Anal. Calc'd for $C_{22}H_{32}O_6$ (392.48): C, 67.32; H, 8.22.

Found: C, 67.23; H, 8.28.

B. By oxidation with chromic acid of I. To a solution of 135.4 mg. of I in 8 cc. of redistilled glacial acetic acid was added gradually 94.0 mg. of chromium trioxide (4.0 equivalents) dissolved in 4.7 cc. of 95% acetic acid. After standing at room temperature overnight, the mixture was brought to dryness *in vacuo* (40°) and the residue repeatedly evaporated with ethyl alcohol until all traces of acetic acid had disappeared. Thereafter 5 cc. of water was added and the gummy mass taken up in 100 cc. of ethyl acetate. This solution was washed with *N* sulfuric acid and several times with small amounts of water. It was extracted four times with 3 cc. of *N* sodium carbonate and four times with small quantities of water. After drying with sodium sulfate and evaporating the solvent 31.7 mg. of resinous neutral material was obtained. The combined carbonate extracts and aqueous washings were made acid to Congo Red with 4 *N* sulfuric acid. The turbid solution was extracted with 100 cc. of ethyl acetate and the extract washed four times with small amounts of water. After drying with sodium sulfate and evaporating the solvent 84.4 mg. of resinous acid material resulted. Crystallization from ether-petroleum ether gave 27.6 mg. of crystals melting between 105 and 155°. Repeated recrystallization from ether yielded 10.5 mg. of clusters of stout rectangular crystals; m.p. 152–156° (effervesc.). The substance was apparently not quite pure. However, there was no depression of the melting point when it was mixed with a sample of VII (*vide supra*). $[\alpha]_D^{22}$ +18.1° (6.6 mg. in 2.0 cc. of chloroform; *l*, 1.51 dm., α +0.09°).

Anal. Calc'd for $C_{22}H_{32}O_6$ (392.48): C, 67.32; H, 8.22.

Found: C, 67.32; H, 8.31.

On repeating this experiment by oxidizing I with 3.2 equivalents of chromic acid, unsatisfactory results were obtained.

Ethyl 3-keto-5-hydroxy-19-tritoxyetiocholanate (X). To a solution of 62.2 mg. of II in 0.5 cc. of *tert*-butyl alcohol was added 33.0 mg. (2 moles) of N-bromoacetamide[11] (95% pure) and 0.05 cc. of pyridine and the colorless mixture was allowed to stand at room temperature for about 24 hours, during which it turned yellow. After the addition of a solution of 100 mg. of sodium sulfite in 1.0 cc. of water, the mixture was extracted with 10 cc. of ether which was followed by washing of the ethereal phase with three 0.5-cc. portions of water and subsequently drying with sodium sulfate. Eventually 66.5 mg. of a yellow resin was obtained which was recrystallized from a mixture of ether and petroleum ether. Clusters of long thin rectangular crystals; total yield: 49.1 mg.; m.p. between 160 and 174°. Recrystallization furnished 26.4 mg. of small rectangular crystals; m.p. 178–181°. (Mixed

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[11] Method of preparation: To a solution of 16.68 g. (0.05 mole) of mercuric nitrate in water was added 25 cc. of 4 *N* sodium hydroxide and the precipitated mercuric oxide washed with water by decantation until all traces of alkali had been removed (litmus). The remaining thick slurry of mercuric oxide was added portionwise to an ice-salt cooled solution of 5.91 g. (0.1 mole) of acetamide in 5.11 cc. (0.1 mole) of bromine. As soon as all of the bromine was consumed, the mixture was filtered from mercuric oxide (no water for rinsing), and the clear, straw-colored filtrate cooled to 0°. The N-bromoacetamide monohydrate was filtered and washed with a minimum amount of ice-water. Yield: 2.37 g.; m.p. 79–81°. Recrystallization from ten times its weight of boiling chloroform, filtering from a fine precipitate of mercuric bromide, and subsequent cooling gave white platelets; m.p. between 90 and 102° (mixture of monohydrated and anhydrous form). The purity was determined by dissolving 30 mg. of the substance in 20 cc. of water, adding 4 cc. of glacial acetic acid, and 300 mg. of potassium iodide and subsequently allowing the mixture to stand in the dark for a period of 10 minutes. After the addition of 25 cc. of water, the filtrate was titrated with 0.1 *N* sodium thiosulfate in the established fashion.

m.p. with starting material: 167–174°). From the filtrate additional, less pure material resulted. $[\alpha]_D^{26}$ −2.5° (17.0 mg. in 2.0 cc. of chloroform; *l*, 1.51 dm., α −0.03°).

Anal. Calc'd for $C_{41}H_{48}O_5$ (620.79): C, 79.32; H, 7.79.

Found: C, 78.88; H, 7.88.

In case of a repetition of this experiment purification of the reaction product by chromatography is indicated.

Ethyl 3-keto-5,19-dihydroxyetiocholanate (XI) *A. By cleavage of the ether linkage of* X. To a solution of 52.8 mg. of crude X in 1.7 cc. of glacial acetic acid was added 4.7 cc. of 95% acetic acid and the mixture allowed to stand at room temperature overnight. After bringing the solution to dryness *in vacuo*, 2 cc. of water was added and the white gummy precipitate taken up in 50 cc. of ether. This was washed with four 1-cc. portions of water, four 1-cc. portions of *N* sodium carbonate, and again four 1-cc. portions of water. After drying over sodium sulfate and evaporation of the ether, 45.4 mg. of a residue was obtained. Crystallization from ether furnished several fractions of long silky needles, totaling 17.1 mg., with melting points of 168–170° (decomp.). Repeated crystallization from ether furnished 4.2 mg. of highest melting material; m.p. 190–195° (decomp.). Because of this small yield the total crude reaction product (45.4 mg.) was subjected to purification by chromatography (3.0 g. of alkali-free alumina;[12] diam. of column: 1 cm.). About 19 mg. of triphenylcarbinol was isolated from the benzene eluates. No appreciable material resulted from the benzene-ether and ether eluates. Essentially identical crystalline material was isolated from eluates consisting of chloroform alone or chloroform containing 0.2% of methanol; total crude yield: about 25 mg. Recrystallization of each of these chromatographic fractions yielded fine needles with melting points ranging between 180 and 190° (decomp.), which were not analyzed, however, since treatment with Girard's Reagent T was attempted for further purification. This procedure was accompanied by dehydration of at least part of the ketonic fraction, *i.e.*, conversion into the α,β-unsaturated ketone (*vide infra*).

B. By oxidation of I *with N-bromoacetamide.* To a solution of 38.0 mg. of I in 0.5 cc. of *tert*-butyl alcohol were added 33.0 mg. (2 moles) of N-bromoacetamide (95% pure) and 0.05 cc. of pyridine. The originally colorless mixture turned yellow on standing at room temperature (27°) overnight. After the addition of a solution of 100 mg. of sodium sulfite in 1 cc. of water the mixture was extracted with 100 cc. of ether and the latter phase washed with three 0.5-cc. portions of water. After drying with sodium sulfate and evaporating the ether, 39.5 mg. of a residue (not quite dry) was obtained. Recrystallization from ether gave fractions of long, silky needles, totaling 18 mg., with melting points between 185 and 190° (decomp.). There was no depression of the melting point when mixed with similarly melting material of the preceding experiment (A). In order to achieve a better purification, all crystalline fractions including the mother liquors were combined (39.5 mg.), dissolved in 25 cc. of benzene, and subjected to chromatographic separation (3.0 g. of alkali-free alumina;[12] diam. of column: 1 cm.). Only traces of residues were obtained from the benzene, benzene-ether, ether, ether-chloroform, and chloroform eluates. The major part of the material (27.2 mg.) was recovered from a single eluate (20 cc. of chloroform +0.5 cc. of methanol). Recrystallization of this fraction from ether gave long fine needles of m.p. 184–190° (decomp.) The total fraction (27.2 mg.) was dissolved in 20 cc. of chloroform and subjected to a renewed chromatographic separation (2.0 g. alkali-free alumina;[12] diam. of column: 1 cm.). Negligible amounts were obtained from eluates consisting of 20 cc. of chloroform or of 20 cc. of chloroform containing up to 0.05 cc. of methanol. The major part of the material was recovered from two successive eluates, both consisting of 20 cc. of chloroform +0.1 cc. of methanol (residues: 11.5 +5.3 mg.). On recrystallizing from ether, both of these residues gave long, fine needles with melting points between 185 and 190° (decomp.). Though unanalyzed, this was probably fairly pure XI. Further purification of

[12] One part of Alumina Adsorption (Fisher), 80–200 MM, 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 with methanol and dried at 200° for four hours.

the total material recovered from the second chromatogram (26.1 mg.) was effected with Girard's Reagent T. This yielded 8 mg. of non-ketonic material, possibly consisting of unchanged I. The ketonic fraction was quite uniform and had obviously largely undergone dehydration to the α,β-unsaturated ketone; melting points (from ether): between 176 and 184° (*vide infra*).

Ethyl 3-keto-19-hydroxy-Δ^4-etiocholenate (XII) *by oxidation with N-bromoacetamide of* I *followed by treatment with Girard's Reagent T.* In orienting experiments the oxidation was performed with varying amounts of N-bromoacetamide, ranging between 1.2 and 2 moles. Though in the following experiment an adequate yield of ketonic material resulted with 1.33 moles of the oxidizing agent, it is considered safer to apply 2 moles.

TABLE I

CHROMATOGRAPHIC FRACTIONATION

NO. OF FRACTION	SOLVENT	WEIGHT OF RESIDUE, MG.	APPEARANCE OF RESIDUE
1	50 cc. of benzene (Original Solution)	2.3	Resinous
2	55 cc of benzene-ether (9:2)	3.5	Resinous
3–5	50 cc. each of benzene-ether (4:1), (7:3), (7:3)	62.1	Crystalline
6–12	50 cc. each of benzene-ether (7:3), (3:2), (1:1), (2:3), (3:7), (1:4), (1:9)	39.0	Crystalline
13–15	50 cc. each of ether	7.7	Crystalline
16	50 cc. of ether + 0.1 cc. of methanol	3.6	Crystalline
17	50 cc. of ether + 0.3 cc. of methanol	9.2	Crystalline
18	50 cc. of ether + 0.3 cc. of methanol	4.0	Crystalline
19	50 cc. of ether + 0.3 cc. of methanol	1.8	Crystalline
20–22	50 cc. each of ether + 0.5 cc., 0.5 cc., 1 cc. of methanol respectively	1.2	Resinous
23	50 cc. of ether-methanol (9:1)	3.4	Resinous
24	25 cc. of methanol	0.9	Resinous
Total		138.7	

To a solution of 171 mg. of I in 9.0 cc. of *tert*-butyl alcohol there were added successively 3.9 cc. of water and 99 mg. (about 1.33 moles) of N-bromoacetamide (95% pure). The originally colorless mixture gradually turned yellow upon standing at room temperature overnight. After the addition of a solution of 450 mg. of sodium sulfite in 4.5 cc. of water, it was extracted with 400 cc. of ether, the latter phase washed with three 5-cc. portions of water and dried over sodium sulfate. Evaporation of the ether yielded 176.9 mg. of a crystalline residue which was separated into ketonic and non-ketonic material by dissolving it in 3.5 cc. of methanol, adding 287 mg. of Girard's Reagent T followed by 0.21 cc. of glacial acetic acid, and refluxing this mixture for one hour on a water-bath. After cooling to −5°, a small piece of ice and an ice-cold solution of 175 mg. of sodium carbonate in 3.5 cc. of water was added. The mixture was extracted quickly with 35 cc. of cold ether and the latter washed with 0.5 cc. of ice-water, 3.5 cc. of *N* sodium carbonate, and four 0.5-cc. portions of water. After drying with sodium sulfate and evaporating, 20.4 mg. of non-ketonic residue was obtained. The first carbonate phase and aqueous washing were combined and made acid to Congo Red with 3.5 cc. of 4 *N* sulfuric acid. After standing for a short while at room temperature, the precipitate was extracted with four 50-cc. portions of ether. The combined ether fractions were washed with 3.5 cc. of *N* sodium carbonate and four 1-cc. portions of water. After drying with sodium sulfate and evaporating the ether, 135.3 mg. of crystalline ketonic residue resulted.

The ketonic residue was chromatographed (8.0 g. alkali-free alumina;[12] diam. of column: 1 cm.). The original solution was passed through within one hour and the subsequent eluates between 15 and 20 minutes each.

By separately recrystallizing the residues of fractions 3–12 (total 101.1 mg.) from ether, a number of fractions of short, fine needles were obtained, melting between 182 and 185°. They represented identical material and were therefore combined (78.0 mg.). Renewed recrystallization from ether did not change the melting point. $[\alpha]_D^{21}$ +137.3° (19.0 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +1.97°). The microanalyses refer to the above material and to that originating from another, similarly conducted experiment, respectively.

Anal. Calc'd for $C_{22}H_{32}O_4$ (360.25): C, 73.28; H, 8.95.

Found: C, 73.31, 73.28; H, 9.05, 9.32.

The ultraviolet absorption spectrum of this substance (λ_{max}^{alc} 243 mμ; ϵ 14,858) was practically identical with that obtained with material (m.p. 180–184°; λ_{max}^{alc} 243mμ; ϵ 15,041) which had been secured by ether cleavage of X, subsequent Girard separation of the steroid material, and repeated recrystallization of the ketonic fraction from ether (*vide supra*). The absorption curve is given in the theoretical part.

The chromatographic fractions 16–18 probably contained XI. This substance was isolated in a pure form from the corresponding chromatographic fractions of another, analogous experiment after recrystallization from ether; silky needles; m.p. 189–191° (melts to a yellow liquid with decomp.). A sample (1.367 mg.) was analyzed.

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

Found: C, 69.67; H, 9.74.

Oxime of ethyl 3-keto-19-hydroxy-Δ^4-etiocholenate. To 9.4 mg. of XII dissolved in 1.7 cc. of absolute alcohol was added a mixture of 20 mg. of hydroxylamine hydrochloride and 30 mg. of sodium acetate in 0.15 cc. of water. After refluxing on a water-bath for three hours, an amount of water was added sufficient to cause the separation of white crystals; wt., 8.0 mg.; m.p. 94–99°. Removal of the ethanol from the filtrate *in vacuo* yielded a second crop of crystals; wt., 0.7 mg.; m.p. 93–99° solidif. and remelt. at 157–162°. Recrystallization of the combined material from aqueous ethanol gave a total of 6.0 mg. of fine needles; m.p. 93–99° solidification at 107°, remelting at 159–162°.

Anal. Calc'd for $C_{22}H_{33}NO_4$ (375.27): N, 3.73. Found: N, 3.94. (Dried at 95°.)

Ethyl 3-keto-19-acetoxy-Δ^4-etiocholenate (XIII). To a solution of 18.3 mg. of XII in 0.6 cc. of pyridine was added 0.6 cc. of acetic anhydride and the mixture allowed to stand at room temperature (25°) overnight. After evaporating to dryness *in vacuo*, the residue was dissolved in 75 cc. of ether and washed successively with two 1-cc. portions of *N* hydrochloric acid, four 1-cc. portions of *N* sodium carbonate, and five 3-cc. portions of water. After drying over sodium sulfate and evaporating the ether, 19.5 mg. of a resinous residue was obtained which resisted attempts at crystallization, and was distilled in a high-vacuum. The temperature was gradually raised to about 200° and then briefly to 250°. The distillate was a sticky colorless resin. $[\alpha]_D^{25}$ +159.2° (9.9 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +1.19°).

Anal. Calc'd for $C_{24}H_{34}O_5$ (402.27): C, 71.60; H, 8.52.

Found: C, 71.50; H, 8.67.

The ultraviolet absorption curve (λ_{max}^{alc} 239 mμ; ϵ 15,465) is presented in the theoretical part.

Partial oxidation of ethyl 3β,5,19-trihydroxyetiocholanate (I) *with chromic acid. (cf. 1, pages 277–280). A. Ethyl 3β,5-dihydroxy-19-oxoetiocholanate* (XVI). The compound previously (1, p. 278) designated ethyl 3-keto-5,19-dihydroxyetiocholanate was subjected to treatment with Girard's Reagent as follows: To a solution of 14.9 mg. of material (melting points between 196 and 201°; no decomp.) in 0.5 cc. of methanol there was added 25.0 mg. of Girard's Reagent T followed by 0.02 cc. of glacial acetic acid. After refluxing on a water-bath for one hour and subsequent cooling to −5°, a small piece of ice and a solution of 15 mg. of sodium carbonate in 0.25 cc. of water was added followed by extraction with 5 cc. of ether in the cold. Washing the ether phase with water, drying with sodium sulfate,

and evaporating gave 2.7 mg. of crude non-ketonic material, which by crystallization from ether gave only 0.1 mg. of crystals melting between 198 and 210°. While still cold the original aqueous phase was made acid to Congo Red with 4 *N* sulfuric acid and allowed to stand at room temperature for a few hours. The acid phase was extracted with several 10-cc. portions of ether and the combined solutions washed with 0.5 cc. of *N* sodium carbonate and four 0.5-cc. portions of water. After drying with sodium sulfate and evaporating the ether, only 0.8 mg. of "ketonic" residue was obtained. On account of the unsatisfactory recovery, the acidified solution was not discarded but allowed to stand at room temperature. After nine days, long, fine needles had separated; yield, 3.3 mg.; m.p. 214–218°; no depression of the m.p. when mixed with material which had not been subjected to a Girard separation. By recrystallization from a large amount of ether short, fine needles melting at 210–216° were obtained. For infrared spectrum see theoretical part.

B. Compound $C_{22}H_{34}O_5$ [*Ethyl 3β,5-dihydroxy-19-oxoetiocholanate 19 → 3-lactol* (XVI)]? The compound previously (1, page 279) tentatively formulated $C_{24}H_{36}O_6$ or $C_{20}H_{30}O_5$ was subjected to purification with Girard's reagent as follows:[13] To a solution of 17.8 mg. of crystalline material (melting points between 165 and 172°; no decomp.) in 0.5 cc. of absolute ethyl alcohol was added 36 mg. of Girard's Reagent T, then 0.03 cc. of glacial acetic acid, and the mixture refluxed for one hour on a water-bath. After cooling to 0°, adding a small piece of ice and a solution of 25 mg. of sodium carbonate in ice-water, the mixture was extracted twice with 5-cc. portions of cold ether and the combined ether phases washed with 2 cc. of water. After drying with sodium sulfate and evaporating, 16.7 mg. of crystalline non-ketonic material was obtained. The combined aqueous phases were made acid to Congo Red with hydrochloric acid. After standing at room temperature for two hours, the mixture was extracted with four 10-cc. portions of ether. The combined ether extracts were washed with water, dried with sodium sulfate, and brought to dryness *in vacuo*; wt. of ketonic (?) material: 1.2 mg. The non-ketonic fraction (16.7 mg.) was recrystallized by dissolving it in 10 cc. of ether and concentrating to a volume of 3 cc. Feltlike mass of very fine needles; wt. 11.8 mg.; m.p. 172.5°. By further concentrating, an additional crop of similar needles was obtained: wt. 2.6 mg.; m.p. 165.5–170°. The crystalline residue weighed 2.0 mg. A solution of the purest material in chloroform gave no yellow color with tetranitromethane. $[\alpha]_D^{23}$ −4° (7.12 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α −0.02°).

Anal. Calc'd for $C_{22}H_{36}O_5$ (380.51): C, 69.44; H, 9.54. (Starting material)
$C_{22}H_{34}O_5$ (378.49): C, 69.81; H, 9.05.
$C_{21}H_{32}O_5$ (364.47): C, 69.20; H, 8.85.
Found: C, 69.36; H, 8.98.

Molecular Weight (Cryoscopic):
Wt. of solvent (camphor): 4.31 mg., wt. of sample: 0.392 mg., K = 40°; Δt = 9.4°, Mol. wt. 387.
Wt. of solvent (camphor): 2.36 mg., wt. of sample: 0.211 mg., K = 40°; Δt = 9.5°, Mol. wt. 376.

Benzoate (XVII):[13] To a solution of 9.7 mg. of the material in 1 cc. of dry pyridine was added 0.06 cc. of benzoyl chloride (tenfold of theoretical for two hydroxyl groups) and the mixture allowed to stand at room temperature for 48 hours. After the addition of ice to yield a total of about 5 cc. when melted, the mixture was acidified to Congo Red with 2.5 cc. of 6 *N* hydrochloric acid and subsequently extracted with four 5-cc. portions of ether. The combined ether extracts were washed twice with 5 cc. of 0.1 *N* hydrochloric acid, once with 5 cc. of water, twice with 2 cc. of 0.5 *N* sodium bicarbonate, and once more with 5 cc. of water. After drying with sodium sulfate and evaporation of the ether *in vacuo* 12.9 mg. of a crystalline residue was obtained. Recrystallization from ether-petroleum ether gave 9.3 mg. of very small, short needles, m.p. 154–155°; recrystallized by solution in 0.5 cc. of methanol to which was added 1 cc. of water; long, shining needles, yield, 8.6 mg., m.p. 155.5° sharply.

[13] Experiment performed by Dr. George W. Barber.

Anal. Calc'd for $C_{29}H_{40}O_6$ (484.61): C, 71.87; H, 8.32.
(Monobenzoate of cpd. $C_{22}H_{36}O_5$)
$C_{29}H_{38}O_6$ (482.59): C, 72.17; H, 7.94.
(Monobenzoate of cpd. $C_{22}H_{34}O_5$)
$C_{28}H_{36}O_6$ (468.47): C, 71.78; H, 7.75.
(Monobenzoate of cpd. $C_{21}H_{32}O_5$)
Found: C, 71.67; H, 7.90.
Molecular Weight (Cryoscopic):
Wt. of solvent (camphor): 5.27 mg., wt of sample: 0.456 mg.; K = 40°; Δt = 7.8°; Mol. wt. 444.
Wt. of solvent (camphor): 4.17 mg., wt. of sample: 0.354 mg.; K = 40°; Δt = 7.5°; Mol. wt. 453.

Ethyl 3β-acetoxy-5-hydroxy-19-oxoetiocholanate (XV). A total of 6.1 mg. of XIV (melting points between 202 and 216°), previously (1, p. 278) claimed to be ethyl 3-keto-5,19-dihydroxyetiocholanate, was dissolved in 0.1 cc. of pyridine. After the addition of 0.1 cc. of acetic anhydride the solution was allowed to stand at room temperature overnight and brought to dryness *in vacuo*. The residue was taken up in 50 cc. of ether and this solution washed with two 0.2-cc. portions of 2 *N* hydrochloric acid, two 0.2-cc. portions of *N* sodium carbonate, and five portions of small amounts of water. After drying with sodium sulfate and evaporating the ether, 7.1 mg. of a residue was obtained which was recrystallized from ether, a cluster of long thin needles (2.5 mg.); m.p. 136–137°.

3β-Acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (V) *by oxidation with chromic acid of* XV. To a solution of 6.8 mg. of XV (crystalline and noncrystalline material), as obtained in the preceding experiment, in 1.0 cc. of redist. glacial acetic acid there was added in four installments 1.61 mg. of chromium trioxide (1.5 equivalents) in 2.41 cc. of 95% acetic acid. After standing at room temperature for 17 hours, two drops of ethyl alcohol were added and the mixture brought to dryness *in vacuo*. The last traces of acetic acid were removed by repeatedly treating the residue with small amounts of alcohol and bringing to dryness. The residue was treated with 1.5 cc. of water and 15 cc. of ether. The ether phase was washed with 1.5 cc. of *N* sulfuric acid and four 1-cc. portions of water, and extracted with three 1-cc. portions of *N* sodium carbonate, followed by four washings with 0.5 cc. of water. After drying with sodium sulfate and evaporating, 4.6 mg. of crude, resinous neutral material was obtained. The combined carbonate phases including the subsequent aqueous washings were cooled with ice and made acid to Congo Red with 4 *N* sulfuric acid. Extraction with 50-cc., 40-cc., and 10-cc. portions of ether, washing the combined ether extracts five times with water, drying with sodium sulfate, and evaporating, yielded 2.4 mg. of acid material. By recrystallizing from ether-petroleum ether, 1.2 mg. of cubic crystals resulted; m.p. 190–194° (effervesc.). No depression of the m.p. when mixed with an authentic sample of V. From the mother liquors additional crystalline material could be isolated; m.p. 190–197° (effervesc.). On reoxidizing the neutral fraction (4.6 mg.), 2.6 mg. of additional acid material was obtained which resisted attempts at crystallization.

SECTION *II*

3β,5,14-Trihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (XVIII). The preparation from strophanthidin of 3β,5,14-trihydroxy-20-keto-14-isopregnane-19,21-dioic acid 21 → 14-lactone[3] was essentially performed as stated previously (5, p. 444).[14] In transforming the pure ketolactonecarboxylic acid into XVIII, the simplification by Butenandt and Gal-

[14] The intermediate in the preparation of this substance is strophanthidinic acid. The recently published method by Buzas and Reichstein (3) for the preparation of the acetate of strophanthidinic acid could not be utilized in the present instance, because optimal yields were apparently obtained only by transforming part of the reaction product into the methyl ester. For the present work it was essential to leave the angular carboxyl group intact.

lagher (14) was used which gave yields (pure, recrystallized material) ranging between 57.1 and 66.7% (5 expts.).

Dehydration of 3β,5,14-trihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (XVIII). This experiment was usually performed with approximately 3 g. of starting material. The reaction product was subjected to a crude separation. For purification analogous fractions resulting from several experiments were pooled.

A solution of 3.1087 g. of XVIII in 310 cc. of 0.101 *N* absolute alcoholic hydrogen chloride was distilled slowly at atmospheric pressure (bath temperature: 90–95°) down to approximately 40 cc. This required about two hours. After the addition of 45 cc. of water, the distillation was continued *in vacuo* (50°) until a turbidity began to appear. Subsequent heating on a water-bath gave a clear solution. On standing at room temperature overnight, a yellow-brown oil separated which became crumbly and finally powdery on kneading it with water. It was washed with water and dried in a vacuum desiccator; wt. (a): 1.0737 g. To the mother liquor was added water until the precipitate or turbidity was not further increased (total volume approx. 200 cc.). After some standing, the flocculent crystalline, white precipitate was filtered and dried; wt. (b): 1.5608 g. The mother liquor was concentrated *in vacuo* to a volume of about 50 cc. and allowed to cool. A white, mealy precipitate separated which was dried; wt. (c): 0.1162 g. The mother liquor was now extracted with eight 50-cc. portions of ether. After drying with sodium sulfate, the combined ether extracts were brought to dryness; white brittle foam; wt. (d): 0.1888 g. In a similar fashion the aqueous phase was extracted with three 50-cc. portions of ethyl acetate. After drying and evaporating the solvent, a white brittle foam was obtained; wt. (e): 0.0362 g. The aqueous phase was now taken completely to dryness which yielded a yellow resin; wt. (f): 0.0529 g. Total recovery: 3.0286 g.

For a further purification, the fractions resulting from three experiments (total starting material: 9.1237 g.) were combined in four groups as follows:

Precipitates of series (a):	4.0604 g.
Precipitates of series (b + c):	4.1353 g.
Ether and ethyl acetate extracts (d + e):	0.6766 g.
Final residues (f):	0.1096 g.
Total recovery:	8.9819 g.

3β,5-Dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid (XIX). A suspension of the precipitates of series (a) in 25 cc. of methanol was evaporated *in vacuo* leaving a brittle foam to which was added 20 cc. of boiling ethyl acetate. The resulting white powdery precipitate, (a) 1, was filtered after standing at room temperature overnight; wt. 1.0469 g. It was recrystallized by dissolving it in 50 cc. of methanol, concentrating to 20 cc. and adding to the hot solution 40 cc. of boiling water. Pale yellow needles, representing XIX; wt. 0.9460 g.; m.p. 262–263°.

In a like fashion a suspension of the precipitates of series (b + c) in 25 cc. of methanol was evaporated to dryness and the resulting brittle foam treated with 20 cc. of boiling ethyl acetate. The resulting white powdery precipitate, (b + c)1, was filtered the following day; wt. 1.6562 g. Recrystallization from aqueous methanol as in the preceding instance gave 1.5462 g. of white needles; m.p. 263–263.5°.

The material of series (d + e) was dissolved in 20 cc. of methanol and the solution evaporated to dryness *in vacuo*. To the resulting white brittle foam was added 20 cc. of boiling ethyl acetate. From the clear solution crystals, (d + e)1, formed slowly. They were filtered after three days and recrystallized from aqueous methanol; long, shining needles, wt. 0.0698 g.; m.p. 263.5°.

Titration:[15] Calc'd for $C_{20}H_{28}O_6$ (364.4): Neut. equiv., 182.2. Found: Neut. equiv., 202.

No crystalline material resulted from series (f).

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[15] The sample (in each case between 10 and 13 mg.) was dissolved in 5–10 cc. of neutralized ethanol and titrated to a phenolphthalein end point with 0.015 *N* sodium hydroxide. Estimated limit of error ±3.5%.

3β,5-Dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid 20-ethyl ester (XX). The ethyl acetate mother liquor of (a)1 (*vide supra*) was evaporated to dryness *in vacuo* and the resulting brittle foam taken up in 20 cc. of ether. After reducing this solution to about one-half of its volume, crystals separated after some standing. 1st crop: brownish prisms, wt. 1.3489 g.; m.p. 174–176°. 2nd crop: light yellow prisms, wt. 0.4771 g.; m.p. 173–174°. By diluting the filtrate with ether to about 10 cc. and adding 10 cc. of petroleum ether, additional crystalline material was obtained. 3rd crop: small, flat prisms, wt. 0.0248 g.; m.p. 171–174°. 4th crop: colorless prisms, wt. 0.0851 g.; m.p. 168–172°.

The ethyl acetate mother liquor of (b + c)1 (*vide supra*) was brought to dryness and the resulting brittle foam dissolved in 25 cc. of ether. After concentrating this solution to approx. 10 cc., crystals appeared slowly overnight. 1st crop: pale yellow prisms, wt. 1.5388 g.; m.p. 174–177°. 2nd crop: white prisms, wt. 0.4480 g.; m.p. 170–171°. Additional crystalline material was obtained from ether-petroleum ether (1:1). 3rd crop: large, colorless prisms, wt. 0.1538 g.; m.p. 168–172°.

An analytical sample was obtained by repeated recrystallization from aqueous ethanol; colorless prisms, m.p. 177°. $[\alpha]_D^{22.5}$ +116.2° (20.06 mg. in 2.0 cc. of absolute ethanol, *l*, 1.51 dm., α +1.76°).

Anal. Calc'd for $C_{22}H_{32}O_6$ (392.5): C, 67.32; H, 8.22; Neut. equiv., 392.5.

Found: C, 67.29, 67.36; H, 8.23, 8.26; Neut. equiv.,[15] 392.

The total yields, obtained in five dehydration experiments from 13.1237 g. of XVIII were: 4.3830 g. (35.1%) of XIX; 5.2882 g. (39.2%) of XX; and approx. 3.4 g. (25.7%) of non-crystalline material.

The combined non-crystalline material (3.4 g.) was subjected to a renewed dehydration experiment: The solution of the substance in 300 cc. of 0.1 *N* absolute alcoholic hydrogen chloride was allowed to stand at room temperature for 20 hours, refluxed on a steam-bath for one hour, and distilled at atmospheric pressure (steam-bath) to about 75 cc. (2 hours). The mixture was then brought to dryness *in vacuo*, yielding a yellow brittle foam which was treated with 100 cc. of *N* sodium bicarbonate and five 50-cc. portions of ether. The bicarbonate phase was extracted with four 50-cc. portions of ethyl acetate. From the bicarbonate phase was recovered 1.3894 g. of amorphous acid material which resisted attempts at crystallization.[16] The ether and ethyl acetate extracts were separately dried with sodium sulfate and evaporated *in vacuo*. Yield of neutral residues: (a) 0.6615 g. (brown brittle foam) and (b) 0.2048 g. (yellow brittle foam) respectively.

3β,5,8-Trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19 → 8-lactone 20-ethyl ester (XXII). The neutral residue from the ether extract (a) was dissolved in 5 cc. of ether. On adding 25 cc. of petroleum ether, a dark brown tar appeared which by kneading was converted into a brown powdery precipitate. On suspending this precipitate in 5 cc. of ether, only a part of it went into solution, leaving light brown granular crystals. On recrystallizing this crystalline material from acetone, several crops of needles were obtained. 1st crop: wt. 38.6 mg. (brown); m.p. 232–235°. 2nd crop: wt. 15.4 mg. (light brown); m.p. 231–233°. 3rd crop: wt. 13.8 mg.; m.p. 229–233°. Repeated crystallization of the combined material from acetone yielded 31.9 mg. of nearly colorless, short rods; m.p. 234–235°. $[\alpha]_D^{25}$ +35° (22.50 mg. in 2.0 cc. of chloroform, containing two drops of ethanol, *l*, 1.51 dm., α +0.60°).

Anal. Calc'd for $C_{22}H_{32}O_6$ (392.5): C, 67.32; H, 8.22.

Found: C, 67.40; H, 8.25. (Dried at 100°).

The non-crystalline part of the ether extract (a) was combined with the residue of the ethyl acetate extract (b) and the mixture chromatographed over 40 g. of alkali-free aluminum oxide[12] (diam. of column: 17 mm.). From two of the late eluates (190 cc. of ether + 10 cc. of methanol, 180 cc. of ether + 20 cc. of methanol) residues were obtained which on recrystallization from ether-petroleum ether and finally from acetone gave 18.7 mg. of

[16] By catalytic hydrogenation with PtO_2 in a solution of absolute ethanol this material could be converted into 0.9561 g. of fairly pure (m.p. 195–196°) 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (XXVII).

colorless needles; m.p. 223–225°. They were apparently identical (mixed m.p.) with the 19 → 8-lactone 20-ethyl ester (XXII) described above. In addition, there were isolated from the final fraction of the chromatogram (195 cc. of methanol + 5 cc. of glacial acetic acid) small amounts of the sodium salt of XX.

3β-Benzoxy-5,8-dihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19 → 8-lactone 20-ethyl ester (XXIII). To a solution of 22.4 mg. of pure XXII in 1 cc. of dry pyridine was added 0.13 cc. of redistilled benzoyl chloride and the mixture allowed to stand at room temperature for 44 hours. After adding some ice, the mixture was acidified to Congo Red with 1 cc. of 6 *N* hydrochloric acid and extracted with four 10-cc. portions of ethyl acetate (product not soluble in ether). The combined ethyl acetate extracts were washed with two 3-cc. portions of 2 *N* hydrochloric acid, 3 cc. of water, two 3-cc. portions of 0.5 *N* sodium bicarbonate, and 3 cc. of water. After drying with sodium sulfate, the solution was brought almost to dryness. On adding to the concentrate 1 cc. of ether, short needles formed; wt. 17.6 mg.; m.p. 230–231°. When mixed with the starting material (XXII), there was a depression of the m.p. (218–224°). The mother liquors yielded a second crop of crystalline material; wt. 3.0 mg., m.p. 228–229°. Recrystallization of the first crop from ethyl acetate-petroleum ether gave a feltlike mass of tiny needles; wt. 14.8 mg., m.p. 230–231°. $[\alpha]_D^{29.5}$ +48° (9.33 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +0.34°).

Anal. Calc'd for $C_{29}H_{36}O_7$ (496.56): C, 70.14; H, 7.30.
Found: C, 69.64; H, 7.26.

3β,5,8-Trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→ 8-lactone 20-methyl ester (XXV). The 3β,5,8-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→ 8-lactone (XXIV) from which this ester is derived was apparently obtained by Ehrenstein previously (*vide* theoretical part). This substance (40 mg., m.p. 275–282° decomp.) was dissolved in 5 cc. of acetone and the solution somewhat concentrated on a water bath. On adding an excess of an ethereal solution of diazomethane, crystals separated at once. After some standing, the excess diazomethane was removed on a water-bath. The crystals were washed with ether; wt. 16.5 mg., m.p. 230–234°. From the mother liquor 14.8 mg. of additional crystalline material, m.p. 229–232°, resulted. Both crops were recrystallized by adding ether to a concentrated solution in acetone; flat, long needles, m.p. 232–237°.

Anal.[17] Calc'd for $C_{22}H_{32}O_6$ (392.25): C, 67.30; H, 8.22. [Dimethyl ester]
$C_{21}H_{30}O_6$ (378.23): C, 66.64; H, 7.99. [Monomethyl ester]
Found: C, 66.29, 66.45; H, 8.03, 7.78.

3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (XXVI). This compound was obtained as described previously (5, 14) by hydrogenating XIX in glacial acetic acid in the presence of platinum oxide. The average yield of pure material (6 expts.) was 92%. The melting point of the purest sample was 255.5°. $[\alpha]_D^{22.5}$ +59° (19.52 mg. in 2.0 cc. of absolute ethanol, *l*, 1.51 dm., α +0.87°); lit. (14) $[\alpha]_D^{16}$ +35° (absolute ethanol), lit. (5) $[\alpha]_D^{20.5}$ +60.0°, $[\alpha]_D^{21.5}$ +64.5° (absolute ethanol).

Anal. Calc'd for $C_{20}H_{30}O_6$ (366.44): C, 65.55; H, 8.25.
Found: C, 65.53, 65.60; H, 8.37, 8.26.

3β,5-Dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (XXVII). *A. By catalytic hydrogenation of* XX. Reduction of 1.0717 g. of XX in a total of 27 cc. of 95% ethanol over 400 mg. of previously reduced platinum oxide resulted in the uptake of one equivalent of hydrogen in one hour (95% was absorbed within one-half hour). The combined hydrogenation products of three such experiments, (a white brittle foam, 3.6781 g. resulting from a total of 3.6094 g. of XX) were dissolved in 10 cc. of benzene. By adding to this solution petroleum ether dropwise at intervals, a turbidity was produced which was gradually transformed into a fine, white granular precipitate; dry wt. 3.6607 g., m.p. 191–193°. This material was recrystallized by dissolving it in 20 cc. of ethanol and gradually adding 30–35 cc. of boiling water. The addition of the water was continued at the rate of the conversion of the turbidity into slender needles. Yield: 3.2520 g., m.p. 200–201°. Con-

[17] Microanalysis (April 1939) by Dr. Ing. A. Schoeller, Berlin-Schmargendorf.

stant m.p. 201°. The substance is soluble in alcohols, acetone, ethyl acetate, chloroform, carbon tetrachloride, and benzene; practically insoluble in ether and petroleum ether. $[\alpha]_D^{22}$ +26° (18.05 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +0.35°). $[\alpha]_D^{29.5}$ +33° (28.87 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +0.72°).

Anal. Calc'd for $C_{22}H_{34}O_6$ (394.49): C, 66.98; H, 8.69; Neut. equiv., 394.49.
Found: C, 66.93, 67.05; H, 8.73, 8.66; Neut. equiv.,[15] 385.

B. By partial esterification of XXVI. In a preliminary experiment 100 mg. of XXVI in 10 cc. of 0.1 *N* absolute ethanolic hydrogen chloride was refluxed on a steam-bath for one hour and distilled at atmospheric pressure to approximately 5 cc. After adding 10 cc. of benzene, the mixture was again concentrated to the same volume and, because there was still a two-phase distillate, this treatment was repeated with 20 cc. of benzene. Crystals separated during the distillation; wt. 78.4 mg., m.p. 253–254° (starting material). The filtrate was brought to dryness; recrystallization of the residue (33.7 mg.) from benzene-petroleum ether gave 27.6 mg. of white granular crystals, m.p. 198–200°, which did not give a depression of the melting point when mixed with XXVII (*vide supra*).

In another experiment 100 mg. of XXVI in 10 cc. of 0.1 *N* absolute ethanolic hydrogen chloride was allowed to stand at room temperature for 16 hours, then refluxed on a steam-bath for 7 hours and finally concentrated at atmospheric pressure to 5 cc. This solution was subjected to distillations with benzene as described above. Only a very small amount of crystalline material precipitated from the final solution on standing overnight. After separating the crystals, the filtrate was brought to dryness; white brittle foam, wt. 89.1 mg.; recrystallization by dissolving in 5 cc. of hot ethanol and adding 10 cc. of boiling water. On cooling tiny, white needles separated, wt. 68.3 mg., m.p. 197–198°; no depression of m.p. when mixed with XXVII (*vide supra*).

Treatment of 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (XXVII) *with Raney nickel in the presence of cyclohexanone.* Fresh, active Raney nickel was prepared from 4.5 g. of nickel-aluminum alloy (16, 17); estimated yield: 1.8 g. A mixture containing the catalyst, 500.0 mg. of XXVII, 25 cc. of toluene, and 10 cc. of redistilled cyclohexanone was stirred and refluxed continuously for a period of 23 hours, bath temperature 130–140°. The solution was filtered from the catalyst and the latter washed with acetone. The combined filtrates were evaporated (water-pump, then oil-pump; bath temp. 65°), leaving about 3 cc. of a yellow liquid. This was dissolved in 80 cc. of ether and washed with 0.5 *N* sodium bicarbonate and 2 *N* sodium hydroxide. From the sodium bicarbonate phase there was recovered approx. 150 to 175 mg. of XXVII. From the sodium hydroxide phase there was obtained a fair amount of phenol and, in addition, about 40 mg. of unchanged XXVII. The total recovery of starting material in this experiment was between 40 and 50%.

Compound $C_{22}H_{28-32}O_5$. The remaining ether layer (neutral fraction) yielded 223.8 mg. of a yellow oil which was purified by chromatography. It was dissolved in a mixture of 15 cc. of benzene and 35 cc. of petroleum ether and the solution passed through a column (diam. 15 mm.) of 10 g. of alkali-free aluminum oxide.[12] Eluting (39 eluates) was performed with combinations of benzene-petroleum ether, benzene, benzene-ether, ether, and ether-methanol. Uniform crystalline material resulted only from four successive eluates (45 cc. of benzene + 5 cc. of ether, 40 cc. of benzene + 10 cc. of ether, 40 cc. of benzene + 10 cc. of ether, 35 cc. of benzene + 15 cc. of ether); total dry residue of these eluates: 55.2 mg. Recrystallization from ether-petroleum ether gave several fractions of granular crystals, melting between 122 and 134°; total: 19.2 mg. Renewed crystallization from ether-petroleum ether gave 15.5 mg. of m.p. 132–134°. Final recrystallization from benzene-petroleum ether gave 11.9 mg. of white needles; m.p. 137–138°. (After a few month's storage, the m.p. of this substance had risen to 142–143°). The substance gave no yellow color with tetranitromethane in chloroform. The ultraviolet absorption spectrum[18] was that of an α,β-unsaturated ketone (λ_{max}^{alc} 233 mμ; ϵ 21,360). The value for ϵ is based on a mol. wt. of 372.44.

[18] Determination by Messrs. Edward H. Unger and K. B. Streeter of the Sharp and Dohme Research Laboratories.

Specific rotation, $[\alpha]_D^{20}$ +96° (4.8 mg. in 2.0 cc. of chloroform; *l*, 1.51 dm., α +0.35° ± 0.03°)

Anal. Calc'd for $C_{22}H_{32}O_5$ (376.47): C, 70.18; H, 8.57.
$C_{22}H_{30}O_5$ (374.46): C, 70.56; H, 8.08.
$C_{22}H_{28}O_5$ (372.44): C, 70.94; H, 7.56.
Found: C, 70.02; H, 8.64.

Molecular Weight (Cryoscopic):
Wt. of solvent (camphor): 3.99 mg., wt. of sample: 0.350 mg.; K = 40°; Δt = 9.7°, mol. wt. 362.
Wt. of solvent (camphor): 2.11 mg., wt. of sample: 0.206 mg.; K = 40°; Δt = 10.6°, mol. wt. 368.

Ethyl 3-keto-14-iso-17-iso-19-nor-Δ^4-etiocholenate (XXX). The material from the mother liquors of the above crystalline product was combined with the residues from the benzene-petroleum ether and benzene eluates (omitting the first few benzene-petroleum ether eluates which contained cyclohexanone condensation products). Total, 63 mg. of resinous material which was dissolved in 20 cc. of benzene-petroleum ether (1:3) and rechromatographed on a column (diam. 10 mm.) of 10 g. of alkali-free alumina. Eluting (32 eluates) was performed as above. Crystallization from dilute methanol of the residues (total weight 15.0 mg.) from four consecutive eluates consisting of benzene-petroleum ether (4:1) and pure benzene gave crops of slowly forming needles with melting points between 72° and 82°. Recrystallization of the total of 7.9 mg. from dilute methanol gave 4.1 mg. of needles; m.p. 78–82°. There was no depression of the melting point on admixture with XXX obtained by simultaneous dehydration and decarboxylation of XXIX (*vide infra*).

In another experiment carried out exactly as described above, except for the absence of cyclohexanone, only unchanged starting material (XXVII) was recovered.

3-Keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (XXIX) *by oxidation with chromic acid*[19] *of* XXVII. To a solution of 150.0 mg. of XXVII in 9 cc. of redist. glacial acetic acid there was added at room temperature in three 5-cc. portions at half-hour intervals a solution of 27.8 mg. (10% excess) of chromium trioxide in 15 cc. of 95% acetic acid. The mixture was allowed to stand overnight and then brought to dryness *in vacuo* (20°). The green residue was evaporated several times with 5–10 cc. of ethanol. The resulting pale green brittle foam was taken up in 25 cc. of ethyl acetate and the solution washed with 5 cc. of *N* sulfuric acid and 5 cc. of water. The washings were re-extracted with ethyl acetate and the ethyl acetate layers were extracted twice with 5 cc. of *N* sodium carbonate and 5 cc. of water respectively. After drying with sodium sulfate, the ethyl acetate phase was brought to dryness, yielding 4.6 mg. of a neutral residue.[20] The combined carbonate phase and aqueous washings were cooled with ice and acidified to Congo Red with 2 cc. of 6 *N* hydrochloric acid. The white, gummy precipitate was extracted with five 10-cc. portions of ethyl acetate. After drying the combined extracts with sodium sulfate and evaporating the solvent, 144.6 mg. of a white brittle foam resulted. Recrystallization from ether-petroleum ether gave 116.5 mg. of clusters of small needles; m.p. 115–118°. Repeated recrystallization from ether-petroleum ether and acetone-ether finally yielded 44.1 mg. with the constant m.p. 133°. When the melting point was redetermined after several weeks of storage, it had risen to 143–144°. $[\alpha]_D^{20}$ +43.1° (20.90 mg. in 2.0 cc. of chloroform, *l*, 1.51 dm., α +0.68°).

Anal. Calc'd for $C_{22}H_{32}O_6$ (392.48): C, 67.32; H, 8.22.
Found: C, 67.30; H, 8.23.

Attempts at transforming 3-keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (XXIX) *into ethyl 3-keto-14-iso-17-iso-19-nor-Δ^4-etiocholenate* (XXX). *A. By refluxing with glacial acetic acid.* When pure (m.p. 133°) XXIX was refluxed with glacial acetic acid for one hour, it was practically completely converted into neutral matter.

[19] In another, preliminary, experiment the oxidation was attempted with N-bromoacetamide. The result was unsatisfactory.

[20] In attempting the oxidation with a 20% excess of chromic acid, an appreciable proportion of a neutral residue (about 25%) was obtained.

In using lower-melting material, an appreciable amount of an acid fraction was obtained which consisted essentially of XXVII. The neutral material secured by two experiments was combined (54.6 mg.) and chromatographed over 5 g. of alkali-free aluminum oxide[12] (diam. of column: 6 mm.). The major part of the material was contained in twelve consecutive eluates (benzene-petroleum ether combinations); attempts at crystallization failed. Hence these fractions were combined and the resinous residue (23.7 mg.) distilled in a high vacuum (b.p. about 225°). The distillate (18.3 mg.) was a colorless, sticky resin. Though the ultraviolet absorption curve[21] (λ_{max}^{alc} 239 mμ) is in agreement with that of an α,β-unsaturated ketone, the molecular extinction coefficients (ϵ_{max} 8621) are somewhat too low for the expected compound. Also the analysis indicated that the substance was not pure.

Anal. Calc'd for $C_{21}H_{30}O_3$ (330.45): C, 76.32; H, 9.15.
Found: C, 74.78; H, 9.17.

Additional resinous material (total: 8.8 mg.) was secured from the benzene-ether and ether eluates of the chromatogram. Also this product was distilled in a high-vacuum (b.p. about 250°). The oily distillate looked somewhat turbid (decomposition?).

Anal. Calc'd for $C_{21}H_{30}O_3$ (330.45): C, 76.32; H, 9.15.
Found: C, 75.47; H, 8.93.

B. By treatment with Girard's Reagent T. (a) at elevated temperature. To a solution of 25 mg. of XXIX (m.p. 133–134°) in 0.5 cc. of absolute ethanol was added 42.4 mg. of Girard's Reagent T and 0.03 cc. of glacial acetic acid. The mixture was refluxed on a steam-bath for one hour and then cooled in ice. After the addition of a small piece of ice and an ice-cold solution of 25 mg. of sodium carbonate in 1 cc. of water, the mixture was extracted with two 10-cc. portions of ether. The combined ether phases yielded after washing with 1 cc. of water, drying with sodium sulfate, and evaporation of the solvent, 2.6 mg. of a resinous neutral non-ketonic residue. The carbonate phase and aqueous washing were combined, acidified to Congo Red with five drops of 6 *N* hydrochloric acid, and allowed to stand at room temperature for two hours. After extracting with five 7-cc. portions of ether, the combined ether layers were washed twice with 2 cc. of cold *N* sodium bicarbonate and twice with 2 cc. of water. The bicarbonate phases and aqueous washings were combined, acidified to Congo Red, and the acid material isolated in the usual fashion; colorless resin; wt. 1.4 mg. The neutral ketonic ether extract was dried with sodium sulfate and yielded a nearly colorless resin; wt. 13.6 mg. Recrystallization from aqueous methanol gave long, thin needles and some yellow oil which was removed with a spatula; yield: 9.0 mg., m.p. 72–81°.

(b) at room temperature. To a solution of 28 mg. of XXIX (m.p. 133–134°) in 0.5 cc. of absolute ethanol was added 45 mg. of Girard's Reagent T and 0.03 cc. of glacial acetic acid. The mixture was warmed briefly to effect solution of Girard's reagent and then allowed to stand at room temperature for 18 hours. It was subsequently worked up as described under (a). Yields: Neutral non-ketonic: yellow resin, wt. 2.0 mg. Acid: resin, wt. 3.6 mg. Neutral ketonic: yellow resin, wt. 21.0 mg. The neutral ketonic fraction was repeatedly chromatographed over alkali-free aluminum oxide.[12] Two crystalline reaction products resulted. The main substance was obtained from the benzene-petroleum ether eluates. It was obviously identical with the crystalline product described under (a). Repeated recrystallization from aqueous methanol gave needles of m.p. 88.5°. This represented ethyl 3-keto-14-iso-17-iso-19-nor-Δ^4-etiocholenate (XXX). The ultraviolet absorption curve (λ_{max}^{alc} 239 mμ; ϵ 19,856) is presented in the theoretical part.

Anal. Calc'd for $C_{21}H_{30}O_3$ (330.45): C, 76.32; H, 9.15.
Found: C, 75.65; H, 8.98. (Dried at 45°.)

From the residues of the more polar eluates (benzene-ether combinations) there was isolated a small amount of a substance which crystallized from aqueous methanol in clusters of short needles; m.p. 142–143°. There was no depression of the melting point when this

[21] Determination by Mr. K. B. Streeter of the Sharp and Dohme Research Laboratories.

substance was mixed with compound $C_{22}H_{28-32}O_5$ obtained by the Raney nickel reaction (*vide supra*). The ultraviolet absorption spectrum[18] was that of an α,β-unsaturated ketone (λ_{max}^{alc} 235 mμ; ϵ 13,340). The value for ϵ is based on a mol. wt. of 372.44.

Anal. Calc'd for $C_{22}H_{32}O_5$ (376.47): C, 70.18; H, 8.57.
$C_{22}H_{30}O_5$ (374.46): C, 70.56; H, 8.08.
$C_{22}H_{28}O_5$ (372.44): C, 70.94; H, 7.58.
Found: C, 69.61; H, 8.46. (Dried at 45°.)

3-Keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (XXXI). To a solution of 50 mg. of XXVI in 5 cc. of glacial acetic acid there was added at 10-minute intervals in 1-cc. portions a solution of 10.1 mg. of chromium trioxide (10% excess) in 5 cc. of 95% acetic acid. The mixture was then allowed to stand for 22 hours. After the addition of five drops of methanol, the solution was brought to dryness in an oil-pump vacuum (20°). The residue was evaporated several times with ethanol and finally briefly dried in a vacuum desiccator over potassium hydroxide. It was dissolved in 25 cc. of ethyl acetate which was washed with three 2-cc. portions of ice-cold *N* sulfuric acid and three 3-cc. portions of ice-water. After drying with sodium sulfate and evaporating the solvent, 49.6 mg. of a white brittle foam resulted. Recrystallization from ethyl acetate-petroleum ether gave 36.7 mg. of slowly forming granular crystals. Inasmuch as this substance melts with decomposition, the observed melting point is rather dependent on the kind of heating. When allowed to heat from room temperature, the m.p. was 178–182°, but when heating was begun at 160°, the m.p. was 187–189°. The substance was recrystallized three times from ethyl acetate-petroleum ether; slowly forming, colorless prisms; wt. 21.3 mg.; m.p. 194° with foaming (heating started at 187°). $[\alpha]_D^{25}$ +71.5° (16.30 mg. in 2 cc. of absolute ethanol, *l*, 1.51 dm., α +0.88°).

Anal. Calc'd for $C_{20}H_{28}O_6$ (364.42): C, 65.91; H, 7.75.
Found: C, 66.02; H, 7.71. (Dried at 50°.)

SUMMARY

Investigationss have been performed to transform strophanthidin into substances structurally related to steroid hormones. Compounds with normal (Section *I*) as well as iso configurations (Section *II*) at carbon atoms 14 and 17 have been prepared.

I

1. Improvements in the conversion of ethyl 3β,5,19-trihydroxyetiocholanate (I) (1, 2) through the intermediates II, III, and IV into 3β-acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (V) (1) have been described. Ethanolysis of V yielded 3β,5-dihydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (VI) which was transformed by oxidation with chromic acid into 3-keto-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (VII). An attempt to convert I into VII by direct oxidation gave unsatisfactory results. The significance of V and VII as possible intermediates in the preparation of 19-nor compounds (IX and VIII respectively) has been discussed.

2. Ethyl 3β,5-dihydroxy-19-tritoxyetiocholanate (II) as obtained by tritylation of I was oxidized with N-bromoacetamide to ethyl 3-keto-5-hydroxy-19-tritoxyetiocholanate (X). By cleavage of the ether linkage, ethyl 3-keto-5,19-dihydroxyetiocholanate (XI) was obtained. On treating XI with Girard's Reagent T, dehydration occurred leading to ethyl 3-keto-19-hydroxy-Δ^4-etiocholenate (XII). The primary alcohol group at carbon atom 10 is inert to oxidation with

N-bromoacetamide. Thus I can be directely oxidized to XI. Purification of the crude reaction product XI by means of Girard's Reagent T yielded the α,β-unsaturated ketone XII which was characterized by its acetate (XIII).

3. Evidence is presented showing that a partial oxidation product of ethyl $3\beta,5,19$-trihydroxyetiocholanate (I), previously (1) considered to be ethyl 3-keto-5,19-dihydroxyetiocholanate, actually is ethyl $3\beta,5$-dihydroxy-19-oxo-etiocholanate (XIV). Oxidation with chromic acid of the corresponding acetyl derivative (XV) gave the known 3β-acetoxy-5-hydroxy-21-norpregnane-19,20-dioic acid 20-ethyl ester (V) (1). Another previously (1) described partial oxidation product of I was found to be nonketonic and yielding a monobenzoate. The structures of ethyl $3\beta,5$-dihydroxy-19-oxoetiocholanate 19 $\rightarrow$ 3-lactol (XVI) and its benzoate (XVII) are tentatively considered.

II

1. $3\beta,5,14$-Trihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (XVIII) was prepared from strophanthidin as described earlier (5) with minor modifications.

2. Treatment of XVIII with ethanolic hydrogen chloride yielded as main products the previously (5, 14) reported $3\beta,5$-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid (XIX) and, in addition, $3\beta,5$-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid 20-ethyl ester (XX). To a neutral by-product was assigned the structure of $3\beta,5,8$-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19$\rightarrow$ 8-lactone 20-ethyl ester (XXII). It was characterized by the monobenzoate (XXIII). The mechanism of formation of compounds XX and XXII is discussed.

3. A previously described (5) isomer of XIX, obtained from XVIII by the action of sulfuric acid in dioxane, probably has the structure of $3\beta,5,8$-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19$\rightarrow$ 8-lactone (XXIV). It was characterized by the monomethyl ester (XXV).

4. Catalytic hydrogenation of XIX gave the known $3\beta,5$-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (XXVI) (5, 14). Oxidation with chromic acid of XXVI (14) gave improved yields of 3-keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (XXXI).

5. Catalytic hydrogenation of XX gave $3\beta,5$-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (XXVII). The latter was also obtained by partial esterification of XXVI.

6. Treatment of XXVII with Raney nickel in the presence of cyclohexanone (1, 15, 17) yielded an appreciable amount of starting material. In addition small amounts of an α,β-unsaturated ketone $C_{22}H_{28-32}O_5$ and ethyl 3-keto-14-iso-17-iso-19-nor-Δ^4-etiocholenate (XXX) were obtained.

7. By oxidation with chromic acid, XXVII was transformed into 3-keto-5-hydroxy-14-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (XXIX). Treatment of XXIX with Girard's Reagent T was accompanied by simultaneous dehydration and decarboxylation leading to ethyl 3-keto-14-iso-17-iso-19-nor-Δ^4-etiocholenate (XXX).

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