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

INVESTIGATIONS ON STEROIDS. XV. FURTHER STUDIES ON 17-ISOCARBOXYLIC ACIDS[1]

G. WINSTON BARBER AND MAXIMILIAN EHRENSTEIN

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The degradation of strophanthidin to 3β,5,14-trihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid (I) has been reported in several publications (1–4). This compound can be subjected to a selective dehydration in such a fashion that only the 14-hydroxyl group is removed. Thus, treatment of I with 0.1 *N* absolute alcoholic hydrogen chloride yielded mainly 3β,5-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid (II) (2–4) and the corresponding 20-ethyl ester (III) (4). In addition, there was isolated a very small amount of a neutral substance to which was assigned the structure of 3β,5,8-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone 20-ethyl ester (IV) (4).

The mode of formation of the latter compound (IV) and the corresponding lactone-acid (V) has been studied and it has now been found that Δ^{14}-unsaturated 10-carboxylic acids can be smoothly converted into the saturated 19→8-lactones by treatment with concentrated hydrochloric acid at room temperature. Thus, 3β,5-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid 20-ethyl ester (III) was converted in excellent yield into IV. In a like fashion, II was transformed in good yield into 3β,5,8-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone (V).[2] The latter compound (V) was also obtained on saponification of IV with potassium hydroxide in ethanol at room temperature. The saponification of an ester of a 17α-carboxylic acid under such mild conditions might be indicative of the iso-configuration at carbon atom 14 (*cf.* 6).

It was reported earlier (3, 4) that treatment of I with sulfuric acid in dioxane solution yielded a substance, m.p. 275–282° (decomp.), tentatively assigned structural formula V. The correctness of this assumption has now been proven, for the methyl ester by which this product was characterized (4) has been found to be identical with that prepared from the lactone-carboxylic acid V which is obtained by treatment of II with hydrochloric acid.

The structures proposed for IV, V, and VI are supported by the failure of these substances to give any color on treatment with tetranitromethane in chloroform, whereas II and III give definite yellow colors. The reasons for suggesting structures involving a 19→8 rather than a 19→14-lactone ring have been discussed

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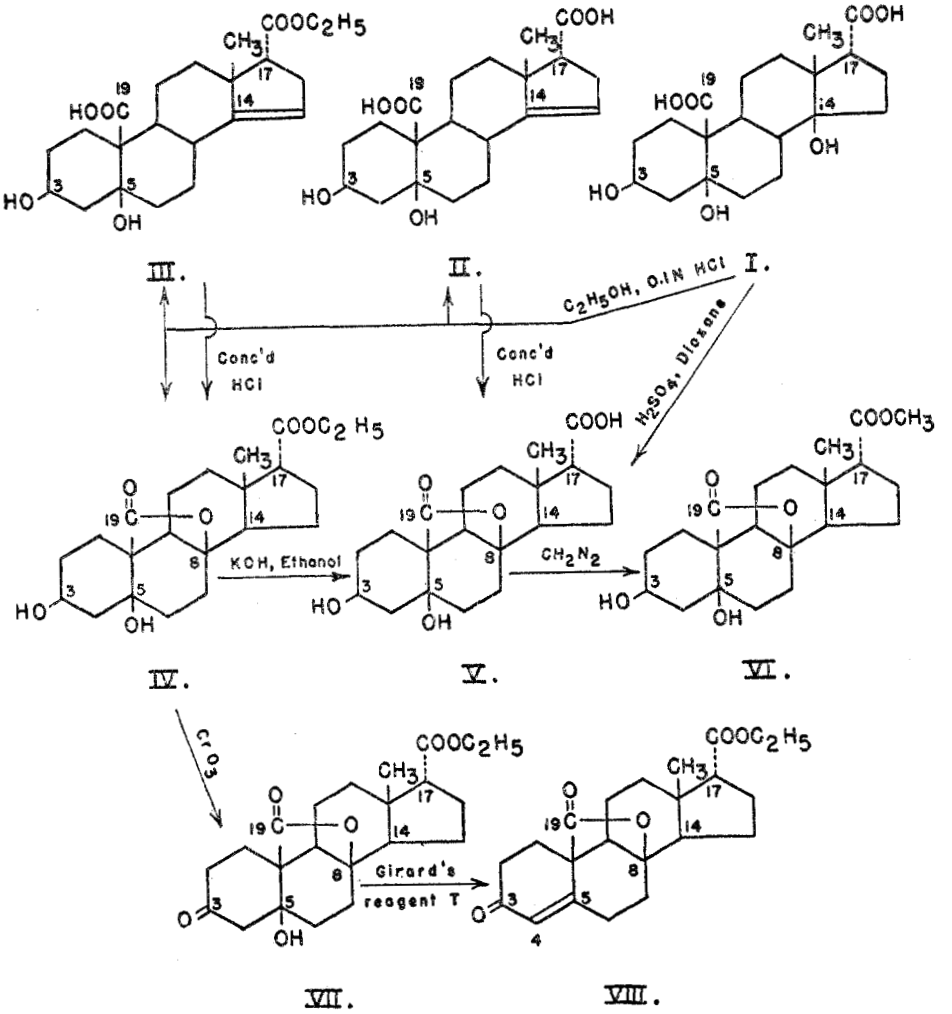
[2] Preliminary experiments to effect in a similar fashion the cyclization of ethyl 3β,5,19-trihydroxy-Δ^{14}-etiocholenate to ethyl 3β,5-dihydroxy-8,19-oxidoetiocholanate (*cf.* 5, p. 336) have not been successful.

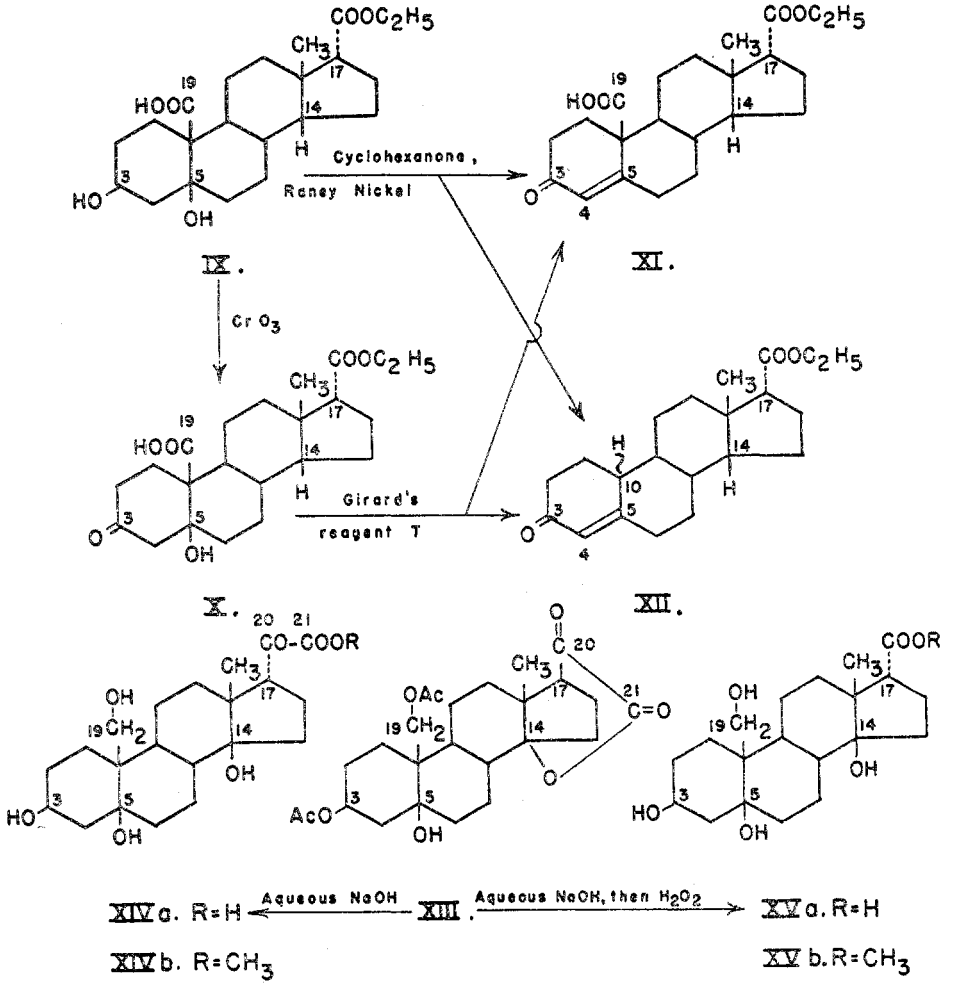
(4, 5, 7). That the lactone ring does not involve the hydroxyl groups at the 3- and 5-positions has been demonstrated by oxidation of IV with chromic acid. The resulting 3-keto-5-hydroxy compound (VII) was treated with Girard's reagent T which, in addition to separating the ketonic material, simultaneously effected dehydration (*cf.* 4, 16) to give 3-keto-8-hydroxy-17-iso-21-nor-Δ^4-pregnene-19,20-



dioic acid 19→8-lactone 20-ethyl ester (VIII). The absorption of VIII in the ultraviolet is characteristic of a Δ^4-3-keto steroid.

As was mentioned previously (4), 3β,5-dihydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (IX), as obtained by catalytic hydrogenation of III, can be oxidized with chromic acid to the corresponding 3-keto compound (X). The latter, on treatment with Girard's reagent T, was simultaneously dehydrated and decarboxylated to ethyl 3-keto-14-iso-17-iso-19-nor-Δ^4-etiocholen-

ate (XII). Another product of this reaction was likewise an α,β-unsaturated ketone of the empirical formula $C_{22}H_{28-32}O_5$ (4), which was believed to be neutral. It has now been established by potenticmetric titration that actually this latter compound is the very weak monocarboxylic acid, 3-keto-14-iso-17-iso-21-nor-Δ^4-pregnene-19,20-dioic acid 20-ethyl ester (XI). The same two products (XI



XIV a. R=H ← Aqueous NaOH — XIII. — Aqueous NaOH, then H_2O_2 → XV a. R=H

XIV b. R=CH_3 XV b. R=CH_3

and XII) were obtained in very small yields from the reaction of IX with cyclohexanone in the presence of Raney nickel (4).

The oxidation of strophanthidol diacetate with potassium permanganate in acetone has been discussed in several publications (8–10). The acid reaction product is 3β,19-diacetoxy-5,14-dihydroxy-14-isoetiocholanic acid. The neutral fraction resulting from the oxidation consists partly of unchanged starting

material. It can be subjected to oxidation again without previous purification, thus furnishing more of the etio acid. With each re-oxidation, however, the neutral fraction becomes more resistant to further oxidation. Recrystallization of neutral material which had been subjected to repeated oxidations yielded 3β,19-diacetoxy-5,14-dihydroxy-20-keto-14-isopregnane-21-oic acid 21→14-lactone (XIII). Ketolactones of this type have been obtained in a number of oxidations of digitaloid aglycones (lit., *cf.* 11) and of structurally related steroids (*cf. e.g.*, 12, 13). Treatment of XIII with aqueous alkali resulted in simultaneous saponification of the acetoxy groups and of the lactone ring. As is known from analogous instances (lit. *cf.* 11), saponification of this type of lactone is accompanied by inversion of the configuration at carbon atom 17, which prevents re-lactonization. The resulting 3β,5,14,19-tetrahydroxy-20-keto-14-iso-17-isopregnane-21-oic acid (XIVa) was characterized by the methyl ester (XIVb). By treating the ketolactone XIII with aqueous alkali and then, without isolating XIVa, oxidizing with hydrogen peroxide (*cf.* 2), 3β,5,14,19-tetrahydroxy-14-iso-17-isoetiocholanic acid (XVa) was obtained and was characterized by the methyl ester (XVb).

EXPERIMENTAL

The melting points were determined with the Fisher-Johns melting-point apparatus. The readings are sufficiently near the true melting points so that no corrections have been made. Unless stated otherwise, the microanalyses were carried out by Dr. E. W. D. Huffman, Denver 2, Colorado, on samples which were dried *in vacuo* over phosphorus pentoxide at 80–90°.

3β,5,8-Trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone 20-ethyl ester (IV) *from 3β,5-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid 20-ethyl ester* (III). Compound III (100 mg.) was suspended in 2 cc. of conc'd hydrochloric acid. On standing overnight the material went slowly into solution. The following day some ice and water were added to the clear colorless solution to make 40 cc. Very minute crystals formed slowly on standing at room temperature overnight. They were filtered, dissolved in 30 cc. of ethyl acetate, and the solution (A) was washed acid-free with *N* sodium bicarbonate and water. The original filtrate was extracted with five 25-cc. portions of ethyl acetate and the combined extracts (B) washed with water. After drying with sodium sulfate both solutions were evaporated to dryness, leaving the white crystalline residues A (58.5 mg.) and B (35.2 mg.). On treatment with ether each residue gave colorless prisms: yield from A: 54.1 mg., m.p. 232–237°; yield from B: 29.7 mg., m.p. 233–238°. Neither sample gave any depression of the melting point upon admixture with authentic IV (4).

3β,5,8-Trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone (V). *A. From 3β,5-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid* (II). A suspension of 17.3 mg. of II in 1 cc. of conc'd hydrochloric acid was kept at room temperature overnight. The following day ice and water were added to the resulting clear, pale yellow solution to make 10 cc. After two more days' standing only a trace of crystalline material had appeared. The reaction mixture was extracted with five 10-cc. portions of ethyl acetate and the combined extracts washed with 5 cc. of water. After drying with sodium sulfate and evaporation of the solvent, 16.3 mg. of a white crystalline residue resulted which was recrystallized from acetone-ether to give 12.3 mg. of small colorless prisms; constant m.p. 305–307° (decomp.). $[\alpha]_D^{25}$ + 68° (7.80 mg. in 2.0 cc. of absol. ethanol; *l*, 1.51 dm.; α + 0.40° ± 0.04°).

B. By saponification of 3β,5,8-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone 20-ethyl ester (IV). Compound IV (28.45 mg.) dissolved in 4.00 cc. of 0.0807 *N* potassium hydroxide in 80% ethanol was kept at room temperature 18 hours, diluted to 10

cc. (ethanol), and titrated (1-cc. aliquots); about 2/3 equivalent of base had been consumed. The remaining solution, evaporated to dryness *in vacuo*, taken up in water (10 cc.), and extracted with ethyl acetate, gave 4.7 mg. of starting material. Acidification of the aqueous solution to Congo Red with 6 *N* HCl, extraction with ethyl acetate, washing with water, and drying (sodium sulfate) gave 17.8 mg. of acidic material. Recrystallized from acetone-ether, this gave 14.5 mg. of colorless prisms, m.p. 305–307° (d.); no depression on admixture with the product obtained in A.

3β,5,8-Trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone 20-methyl ester (VI). This compound was prepared from 7.8 mg. of V by the use of diazomethane. Yield, 9.6 mg. crude, 5.3 mg. of long, thin needles after recrystallization from acetone-ether; m.p. 239° undepressed on admixture with the substance prepared differently, previously (4, p. 369).

Anal. Calc'd for $C_{21}H_{30}O_6$ (378.45): C, 66.64; H, 7.99.
Found: C, 66.51; H, 8.03.

3-Keto-8-hydroxy-17-iso-21-nor-Δ^4-pregnene-19,20-dioic acid 19→8-lactone 20-ethyl ester (VIII). To 18.7 mg. of IV dissolved in 1 cc. of glacial acetic acid, a solution of 3.82 mg. of chromium trioxide (20% excess) in 2 cc. of 95% acetic acid was added in two equal portions 1/2 hour apart. The mixture was kept at room temperature for 20 hours. After the addition of five drops of methanol, the reaction mixture was frozen and the solvent was removed by evacuation on an oil-pump (temp. below 0°). The residue was dissolved in 20 cc. of ethyl acetate and the solution was washed free of chromium with *N* sulfuric acid and water. After drying over sodium sulfate and evaporation of the solvent 21.4 mg. of white brittle foam resulted which was dissolved in 0.5 cc. of absolute ethanol. To this was added 40 mg. of Girard's reagent T and subsequently 0.03 cc. of glacial acetic acid. The mixture was warmed very briefly to effect solution and was then kept at room temperature for 18 hours. Addition of a small piece of ice and an ice-cold solution of 25 mg. of sodium carbonate in 5 cc. of water yielded a turbid aqueous solution which was extracted successively with 5 cc. of ether (aqueous layer still turbid), 5 cc. of ethyl acetate, and once more with 5 cc. of ether. Washing the combined ether-ethyl acetate extracts with 5 cc. of ice-water, drying over sodium sulfate and evaporating gave 4.5 mg. of resinous non-ketonic material which was discarded. The combined aqueous layers were acidified to Congo Red with 2 cc. of 6 *N* hydrochloric acid and, after standing at room temperature for one hour, were extracted with ether. The ether extract was washed with water, dried over sodium sulfate, and evaporated to dryness to give 15.5 mg. of resinous ketonic material. Careful recrystallization from methanol-water gave 9.5 mg. of glistening platelets, m.p. 124–126°. By repeated recrystallization the m.p. was raised to 133°. The ultraviolet absorption spectrum was that of an α,β-unsaturated ketone (λ_{max}^{alc} 243.5 mμ; ε 13,130.$[\alpha]_D^{29}$ + 87° (5.63 mg. in 2.0 cc. of chloroform; *l*, 1.51 dm.; α +0.37° ± 0.02°).

Anal. Calc'd for $C_{22}H_{28}O_5$ (372.44): C, 70.94; H, 7.58.
Found: C, 70.84; H, 7.80. (Dried at 75°)

3β,19-Diacetoxy-5,14-dihydroxy-20-keto-14-isopregnane-21-oic acid 21→14-lactone (XIII). Strophanthidol diacetate was oxidized with potassium permanganate in acetone solution, and the neutral part of the reaction product was subjected to a renewed oxidation. The resulting neutral fraction was oxidized a third time. With each successive oxidation the yield of acid material decreased (*e.g.*, 52.7%, 40.0%, 15%), and the neutral fraction became more refractory to further oxidation (8, 10). The neutral material obtained after the third oxidation, 1.96 g. in the form of a brittle, white foam, was recrystallized from acetone-ether and yielded 0.53 g. of a crystalline product,[3] m.p. between 155° and 167°, which gave a completely negative Legal test (14). This was reacetylated with 2 cc. of acetic anhydride in 4 cc. of dry pyridine at room temperature overnight. The reaction mixture was brought to dryness *in vacuo* (3 mm.) at 0°, the residue was taken up in ethyl acetate, and the solution was washed with cold *N* hydrochloric acid and water. After drying over sodium sulfate

[3] Isolated by Dr. Helmut C. Neuman.

and evaporation of the solvent 0.53 g. of white, brittle foam resulted. Crystallization from acetone-ether gave 462 mg. of pellet-shaped clusters of very small needles. Repeated recrystallization yielded 422 mg. with the constant m.p. 169–170°, resolidification at about 175° and remelting at 208–209°. $[\alpha]_D^{27}$ −16° (10.32 mg. in 2.0 cc. of chloroform; *l*, 2 dm.; α −0.17° ± 0.04°).

Anal. Calc'd for $C_{25}H_{34}O_8$ (462.52): C, 64.92; H, 7.41.

Found: C, 65.07; H, 7.49.

3β,5,14,19-Tetrahydroxy-20-keto-14-iso-17-isopregnane-21-oic acid (XIVa). The ketolactone XIII (49.5 mg.) in 1.00 cc. of 0.548 *N* aqueous sodium hydroxide was heated on a steam-bath for 30 minutes. After standing at room temperature overnight the solution required 0.22 cc. of 1.05 *N* sulfuric acid for neutralization (about three equivalents of sodium hydroxide consumed). An additional 1 cc. of 1.05 *N* sulfuric acid gave crystals which were filtered, washed with ice-water, and dried; yield 38.8 mg. Repeated crystallization from methanol-ether gave 20.1 mg. of fine granular crystals, m.p. 247–248° (decomp.). The compound was too insoluble in any of the usual solvents to permit the determination of the optical rotation.

Anal. Calc'd for $C_{21}H_{32}O_7$ (396.47): C, 63.61; H, 8.14.

Found: C, 63.36; H, 8.33.

Methyl ester (XIVb). When 10.2 mg. of the acid (XIVa) was treated with diazomethane and the product recrystallized from acetone-petroleum ether it gave 6.2 mg. of clusters of needles; m.p. 110–113°.

3β,5,14,19-Tetrahydroxy-14-iso-17-isoetiocholanic acid (XVa). A suspension of 206 mg. of the ketolactone XIII in 4.00 cc. of 0.548 *N* aqueous sodium hydroxide was warmed on a steam-bath until the crystals had dissolved, held at room temperature overnight, and diluted with 12 cc. of water. Then 2 cc. of 30% hydrogen peroxide was added, which initiated a slow gas evolution and a gradual fading of the color. The solution was kept at room temperature for 24 hours and then made barely acid to litmus with 1.20 cc. of 1.05 *N* sulfuric acid. This caused the beginning of crystallization. After five more days an additional 1.20 cc. of 1.05 *N* sulfuric acid was added to make the mixture just acid to Congo Red. The crystalline precipitate was filtered after standing in the cold overnight; wt. 134.4 mg. (82%); m.p. 252–255°. Recrystallization from acetone-petroleum ether gave 119.7 mg. of granular crystals; constant m.p. 259–261°. When the determination of the m.p. is begun above 170°, the sample melts immediately, promptly resolidifies and finally melts as before at 259–261°. This behavior is presumably caused by the sudden release of crystal solvent. $[\alpha]_D^{20}$ +3° (8.96 mg. in 2.0 cc. of absol. ethanol; *l*, 2 dm.; α + 0.03° ± 0.02°). For analysis, a sample was dried *in vacuo* over P_2O_5 at 100°, and the special drying and handling procedure of Milner and Sherman (15) was essential.

Anal. Calc'd for $C_{20}H_{32}O_6$ (368.46): C, 65.19; H, 8.76.

Found: C, 64.88; H, 8.84.

Methyl ester (XVb). When 20.0 mg. of the acid (XVa) was methylated with diazomethane, and the product was recrystallized from acetone-ether, there was formed 20.0 mg. of long, fine needles; m.p. 225–226°.

SUMMARY

1. The facile conversion of 3β,5-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid (II) into 3β,5,8-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone (V) and of 3β,5-dihydroxy-17-iso-21-nor-Δ^{14}-pregnene-19,20-dioic acid 20-ethyl ester (III) into 3β,5,8-trihydroxy-17-iso-21-norpregnane-19,20-dioic acid 19→8-lactone 20-ethyl ester (IV) have been demonstrated. The latter compound (IV) has been converted into 3-keto-8-hydroxy-17-iso-21-nor-Δ^4-pregnene-19,20-dioic acid 19→8-lactone 20-ethyl ester (VIII).

2. A product with the empirical formula $C_{22}H_{28-32}O_5$, obtained by subjecting 3β,5-dihydroxy-14-iso-17-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (IX) to reaction with cyclohexanone in the presence of Raney nickel or by treating 3-keto-5-hydroxy-14-iso-17-iso-21-norpregnane-19,20-dioic acid 20-ethyl ester (X) with Girard's reagent T, has been recognized as 3-keto-14-iso-17-iso-21-nor-Δ^4-pregnene-19,20-dioic acid 20-ethyl ester (XI).

3. A neutral oxidation product of strophanthidol 3,19-diacetate has been identified as 3β,19-diacetoxy-5,14-dihydroxy-20-keto-14-isopregnane-21-oic acid 21→14-lactone (XIII) which in turn was transformed into 3β,5,14,19-tetrahydroxy-20-keto-14-iso-17-isopregnane-21-oic acid (XIVa) and 3β,5,14,19-tetrahydroxy-14-iso-17-iso-etiocholanic acid (XVa).

PHILADELPHIA 4, PA.

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