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

# INVESTIGATIONS ON STEROIDS. XIX. FURTHER DEGRADATION PRODUCTS OF STROPHANTHIDOL[1]*

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In attempts to prepare analogs of steroid hormones having the angular carbon atom 19 present in an oxygenated form, strophanthidol (I) was selected as a suitable and easily accessible starting material. In an earlier publication (1) the oxidation in acetone solution of strophanthidol diacetate (Ia) with potassium permanganate was described. This yielded mainly an acid, non-crystalline, reaction product which upon deacetylating gave the crystalline 3$\beta$,5,14,19-tetrahydroxy-14-isoetiocholanic acid (II). This acid has served as an intermediate for subsequent transformations (2–6).

As has been shown in numerous instances by Reichstein and his associates (Lit. *cf.* 7, p. 90), cardiac aglycones may be degraded with ozone to glyoxylates which in turn can be hydrolyzed to ketols of the type of the adrenal cortical steroids. It was decided to apply this degradation to strophanthidol diacetate (Ia) in order to examine the products as possible useful intermediates in the synthesis of analogs of steroid hormones.

Ozonization of Ia and reductive cleavage of the resulting ozonide yielded 3$\beta$,19-diacetoxy-21-glyoxyloxy-14-isopregnane 5,14-diol-20-one (III). This substance resisted crystallization, even after chromatography. Treatment of compounds of this type with aqueous methanolic potassium bicarbonate is reported (Lit. *cf.* 7, p. 90) to produce invariably a partial saponification to a free ketol in the side chain while the acetoxy group at carbon atom 3 is retained. In the present case (III) no uniform major reaction product was isolated. The evidence presented in the experimental section suggests that the reaction product was a mixture of 14-isopregnane-3$\beta$,5,14,19,21-pentol-20-one (IV) and 3$\beta$-acetoxy-14-isopregnane-5,14,19,21-tetrol-20-one (V). This view is supported by observations made in this laboratory [2, p. 284 (c)] that, under these experimental conditions, compounds having acetoxy groups at $C_{19}$ and at $C_3$ are rather easily deacetylated in the 19- and in the 3-position. All attempts to obtain the com-

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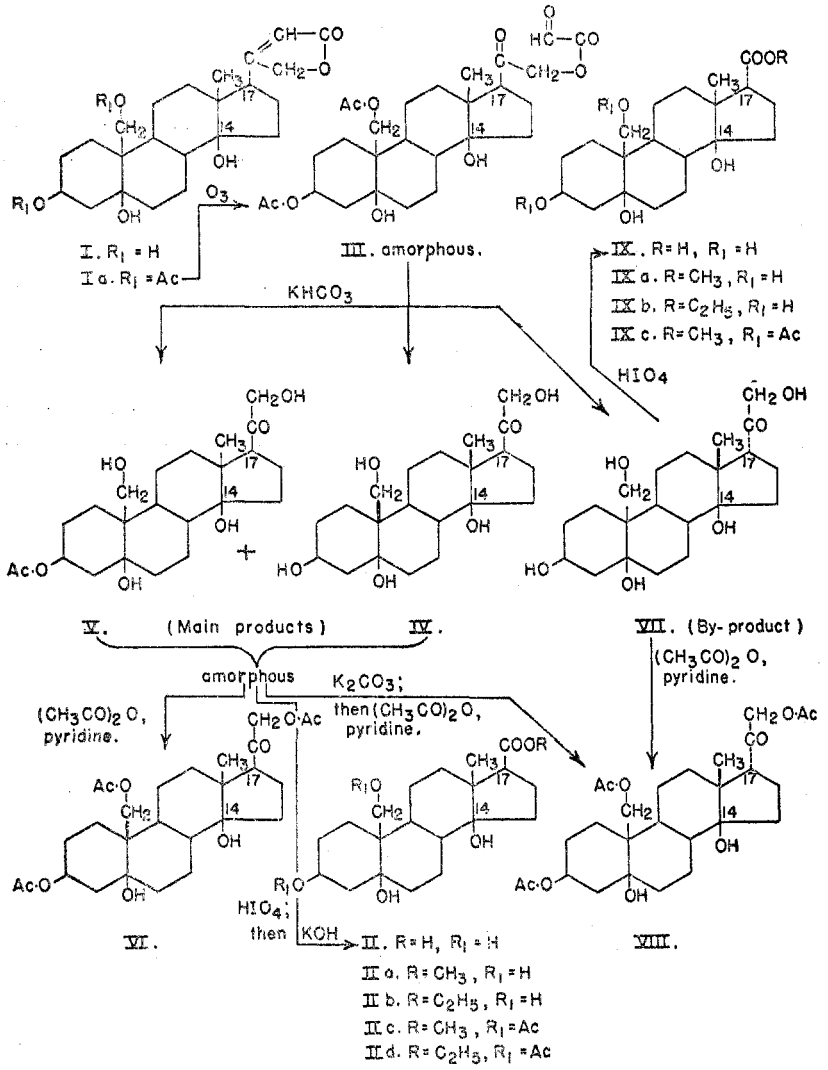
[2] Predoctoral Fellow (1949–1951) of the American Cancer Society, Inc., upon recommendation of the Committee on Growth of the National Research Council. This paper is based on a section of a thesis submitted by Charles P. Balant to the Graduate School of Arts and Sciences of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Physiological Chemistry (June 1952).

pletely saponified ketol IV in a crystalline form failed. It is interesting to note, however, that in one experiment where chromatographically-purified III[3] was hydrolyzed by this method, about 25% of a pure crystalline compound was isolated and recognized as 14-iso-17-isopregnane-3$\beta$,5,14,19,21-pentol-20-one




(VII). It was characterized by preparation of the crystalline acetylation product 3$\beta$,19,21-triacetoxy-14-iso-17-isopregnane-5,14-diol-20-one (VIII), and by conversion to the previously (8) described 3$\beta$,5,14,19-tetrahydroxy-14-iso-17-iso-

[3] The possibility of some inversion at $C_{17}$ under the influence of alumina has to be considered, though it appears unlikely.

etiocholanic acid (IX) by oxidation with periodic acid. Further characterization of IX was made by the hitherto unknown ethyl ester IXb.

As stated, saponification of III with aqueous methanolic potassium bicarbonate probably yielded mainly a mixture of IV and V. In agreement with this view, acetylation of the reaction product gave a satisfactory yield of the crystalline 3β,19,21-triacetoxy-14-isopregnane-5,14-diol-20-one (VI). Furthermore, on oxidizing the mixture with periodic acid and subsequently saponifying with methanolic potassium hydroxide, the known (1) 3β,5,14,19-tetrahydroxy-14-isoetiocholanic acid (II) resulted.[3a] With diazoethane the latter was converted to the ethyl ester IIb, previously (1) obtained only as a by-product when II was treated with ethanolic hydrogen chloride. Acetylation of IIb gave ethyl 3β,19-diacetoxy-5,14-dihydroxy-14-isoetiocholanate (IId).

The formation of the 17-iso compound VII from III under the mild conditions employed was unexpected.[4] It was of interest to determine whether potassium carbonate would effect a more rapid and complete epimerization. For this purpose, the material which had been pretreated with potassium bicarbonate was reacted with potassium carbonate in aqueous dioxane-methanol. This yielded besides a sizable acid fraction[5] mainly neutral material from which VIII was isolated as the only crystalline product after acetylation and chromatographic purification.

The question arose whether ozonization of free strophanthidol (I) would lead to 21-glyoxyloxy-14-isopregnane-3β,5,14,19-tetrol-20-one (X) and after hydrolysis with aqueous methanolic potassium bicarbonate to IV and thus facilitate the isolation of a single substance. Actually no crystalline material was isolated after hydrolysis. Acetylation of the crude reaction product yielded VI by direct crystallization. Chromatography of the mother liquor gave additional VI and a small amount of VIII. These products must have arisen from IV and VII respectively. This demonstrated that hydrolysis of X with aqueous methanolic potassium bicarbonate was associated with some inversion at carbon atom 17.

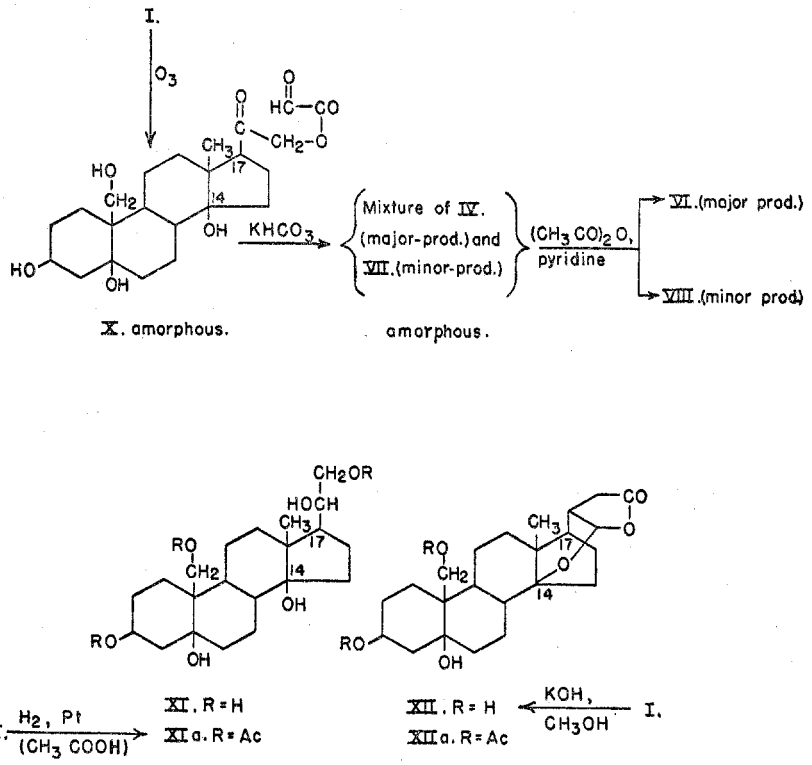
In an orienting experiment, not recorded in the experimental section, crude III was treated with potassium hydroxide in methanol at room temperature in the presence of air. As is known (11, 12), ketol esters of the series of the adrenal cortical hormones (*i.e.* having normal configurations at carbon atoms 14 and 17) under these conditions are almost instantaneously and quantitatively oxidized to the corresponding etio acids. The question arose whether the oxidation of III in alkaline medium would proceed at a much faster rate than the epimerization at carbon atom 17, thus forming exclusively the etio acid with normal configuration at $C_{17}$ (II). In effect, approximately 80% of the resulting

[3a] During the course of these investigations J. Schmutz (16, p. 1451) also reported the transformation of Ia into II by way of the amorphous III.

[4] With the substituent at $C_{14}$ in the iso-position, as in the present instance, epimerization at $C_{17}$ to the thermodynamically more stable iso-configuration is favored. This rearrangement is generally achieved by refluxing in the presence of caustic alkali or of mineral acids. For discussions of these aspects *cf. e.g.* (9, p. 1979; 10, p. 1520).

[5] The acid material probably results from the oxidation in the alkaline medium of the ketol side chain by the oxygen of the air (*cf.* 11, 12).

material was acid and was chromatographically purified in the form of the acetylated methyl esters. Both methyl 3β,19-diacetoxy-5,14-dihydroxy-14-iso-etiocholanate (IIc) and methyl 3β,19-diacetoxy-5,14-dihydroxy-14-iso-17-iso-etiocholanate (IXc) were isolated in about equal amounts, the latter being eluted before the former. The total yield of IIc and IXc represented about 25% of the acid material. The esters IIc and IXc had not been described before. For comparison purposes they were prepared by acetylating the authentic methyl esters IIa and IXa. The orienting experiment indicated that inversion at $C_{17}$ in the presence of caustic alkali proceeds at a very rapid rate. It will be investigated whether: (a) the inversion of the configuration of the ketol side




chain can be made quantitative prior to oxidation; (b) whether the 17-iso ketol can then be oxidized to the isoetio acid as the exclusive product.

In accordance with expectations, the 17-iso compounds VIII, IX (8), IXa (8), IXb, and IXc are more levorotatory than the respective 17-normal compounds VI, II (1), IIa (1), IIb (1), and IIc.

In view of the tendency for inversion at carbon atom 17, compounds with a free or esterified ketol side chain may be too labile to serve as intermediates for further transformations. Such difficulties are not expected with compounds having a hydroxyl at carbon atom 20 instead of an oxo group. Bearing in mind the possible usefulness of such a reduced compound, the easily accessible tri-

acetate VI was hydrogenated over platinum in glacial acetic acid. This gave a uniform crystalline hydrogenation product in about 50% yield, considered to be 3β,19,21-triacetoxy-14-isopregnane-5,14,20β-triol (XIa) with the tentative designation of the $C_{20}$-hydroxyl group in consideration of present views on the stereochemical course of the catalytic hydrogenation of a $C_{20}$-keto group (Lit. *cf.* 13). The non-crystalline portion of the hydrogenation product was oxidized with chromic acid, and reconverted into the crystalline VI. Saponification of the crystalline XIa with aqueous methanolic potassium carbonate yielded the crystalline 14-isopregnane-3β,5,14,19,20β,21-hexol (XI).

For comparison purposes isostrophanthidol (XII) was needed. It was obtained in the usual fashion (Lit. *cf.* 7) by the action of methanolic potassium hydroxide upon strophanthidol (I). It was characterized by the 3,19-diacetate (XIIa).

## 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°.

*Preparation of crude 3β,19-diacetoxy-21-glyoxyloxy-14-isopregnane-5,14-diol-20-one* (III) *by ozonization of strophanthidol diacetate* (Ia). Through a solution of 3 g. of Ia, m.p. 186.5–187.5°, in 150 cc. of dry ethyl acetate was passed at −70° a stream of oxygen containing approx. 4% of ozone for 40 minutes (approx. 10% excess). After keeping the deep blue-colored solution at −70° for an additional 30 minutes, the solvent was removed *in vacuo* at room temperature. The sticky ozonide was dissolved in 60 cc. of glacial acetic acid and zinc dust was added in small portions with shaking until a negative starch-iodide test was obtained (total duration: approx. 1½ hours). After filtering, the reaction mixture was brought to dryness *in vacuo*. The residue was dissolved in chloroform which was washed to neutrality at 0° with 5% sodium carbonate and with water. After drying over sodium sulfate and removal of the solvent *in vacuo*, 3.036 g. of resin was obtained which resisted all attempts at crystallization. From the carbonate phase there was isolated in the usual fashion 106.3 mg. of resinous acidic material.

*Hydrolysis of crude 3β,19-diacetoxy-21-glyoxyloxy-14-isopregnane-5,14-diol-20-one* (III) *with potassium bicarbonate in aqueous methanol.* To 3.036 g. of crude resinous III (preced. expt.) in 120 cc. of redistilled methanol was added 3 g. of potassium bicarbonate in 60 cc. of water. The mixture was kept at room temperature for 18 hours and the methanol was then removed under reduced pressure. The residual solution was extracted repeatedly with a mixture of chloroform-ethanol (3:1) and the extract was washed once with water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. Yield: 2.6417 g. of resin which resisted all attempts at crystallization. It consisted probably mainly of a mixture of 14-isopregnane-3β,5,14,19,21-pentol-20-one (IV) and 3β-acetoxy-14-isopregnane-5,14,19,21-tetrol-20-one (V).

*3β,19,21-Triacetoxy-14-isopregnane-5,14-diol-20-one* (VI). One gram of the crude product (probably a mixture of IV and V) obtained by hydrolysis with potassium bicarbonate of III (*vide supra*) was dissolved in 10 cc. of dry pyridine. After the addition of 6 cc. of acetic anhydride and keeping the mixture at room temperature for 21 hours, the major part of the reagents was removed *in vacuo* (room temperature). The semicrystalline residue was dissolved in ethyl acetate and the solution was washed to neutrality with *N* hydrochloric acid, *N* sodium bicarbonate, and with water. After drying over sodium sulfate and evaporation of the solvent *in vacuo*, 1.029 g. of resin was obtained which crystallized from a concentrated

solution in acetone after the slow addition of some ether and petroleum ether. Total yield (3 crops): 584.1 mg. of needles with melting points between 190.5 and 194°. By chromatographic (alumina) purification of the material contained in the mother liquors, an additional 49.1 mg. (eluted with benzene-ether, 1:1, and with ether alone) of crystalline fractions, m.p. 188–189.5°, was obtained. The product which had been obtained by direct crystallization (584.1 mg.) appeared uniform upon subjecting it to chromatographic purification. Hence all crystalline fractions obtained in this experiment represented the same compound (VI). Prior to analysis the material was once more recrystallized from acetone-petroleum ether; m.p. 188.5–189.5° $[\alpha]_D^{27}$ +71.7° (23.7 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +1.70°).

*Anal.* Calc'd for $C_{27}H_{40}O_9$ (508.59): C, 63.76; H, 7.93.

Found: C, 63.72, 63.82; H, 8.07, 8.01.

*3β,5,14,19-Tetrahydroxy-14-isoetiocholanic acid* (II). One gram of the crude product (probably a mixture of IV and V) obtained by hydrolysis with potassium bicarbonate of III (*vide supra*) was dissolved in 15 cc. of redistilled dioxane. To this was added 1.75 g. of periodic acid ($HIO_4 \cdot 2H_2O$) in 5 cc. of water. After keeping this mixture at room temperature for three hours, the dioxane was removed *in vacuo* and, after the addition of some water, the residue was extracted six times with large quantities of chloroform. The combined chloroform extracts were washed repeatedly with 2 *N* sodium carbonate and with water. The chloroform phase yielded, after drying over sodium sulfate, only 34.3 mg. of resinous neutral material.

The carbonate phase was made acidic to Congo Red by the addition of conc'd hydrochloric acid in the cold and was then extracted seven times with chloroform. The combined extracts were washed with a solution of sodium thiosulfate and with water. After drying over sodium sulfate and evaporating the solvent, 679.7 mg. of resinous acid material was obtained which did not crystallize. It was completely deacetylated by overnight treatment at room temperature with 13 cc. of 1.85 *N* methanolic potassium hydroxide (approx. 4 equiv. of KOH). The excess of potassium hydroxide was then neutralized partially by the addition of glacial acetic acid and the methanol was evaporated under reduced pressure. Extracting the residue with chloroform gave only 25.8 mg. of resinous neutral material. After adding some water, the residual solution was made acidic to Congo Red by the addition of conc'd hydrochloric acid in the cold and was then extracted seven times with chloroform. The combined extracts were washed with water, dried over sodium sulfate, and evaporated to dryness. Yield: 365.8 mg. of resin which was crystallized from acetone-ether; the crystallization was completed by the addition of some petroleum ether. Yield of crystalline material: 305.7 mg.; melting points ranging from 214 to 217.5°. There was no depression of the m.p. when mixed with an authentic sample of II (1).

*Ethyl 3β,5,14,19-tetrahydroxy-14-isoetiocholanate* (IIb). To a suspension of 75 mg. of II in 5 cc. of acetone was added an ethereal solution of diazoethane[6] until the deep yellow color persisted. After the instantaneous reaction, the mixture was kept at room temperature for ten minutes and was then brought to dryness; wt. of foamy residue: 89.5 mg. This was dissolved in the minimum amount of warm acetone, to which was subsequently added ether and petroleum ether. This gave clusters of needles; 1st crop, 67.5 mg., m.p. 184.5–186°; 2nd crop, 13.9 mg., m.p. 173–176°. Constant melting point after further recrystallization: 188–188.5°. $[\alpha]_D^{26}$ +42.3° (13.6 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +0.58°); lit (1) m.p. 186–188.5° $[\alpha]_D^{23.5}$ +45.7° (chloroform).

*Ethyl 3β,19-diacetoxy-5,14-dihydroxy-14-isoetiocholanate* (IId). To 25.6 mg. of IIb in 0.6 cc. of dry pyridine was added 0.3 cc. of acetic anhydride and the solution was kept at room temperature for 22 hours. Evaporation of the mixture to dryness *in vacuo* (room temperature) gave a crystalline residue which was triturated with water and filtered. After drying, it was recrystallized from acetone to which some ether and much petroleum ether

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

was added. Total yield: 24.2 mg. with melting points between 139 and 142.5°. After renewed recrystallization the m.p. was 140.5–141°. $[\alpha]_D^{20}$ +52.3° (11.3 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +0.59°).

*Anal.* Calc'd for $C_{26}H_{40}O_5$ (480.58): C, 64.98; H, 8.39.

Found:[7] C, 64.90; H, 8.42.

*3β,19,21-Triacetoxy-14-isopregnane-5,14,20β-triol* (XIa). A solution of 950 mg. of VI in 30 cc. of redistilled glacial acetic acid was added to a suspension of 100 mg. of previously-reduced platinum oxide in 5 cc. of glacial acetic acid. The mixture was shaken under hydrogen for a period of eight hours. The total absorption of hydrogen, practically complete after five hours, was 54.7 cc.; calc'd for VI under the experimental conditions: 46.4 cc. The solvent was removed *in vacuo* (room temp.), the residue was taken up in 250 cc. of ethyl acetate and the solution was washed to neutrality with 0.5 *N* sodium bicarbonate and with water. After drying over sodium sulfate and evaporation of the solvent 932.4 mg. of colorless resin was obtained. This material was dissolved in a little warm acetone and ether was added to the solution, followed by petroleum ether. A total of 358.0 mg. of colorless needles was obtained, m.p. 131.5–132.5°. Recrystallization from acetone-petroleum ether gave material which showed a double melting point: 104–105°, resolidifying around 110°, remelting at 134–135°. Purification of the mother liquor by chromatography over aluminum oxide gave 136.8 mg. of additional material with somewhat lower melting points (eluted with mixtures of ether-chloroform, the ratios ranging from 7:3 to 5:95). Recrystallization of these chromatographic fractions from acetone-petroleum ether gave 87.7 mg. of crystals with the same double melting point as recorded above, $[\alpha]_D^{25.5}$ +24.7° (20.7 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +0.51°).

*Anal.* Calc'd for $C_{27}H_{42}O_9$ (510.61): C, 63.51; H, 8.29.

Found: C, 63.06; H, 8.25.

A sample of 75 mg. of non-crystalline material resulting from the mother liquors of crystalline chromatographic fractions was oxidized in glacial acetic acid with chromium trioxide in the usual fashion. Recrystallization of the crude reaction product gave 28.0 mg. of needles, m.p. 194–195°; identified as VI (mixture m.p.).

*14-Isopregnane-3β,5,14,19,20β,21-hexol* (XI). To 143 mg. of XIa in 5 cc. of methanol was added 150 mg. of potassium carbonate in 1.5 cc. of water and the mixture was kept at room temperature for 17 hours. After evaporating to dryness, the residue was dissolved in water and was extracted seven times with chloroform-ethanol (3:1). The extract was washed once with water, dried over sodium sulfate, and evaporated to dryness. Yield of crude material: 91.8 mg. This was dissolved in a small volume of warm methanol to which were gradually added ether and finally petroleum ether. A total of 65.8 mg. of crystals was obtained, m.p. 241–243.5°. By four recrystallizations from the same solvents the melting point was raised to 248–248.5°. $[\alpha]_D^{22.5}$ +17.0° (15.36 mg. in 2 cc. of ethanol; l, 2 dm.; $\alpha$ +0.26°).

*Anal.* Calc'd for $C_{21}H_{36}O_6$ (384.50): C, 65.59; H, 9.44.

Found: C, 65.47; H, 9.49 (Dried at 100°).

*14-Iso-17-isopregnane-3β,5,14,19,21-pentol-20-one* (VII) *from crude 3β,19-diacetoxy-21-glyoxyloxy-14-isopregnane-5,14-diol-20-one* (III). Crude III was subjected to two successive chromatographic purifications over alkali-free alumina. A total of 108.4 mg. of resinous material, eluted by benzene-ether and pure ether, was hydrolyzed by dissolving in 5 cc. of methanol to which was added 100 mg. of potassium bicarbonate in 2.5 cc. of water. After keeping the mixture at room temperature for 22 hours, the methanol was removed *in vacuo* and the aqueous phase was extracted repeatedly with chloroform, followed by ethyl acetate. After washing with water and drying over sodium sulfate, evaporation of the solvents *in vacuo* gave a white, partly crystalline residue. This was separated into 51.5 mg. of crystals and 13.2 mg. of resin. Recrystallization of the crystalline material from dioxane-ether gave a total of 20.7 mg.; m.p. 238.5–241.5°. Crystallization of the resin in a like fashion gave 5.5 mg. of crystals with the same m.p. Additional recrystallization from methanol gave hexag-

[7] As a precautionary measure special drying (15) was performed though it proved unnecessary.

onal crystals melting at 243.5–244.5°. $[\alpha]_D^{23}$ −25.1° (10.35 mg. in 2.0 cc. of ethanol; l, 2 dm.; $\alpha$ −0.26°).

*Anal.* Calc'd for $C_{21}H_{34}O_6$ (382.48): C, 65.94; H, 8.96.

Found: C, 66.27, 66.10; H, 8.54, 8.55 (Dried at 100°).

*3β,19,21-Triacetoxy-14-iso-17-isopregnane-5,14-diol-20-one* (VIII). *A. From pure 14-iso-17-isopregnane-3β,5,14,19,21-pentol-20-one* (VII). To 20 mg. of VII (*vide* preced. expt.) in 0.5 cc. of pyridine was added 0.25 cc. of acetic anhydride and the mixture was kept at room temperature for 22 hours. Evaporation to dryness *in vacuo* (room temp.) gave a crystalline residue which was triturated with water, filtered, and dried. Recrystallization from acetone-petroleum ether gave 21.1 mg. of clusters of thin needles, m.p. 220–222.5°. $[\alpha]_D^{28}$ +6.6° (17.8 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +0.12°).

*B. From the amorphous product obtained by hydrolysis with potassium bicarbonate of crude 3β,19-diacetoxy-21-glyoxyloxy-14-isopregnane-5,14-diol-20-one* (III). To 600 mg. of the amorphous product (probably a mixture of IV and V; *vide supra*) in a mixture of 200 cc. of methanol and 10 cc. of dioxane was added in two equal portions, 2½ hours apart, a solution of 500 mg. of potassium carbonate in 10 cc. of water. After keeping the mixture at room temperature overnight, the solvents were removed under reduced pressure and, after the addition of some water, the reaction mixture was repeatedly extracted with chloroform-ethanol 3:1 and with chloroform-ethanol 2:1. After drying the combined extracts over sodium sulfate and evaporating to dryness, 378.6 mg. of resin (neutral) was obtained which resisted all attempts at crystallization. The carbonate phase, upon acidifying and extracting with ethyl acetate, yielded 133.0 mg. of resin (acid) which was soluble in *N* sodium carbonate.

The neutral fraction (378.6 mg.) was treated with 5 cc. of dry pyridine and 3 cc. of acetic anhydride for 24 hours. After evaporating to dryness, the remaining syrup was dissolved in ethyl acetate which was washed with *N* hydrochloric acid, *N* sodium bicarbonate, and with water. After drying over sodium sulfate and removal of the solvent, 375.6 mg. of resin resulted. This was dissolved in a mixture of 40 cc. of benzene and 10 cc. of petroleum ether, and was chromatographed over alkali-free alumina (16 g.; diam. of column: 8 mm.). Elution with mixtures of benzene-ether of various proportions, with ether alone and with mixtures of ether-chloroform 9:1 and 4:1 gave a total of 139.8 mg. of resinous fractions. On dissolving them in a small volume of acetone and adding petroleum ether, a total of 45.6 mg. of clusters of thin needles with melting points between 212 and 229.5° was obtained. After recrystallizing the combined material, the m.p. was 221.5–223.5°. There was no depression of the m.p. upon admixture with a sample of VIII as obtained by acetylation of crystalline VII (see preced. expt.) $[\alpha]_D^{27}$ +7.4° (20.3 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +0.15°).

*Anal.* Calc'd for $C_{27}H_{40}O_9$ (508.59): C, 63.76; H, 7.93.

Found: C, 63.78; H, 7.61.

*Preparation of crude 21-glyoxyloxy-14-isopregnane-3β,5,14,19-tetrol-20-one* (X) *by degradation with ozone of strophanthidol* (I). Through a solution of 1.750 g. of I in 85 cc. of methanol was passed at −70° a stream of oxygen containing approx. 4% of ozone for a period of 20 minutes (approx. 10% excess). After keeping the blue solution at −70° for an additional 20 minutes, the solvent was removed *in vacuo* (room temp.). The residue was dissolved in glacial acetic acid and zinc dust was added in small portions with shaking until a starch-iodide test was negative. After filtering, the solution was brought to dryness *in vacuo* and the residue was dissolved in chloroform which was washed successively with water, with a 5% solution of sodium carbonate, and again with water. After drying over sodium sulfate and removal of the solvent 1.1468 g. of resin (*i.e.* crude X) was obtained. Acidification of the carbonate phase and subsequently extracting it in the usual fashion gave 416.7 mg. of resinous acidic material.

*Hydrolysis of crude 21-glyoxyloxy-14-isopregnane-3β,5,14,19-tetrol-20-one* (X) *in aqueous methanol in the presence of potassium bicarbonate.* To 1.1468 g. of crude resinous X (preced. expt.) in 50 cc. of methanol was added 1.250 g. of potassium bicarbonate in 25 cc. of water and the mixture was kept at room temperature for 20 hours. After removal of the methanol *in vacuo*, the reaction mixture was extracted successively (a) six times with chloroform and

(b) six times with chloroform-ethanol 3:1. After washing with water, the extracts were dried separately over sodium sulfate and were evaporated to dryness. Yields: (a) 887.7 mg. and (b) 160.7 mg. of resin which could not be made to crystallize. On the basis of the subsequent acetylation experiment, the major fraction (a) contained, besides unidentified material a larger proportion of 14-isopregnane-3β,5,14,19,21-pentol-20-one (IV) and a smaller proportion of 14-iso-17-isopregnane-3β,5,14,19,21-pentol-20-one (VII).

*Acetylation of the reaction product obtained by potassium bicarbonate hydrolysis of crude 21-glyoxyloxy-14-isopregnane-3β,5,14,19-tetrol-20-one* (X): *3β,19,21-triacetoxy-14-isopregnane-5,14-diol-20-one* (VI) *and 3β,19,21-triacetoxy-14-iso-17-isopregnane-5,14-diol-20-one* (VIII). To 887.7 mg. of starting material [residue (a) of preced. expt., containing IV and VII] in 9 cc. of dry pyridine was added 5 cc. of acetic anhydride and the mixture was kept at room temperature for 24 hours. After removal of the solvents *in vacuo*, the residue was dissolved in 250 cc. of ethyl acetate which was washed to neutrality, dried over sodium sulfate, and evaporated to dryness; yield: 1.007 g. of resin. Upon dissolving it in a small volume of acetone and adding some ether and much petroleum ether, 169.5 mg. of crystalline material, m.p. after recrystallizing 193.5–194°, was obtained which was identified as 3β,19,21-triacetoxy-14-isopregnane-5,14-diol-20-one (VI) (mixture m.p.).

From the mother liquors there was obtained 886.2 mg. of resin which was dissolved in a mixture of 25 cc. of benzene and 50 cc. of petroleum ether and was chromatographed over alkali-free alumina (35 g.; diam. of column: 19 mm.). Fractions 1 to 9 (eluted with various combinations of benzene-ether, with ether, and with ether-chloroform 14:1) yielded a total of 376.1 mg. of material. Fractions 4 to 6 (containing 137.7 mg.) were crystallized from ether-petroleum ether, yielding 30.1 mg. of crystals, of m.p. 219–224°. Recrystallization from acetone-petroleum ether gave 23.3 mg. of m.p. 221–223.5°. This was identified as 3β,19,21-triacetoxy-14-iso-17-isopregnane-5,14-diol-20-one (VIII) by determination of the mixture m.p. Fractions 7 to 9 (containing 104.7 mg.) yielded crystalline material which was recognized as a mixture of VI and VIII. Fractions 10 and 11 (eluted with ether-chloroform 1:1, 1:3) gave 270.5 mg. of material which upon recrystallization from acetone-petroleum ether furnished 201.0 mg. of needles, melting points between 187 and 193.5°; identified as additional 3β,19,21-triacetoxy-14-isopregnane-5,14-diol-20-one (VI) (mixture m.p.).

*3β,5,14,19-Tetrahydroxy-14-iso-17-isoetiocholanic acid* (IX) *and ethyl 3β,5,14,19-tetrahydroxy-14-iso-17-isoetiocholanate* (IXb) *from 14-iso-17-isopregnane-3β,5,14,19,21-pentol-20-one* (VII). To 13.3 mg. of VII in 3 cc. of methanol was added 25 mg. of periodic acid ($HIO_4 \cdot 2H_2O$) in 0.8 cc. of water and the mixture was then kept at room temperature for 3 hours. On removing the methanol *in vacuo*, crystals separated which were washed with water and dried; wt. 9.4 mg. Recrystallization from acetone gave 7.2 mg.; m.p. 247–248.5°. There was no depression of the m.p. when mixed with an authentic sample of IX (8).

A solution of 9.4 mg. of IX (obtained from VII as presented above) in acetone was treated with ethereal diazoethane.[6] This yielded 11.0 mg. of crude ester which upon crystallization from acetone-petroleum ether gave 8.1 mg. of small needles, m.p. 228–229.5°. No depression of m.p. when mixed with an authentic sample of IXb (*vide* subseq. expt.).

*Ethyl 3β,5,14,19-tetrahydroxy-14-iso-17-isoetiocholanate* (IXb) *from 3β,5,14,19-tetrahydroxy-14-iso-17-isoetiocholanic acid* (IX). To 15 mg. of authentic IX (8) in acetone was added a solution of diazoethane[6] in ether until the yellow color persisted. After keeping the mixture at room temperature for 10 minutes, it was evaporated to dryness and the residue was recrystallized from acetone-ether, to which finally petroleum ether was added. Yield: 12.7 mg. of needles; m.p. 231–232.5°. $[\alpha]_D^{28}$ +8.9° (7.9 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +0.07°).

*Anal.* Calc'd for $C_{22}H_{36}O_6$ (396.51): C, 66.64; H, 9.15.

Found:[8] C, 66.24; H, 9.17.

*Methyl 3β,19-diacetoxy-5,14-dihydroxy-14-isoetiocholanate* (IIc). A solution of 50 mg. of methyl 3β,5,14,19-tetrahydroxy-14-isoetiocholanate (IIa) (1) in 1 cc. of dry pyridine was treated with 0.5 cc. of acetic anhydride at room temperature for 20 hours and the reaction

[8] Special drying (15) was essential.

product was isolated in the usual fashion. The crude material (64.4 mg.) was dissolved in a little ether and petroleum ether was added to the solution until it became turbid. On seeding with a sample which had been obtained in another experiment after chromatography (*vide* theoretical part), a total of 34.5 mg. of crystals of m.p. 103–104.5° was obtained; m.p. after recrystallization: 105–105.5°. $[\alpha]_D^{27}$ +57.0° (20.2 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +1.15°).

*Anal.* Calc'd for $C_{25}H_{38}O_8$ (466.55): C, 64.36; H, 8.21.

Found:[7] C, 64.28; H, 8.26.

*Methyl 3β,19-diacetoxy-5,14-dihydroxy-14-iso-17-isoetiocholanate* (IXc). To 15 mg. of IXa (8) in 0.5 cc. of dry pyridine was added 0.2 cc. of acetic anhydride and the mixture was kept at room temperature for 20 hours. The reaction product was isolated in the usual fashion. Crystallization from ether-petroleum ether gave 14.0 mg. of clusters of thin needles; m.p. 69–77.5°, resolidifying at 81°, remelting at 178–179°. $[\alpha]_D^{23}$ +1.8° (13.1 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +0.02°).

*Anal.* Calc'd for $C_{25}H_{38}O_8$ (466.55): C, 64.36; H, 8.21.

Found:[8] C, 63.85; H, 8.15 (Dried at 45°).

*Isostrophanthidol* (XII) *from strophanthidol* (I). A solution of 1 g. of I in 10 cc. of methanol containing 240 mg. of potassium hydroxide was kept at room temperature for 1½ hours and, after pouring it into 40 cc. of a mixture of ice and water, the resulting turbid solution was placed in the refrigerator overnight. After acidification to Congo Red with conc'd hydrochloric acid, the mixture was warmed on a steam-bath to 45–50° and was then saturated with sodium chloride. Upon standing in the refrigerator, a voluminous crystalline precipitate was obtained; wt. 559.0 mg. An additional crop, wt. 197.7 mg., resulted from concentrating the mother liquor. Recrystallization from dilute methanol gave a total of 631.5 mg. of crystalline material with melting points ranging from 218 to 230°. Recrystallization from ethyl acetate gave clusters of very small, colorless needles; m.p. 226.5–227.5°. They depressed the m.p. of strophanthidol and failed to give a positive Legal test. $[\alpha]_D^{28.5}$ +36.2° (20.2 mg. in 2.0 cc. of methanol; l, 2 dm.; $\alpha$ +0.73°).

*Anal.* Calc'd for $C_{23}H_{34}O_6$ (406.50): C, 67.94; H, 8.43.

Found:[8] C, 68.08; H, 8.41.

*Isostrophanthidol 3,19-diacetate* (XIIa). To XII (205.5 mg.) in 2 cc. of dry pyridine was added 1 cc. of acetic anhydride and the mixture was kept at room temperature for 20 hours. After evaporating to dryness *in vacuo*, the residue was taken up in ethyl acetate and the solution was washed with *N* hydrochloric acid, *N* sodium bicarbonate, and with a saturated sodium chloride solution. After drying over sodium sulfate, the solvent was removed *in vacuo* and the residue was recrystallized from acetone-petroleum ether. Yield: 216.0 mg. of stout colorless needles; m.p. 225–226°; no change of m.p. after renewed recrystallization; there was a depression of the m.p. when mixed with a specimen of XII. $[\alpha]_D^{23}$ +32.6° (21.5 mg. in 2.0 cc. of chloroform; l, 2 dm.; $\alpha$ +0.70°).

*Anal.* Calc'd for $C_{27}H_{38}O_8$ (490.57): C, 66.10; H, 7.81.

Found:[7] C, 66.08; H, 7.90.

## SUMMARY

1. The ozonization of strophanthidol (I) and its 3,19-diacetate (Ia) has been studied. The reaction products, 21-glyoxyloxy-14-isopregnane-3β,5,14,19-tetrol-20-one (X) and 3β,19-diacetoxy-21-glyoxyloxy-14-isopregnane-5,14-diol-20-one (III) respectively, are amorphous.

2. Hydrolysis of X and III in alkaline media leads, even under very mild conditions (potassium bicarbonate), to some inversion of the configuration of the ketol side chain at carbon atom 17. Thus it was possible to prepare compounds derived from 14-isopregnane-3β,5,14,19,21-pentol-20-one (IV) and 14-iso-17-isopregnane-3β,5,14,19,21-pentol-20-one (VII) and their respective oxida-

tion products, 3$\beta$,5,14,19-tetrahydroxy-14-isoetiocholanic acid (II) and 3$\beta$,5,14,19-tetrahydroxy-14-iso-17-isoetiocholanic acid (IX).

3. 14-Isopregnane-3$\beta$,5,14,19,20$\beta$,21-hexol (XI) and its 3,19,21-triacetate (XIa) have been described.

4. Strophanthidol was converted into isostrophanthidol (XII) and its 3,19-diacetate (XIIa).

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## REFERENCES

(1) EHRENSTEIN AND JOHNSON, *J. Org. Chem.*, **11**, 823 (1946).
(2) EHRENSTEIN, JOHNSON, OLMSTED, VIVIAN, AND WAGNER, *J. Org. Chem.*, **15**, 264 (1950).
(3) EHRENSTEIN AND NEUMANN, *J. Org. Chem.*, **16**, 335 (1951).
(4) EHRENSTEIN, BARBER, AND GORDON, *J. Org. Chem.*, **16**, 349 (1951).
(5) HERZIG AND EHRENSTEIN, *J. Org. Chem.*, **17**, 713 (1952).
(6) HERZIG AND EHRENSTEIN, *J. Org. Chem.*, **17**, 724 (1952).
(7) HEUSSER (Konstitution, Konfiguration und Synthese digitaloider Aglykone und Glykoside), *Progress in the Chemistry of Organic Natural Products*, Springer Verlag, Wien, **7**, 87 (1950).
(8) BARBER AND EHRENSTEIN, *J. Org. Chem.*, **16**, 1615 (1951).
(9) MEYER, *Helv. Chim. Acta*, **30**, 1976 (1947).
(10) LARDON, *Helv. Chim. Acta*, **32**, 1517 (1949).
(11) VELLUZ, PETIT, PESEZ, AND BERRET, *Bull. soc. chim. France*, 123 (1947); *Chem. Abstr.*, **41**, 5537 (1947).
(12) HERZIG AND EHRENSTEIN, *J. Org. Chem.*, **16**, 1050 (1951).
(13) HIRSCHMANN, DAUS, AND HIRSCHMANN, *J. Biol. Chem.*, **192**, 115 (1951).
(14) BLATT, *Org. Syntheses*, Coll. Vol. II, 165, 461 (1943).
(15) MILNER AND SHERMAN, *Ind. Eng. Chem., Anal. Ed.*, **8**, 427 (1936).
(16) SCHMUTZ, *Helv. Chim. Acta*, **32**, 1442 (1949).