[CONTRIBUTION FROM WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY]

Degradation of Corticosteroids. III.[1,2] Catalytic Hydrogenation of Cortisol

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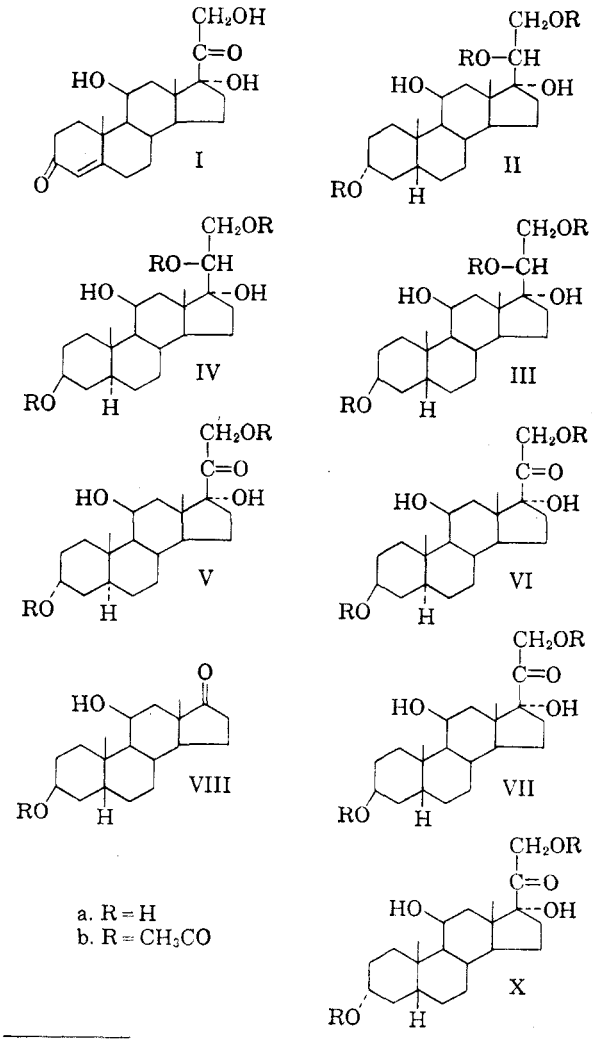
Received October 29, 1958

The catalytic hydrogenation of cortisol gave complicated mixtures, which were difficult to resolve, instead of the expected equatorial allo isomer. Hydrogenation in glacial acetic acid with platinum oxide gave 39% of 3α,11β,17α,20β,21-pentahydroxypregnane, 27% of 3β,11β,17α,20β,21-pentahydroxypregnane, and only 9.5% of 3β,11β,17α,20β,21-pentahydroxyallo pregnane. Hydrogenation of cortisol in glacial acetic acid with rhodium (5%) on alumina gave 13.5% of 3β,11β,17α,21-tetrahydroxyallopregnan-20-one, 32% of 3β,11β,17α,21-tetrahydroxypregnan-20-one, 26.5% of 3α,11β,17α,21-tetrahydroxyallopregnan-20-one, 18% of 3α,11β,17α,21-tetrahydroxypregnan-20-one and 1% of 3β,11β-dihydroxyetiocholan-17-one. Evidence is presented for the hydrogen bonding of carbonyls at C-20 with water of crystallization.

For the past few years we have been investigating the biosynthetic origin of carbon atoms of the corticosteroid nucleus. Cortisol-C^{14} was biosynthesized from acetate-1-C^{14} and methods had to be devised for the degradation and isolation of individual carbons of the steroid nucleus. One of the approaches explored for the opening of ring D was to hydrogenate cortisol to an alcohol saturated in ring A, and to cleave the side chain and open ring D of the derived 17-ketosteroid. It has been reported that steroidal 4-en-3-ones having an 11β-hydroxy, an 11-ketone or unsaturation at $C^{9(11)}$ or C^{11} on hydrogenation yield almost exclusively allo-isomers.[3] When the reduction proceeds to the alcohol stage, an equatorial hydroxyl (3β) group is formed. It was hoped that on hydrogenation of cortisol satisfactory yields of a single isomer of the allo series would be obtained. With this in mind studies were undertaken on the catalytic hydrogenation of cortisol in glacial acetic acid with two catalysts: platinum oxide and rhodium (5%) on alumina. Contrary to expectation, in both cases, complex mixtures, difficult to resolve, were obtained. Certain of our results differ profoundly from those previously reported.[3]

A solution of cortisol (I) in glacial acetic acid was shaken for 16 hours in an atmosphere of hydrogen with platinum oxide.[4] The reaction



(1) (a) Paper I: E. Caspi, G. Rosenfeld, and R. I. Dorfman, *J. Org. Chem.*, **21**, 814 (1956). (b) Paper II. E. Caspi, F. Ungar, and R. I. Dorfman, *J. Org. Chem.*, **22**, 326 (1957).

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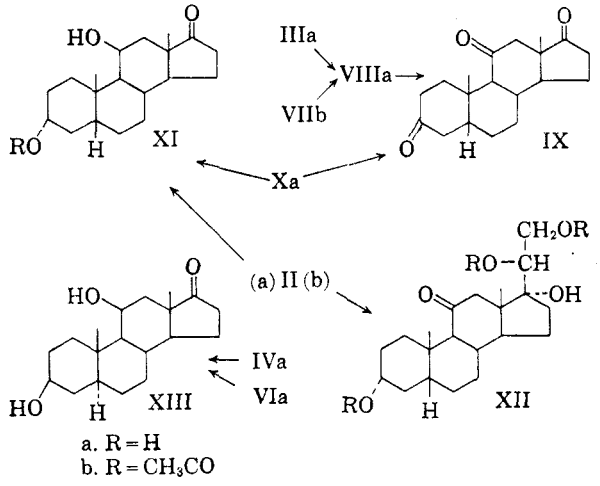
(4) Purchased from Baker and Co., Inc., Newark, N. J.

mixture was processed as described in the experimental part and 3α,11β,17α,20β,21-pentahydroxypregnane (IIa, 39%), 3β,11β,17α,20β,21-pentahydroxypregnane (IIIa, 27%), 3β,11β,17α,-20β,21-pentahydroxyallopregnane (IVa, 9.7%) were isolated.

The major product (IIa) was obtained in two forms as the anhydrous pentol $C_{21}H_{36}O_5$ m.p. 261–263° (19%), and the monohydrate $C_{21}H_{36}O_5 \cdot H_2O$ m.p. 166–170° (20%). On recrystallization from methanol the monohydrate was converted to the anhydrous pentol. Acetylation of IIa gave a sirupy triacetate IIb which was oxidized[5] to XIIb and then saponified[6] to the free alcohol XIIa. The rotational increment on acetylation XIIb → XIIa $\Delta[M]_D$ + 409° is consistent with a 20β hydroxyl.[7] Finally, cleavage of the side chain[8] of IIa gave the known 3α,11β-dihydroxyetiocholan-17-one and completes the proof of the assigned structure.



The second product was the previously undescribed pentol IIIa. The substance (m.p. 242–244°) analyzed for $C_{21}H_{36}O_5$ and its infrared spectrum had bands at 3600, 3450, and 1022 (axial hydroxyl) cm.$^{-1}$. Acetylation gave a triacetate IIIb ($C_{21}H_{42}O_8$, m.p. 153–155°) which had a complex group of bands in the C—O—C stretching region, 1285, 1270, 1239, 1205 cm.$^{-1}$, characteristic of axial acetoxy compounds.[9–11] The rotational increment on acetylation IIIb → IIIa $\Delta[M]_D$ + 285, is consistent with a 20β hydroxy function. Oxidation of IIIa with sodium bismuthate gave VIIIa (m.p. 259–261°), the infrared spectrum of which differed from those of the known 3α,11β-dihydroxyetiocholan-17-one, 3α,11β-dihydroxyandrostan-17-one and 3β,11β-dihydroxyandrostan-17-one. Oxidation of VIIIa with chromium trioxide pyridine complex[5] gave IX identical in every respect with a sample of etiocholane-3,11,17-trione prepared from 3α,11β,17α,21-tetrahydroxypregnan-20-one.

The pentol IVa was obtained as the monohydrate (9.7%) $C_{21}H_{36}O_5 \cdot H_2O$ and showed a double melting point at 159–163° with resolidification and remelting at 215–216°. The structure of the substance was proven by the identity of the infrared spectra of the free alcohol IVa and of the triacetate IVb (m.p. 204–208°) with those of authentic samples.[12]

The preponderance of cis isomers formed on hydrogenation of cortisol with platinum oxide in acetic acid was surprising and to our knowledge has not been previously reported. It has been assumed that 11β-hydroxy functions prevent the adsorption of the β side of the steroids on the surface of the catalyst[3e,f] and consequently the hydrogen atoms must enter from the α side and form the alloisomers. The results reported in this paper seem to indicate that other factors, besides the steric hindrance of the 11β-hydroxyl, may be influencing the course of the reaction. This assumption finds support in the reported observations that modification of conditions of hydrogenation profoundly influences the stereochemistry of the resulting products.[13–16] The isolation of relatively large amounts of 3β,11β,17α,20β,21-pentahydroxypregnane is of interest. Although the formation of the thermodynamically less stable axial compounds could be expected according to Barton's modified Auwers-Skita rule,[17] the substance seems not to have been previously described.

A solution of cortisol (I) in glacial acetic acid was shaken for 3 hr. in an atmosphere of hydrogen with

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rhodium (5%) on alumina until the gas uptake became very slow. The reaction mixture was processed as described in the epxerimental part and 3β,11β,17α,21-tetrahydroxyallopregnan-20-one (VIa, 13.5%), 3β,11β,17α,21-tetrahydroxypregnan-20-one (VIIa, 32%), 3α, 11β, 17α, 21-tetrahydroxyallopregnan-20-one (Va, 26.5%), 3α,11β,17α,21-tetrahydroxypregnan-20-one (Xa, 18%) and 3β,11β-dihydroxyetiocholan-17-one (VIIIa,1%) were isolated.

The equatorial alcohol VIa (13.5%) was obtained in two form as the tetrahydro derivative $C_{21}H_{34}O_5$, m.p. 221–224°, and as the hemihydrate $C_{21}H_{34}O_5 \cdot {}^1/_2\ H_2O$, m.p. 211–214°. The infrared spectra of the two forms differed in the carbonyl region and in the 1450–1200 cm.$^{-1}$ region. However, both products moved identically when chromatographed on paper.[18] Their solutions in sulfuric acid gave identical spectra,[19] on acetylation identical esters were obtained and finally on cleaving the side chain both gave XIII establishing thus the assigned structures. The infrared spectrum of the anhydrous sample had a single carbonyl band at 1705 cm.$^{-1}$ and the hemihydrate had two distinct bands at 1710 and 1690 cm.$^{-1}$. The intensity of the 1690 cm.$^{-1}$ band varied from 80–100% of the 1710 cm.$^{-1}$ band. The presence of the 1690 cm.$^{-1}$ band in the hydrated sample indicates a partial hydrogen bonding of the ketone at C-20 with the water of hydration.

The apparently not yet described VIIa was isolated as the hemihydrate $C_{21}H_{34}O_5 \cdot {}^1/_2H_2O$, m.p. 135–138°, in 32% yield. The structure assignment of the substance is based on elementary analysis, purple coloration with blue tetrazolium,[20] infrared spectrum and on oxidative cleavage of the side chain to VIIIa.

In addition to the two described products the known 3α,11β,17α,21-tetrahydroxyallopregnan-20-one (Va), m.p. 244–245°, (26.5% yield) and 3α,11β,17α,21-tetrahydroxypregnan-20-one (Xa), m.p 207–209°, (18% yield) were isolated and identified by comparison of their infrared spectra with those of authentic samples. In all hydrogenation experiments with rhodium (5%) on alumina small amounts 1–1.5% of 3β,11β-dihydroxyetiocholan-17-one (VIIIa) were formed.

Hydrogenation of cortisol in acetic acid solution with rhodium (5%) on alumina catalyst led to the reduction of the conjugated carbonyl function in ring A and the formation of the "tetrahydro" derivatives. The dihydroxy acetone moiety remained essentially unchanged. In some experiments up to 9% of various 20β-hydroxy pentols were obtained. It seems possible that the formation of the small amounts of 17-ketosteroid was an artifact and the cleavage of the dihydroxyacetone moiety occurred on the alumina which supported the rhodium catalyst. Transformations of steroids on alumina were previously observed.

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EXPERIMENTAL[21]

Hydrogenation of cortisol with platinum oxide catalyst. A mixture of 4 g. of cortisol, 150 ml. of glacial acetic acid (Mallinkrodt Analytical Reagent, Dichromate Test) and 560 mg. of platinum oxide[4] was shaken 16 hr. in an atmosphere of hydrogen. The catalyst was filtered, washed with acetone, and the solvents removed *in vacuo*. The residue was dissolved in ethyl acetate, washed with 2*N* sodium carbonate, water, then dried over sodium sulfate, and the solvent distilled leaving 4.4 g. of a glass. A portion of the glass (1.1 g.), equivalent to 1 g. of cortisol, was dissolved in ethyl acetate and on concentration of the solution a white semi-solid residue separated out. The residue was digested three times with small amounts of hot ethyl acetate leaving 424 mg. of a mixture of solids (*Fraction A*), m.p. 147–159° which was subsequently fractionally crystallized. Concentration of the combined mother liquors gave 206 mg. (in four crops) of a homogeneous product, m.p. 212–222° (*Fraction B*). Finally, the remaining mother liquor was evaporated to dryness and carefully chromatographed on silica gel (*Chromatography 1*). The column (60 × 3 cm.) was eluted with benzene, mixtures of benzene-ethyl acetate, ethyl acetate, mixtures of ethyl acetate-methanol and methanol. Eluates of 50 ml. were collected.

Hydrogenation of cortisol with rhodium (5%) on alumina catalyst. To a pre-reduced mixture of 100 ml. of glacial acetic acid (Mallinkrodt Analytical Reagent, Dichromate Test) and 550 mg. of rhodium (5%) on alumina catalyst[4] 2 g. of cortisol was added and the suspension was shaken in an atmosphere of hydrogen. The reaction was stopped after 3 hr. after 2.1 mols. of hydrogen had been absorbed and the gas uptake had almost ceased. The reaction mixture was processed as described above and on concentration of the ethyl acetate solution 535 mg. of solids, m.p. 205–233° was obtained. The mother liquor was distilled to dryness and the residue was chromatographed on silica gel (*Chromatography 2*). The column (50 × 2 cm.) was eluted with benzene, mixtures of ethyl acetate-benzene, ethyl acetate, mixtures of methanol-ethyl acetate and methanol. Eluates of 50 ml. were collected.

3α,11β,17α,20β,21-Pentahydroxypregnane (IIa). The above described *Fraction A* was fractionally crystallized from mixtures of methanol-ethyl acetate and eight crops of crystalline solids were collected. The first crop was IIa monohydrate and the second the anhydrous IIa. The subsequently obtained crops 3–8 were 3β,11β,17α,20β,21-pentahydroxyallopregnane (IVa).

(21) The eluates from the chromatography columns were assayed by infrared spectroscopy on a Perkin-Elmer Infracord Spectrometer. The yields reported are based on the weights of products eluted from the chromatography columns. Melting points were determined on a Fisher-Johns hot stage and are reported as read. Analyses were performed by Drs. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England. Ultraviolet absorption spectra were determined by means of a Cary Model 11 MS spectrophotometer. Optical rotations were determined in methanol in a 1-dm. semimicro tube. Infrared spectra were obtained from material incorporated into rectangular potassium bromide prisms[11] on a Perkin-Elmer 12C spectrometer.

A. Isolation of IIa monohydrate. *a.* The first crop obtained on fractional crystallization of *Fraction A* gave 200 mg. of IIa, m.p. 164–168°.

The sample was recrystallized twice from methanol-ethyl acetate, m.p. 166–170°; $[\alpha]_D^{20}$ +28.8° (*c*, 0.5618), $[M]_D$ +111°; ultraviolet $\lambda_{max.}^{MeOH}$ none; infrared $\nu_{max.}^{KBr}$ 3550, 1039 (equatorial hydroxyl) cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{36}O_5 \cdot H_2O$: C, 65.25; H, 9.91. Found: C, 65.02; H, 9.71.

B. Isolation of anhydrous IIa. *a.* The second crop obtained on fractional crystallization of *Fraction A* gave 98 mg. of IIa, m.p. 230–235°. *b. From Chromatography 1.* Fractions 128–138, which were eluted with a mixture of methanol-ethyl acetate (1:9) yielded 94.2 mg. of IIa. The combined residue was crystallized from methanol and gave IIa, m.p. 253–257°.

The infrared spectra of both forms of IIa were essentially identical.

A sample was recrystallized from methanol, m.p. 261–263°; $[\alpha]_D$ +28° (*c*, 0.6433); $[M]_D$ +103°; ultraviolet: $\lambda_{max.}^{MeOH}$ none; infrared $\nu_{max.}^{KBr}$ 3580, 1039 (equatorial hydroxyl) cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85; Found: C, 68.14; H, 9.63.

The triacetate IIb was prepared in the usual manner but could not be crystallized.

3β,11β,17α,20β,21-Pentahydroxypregnane (IIIa). A. The previously described *Fraction B* gave 206 mg. of **IIIa**, m.p. 212–222°.

B. *From Chromatography 1.* Fractions 88–144 which were eluted with a mixture of ethyl acetate-benzene (17:3), gave 68.9 mg. of IIIa, m.p. 210–224°.

A sample was recrystallized from methanol-ethyl acetate, m.p. 242–244°. $[\alpha]_D^{22}$ +27.6° (*c*, 0.6450); $[M]_D$ +102°; ultraviolet $\lambda_{max.}^{MeOH}$ none; $\nu_{max.}^{KBr}$ 3600, 3450, 1022 (axial hydroxyl) cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 69.10; H, 9.78.

The triacetate IIIb was prepared in the usual manner and was recrystallized from ethyl acetate, m.p. 153–155°. $[\alpha]_D^{22}$ +78.3° (*c*, 0.8406); $[M]_D$ +387°; ultraviolet: $\lambda_{max.}^{MeOH}$ none; $\nu_{max.}^{KBr}$ 3600, 1285, 1270, 1239, 1205 (axial acetate), 1028 (axial acetoxy) cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{42}O_8$: C, 65.56; H, 8.56. Found: C, 65.59; H, 8.25.

3β,11β,17α,20β,21-Pentahydroxyallopregnane (IVa). A. Crops 3–8 obtained on fractional crystallization of *Fraction A* yielded 72 mg. of IVa, m.p. 200–208°.

B. *Chromatography 1.* Fractions 116–124, which were eluted with ethyl acetate, yielded 24.5 mg. of IVa, m.p. 140–144°. The infrared spectra of both samples were identical with that of authentic IVa.[12]

A sample was recrystallized from methanol and showed a double melting point first at 159–163° then resolidified and melted again at 215–216°. $[\alpha]_D^{22}$ +9.8 (*c*, 0.5555) $[M]_D$ +38°; *ultraviolet:* $\lambda_{max.}^{MeOH}$ *none; infrared:* $\nu_{max.}^{KBr}$ 3530, 1043 (equatorial hydroxyl) cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{36}O_5 \cdot H_2O$: C, 65.25; H, 9.91. Found: C, 65.18; H, 10.01.

The triacetate IVb was prepared in the usual manner; m.p. 204–208°; infrared: $\nu_{max.}^{KBr}$ 3600, 1270, 1028 (equatorial hydroxyl) cm.$^{-1}$. The infrared spectrum was identical with that of authentic IVb.[12]

3α,11β,17α,21-Tetrahydroxyallopregnan-20-one (Va). The three crystalline crops (535 mg.) obtained following the hydrogenation of cortisol in the presence of rhodium (5%) on alumina were Va, m.p. 210–233°.

A sample was recrystallized from methanol, m.p. 244–245°. $[\alpha]_D^{21}$ +59.7° (*c*, 0.3356); $[M]_D$ +219°; ultraviolet: $\lambda_{max.}^{MeOH}$ no selective absorption in the 200–240 mμ region; infrared: $\nu_{max.}^{KBr}$ 3500, 1715, 1005 (axial hydroxyl) cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.24; H, 9.26.

The diacetate Vb was prepared in the usual manner; m.p. 188–190°, $[\alpha]_D^{19}$ +65.3° (*c*, 0.5605 in methanol); $[M]_D$ +295°; infrared: $\nu_{max.}^{KBr}$ 3600, 1750, 1720, 1265, 1028 (axial acetoxy) cm.$^{-1}$.

3β,11β,17α,21-Tetrahydroxyallopregnan-20-one (VIa). *Chromatography 2.* Fractions 96–107, which were eluted with a mixture of benzene-ethyl acetate (1:1), gave 274 mg. of VIa. Crystallization of the product from acetone gave a first crop m.p. 211–215° and a second crop m.p. 220–222°.

A. The lower melting product was recrystallized, m.p. 215–216°; ultraviolet: $\lambda_{max.}^{MeOH}$ no specific absorption in the 200–240° mμ region: infrared: $\nu_{max.}^{KBr}$ 3540, 3480, 1705, 1690, 1048 (equatorial hydroxyl) cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{34}O_5 \cdot {}^1/_2H_2O$: C, 67.17; H, 9.40. Found: C, 67.40; H, 9.55.

Drying of a sample of the hydrate for 16 hr. at 80° at 0.01 mm. pressure or 3 hr. at 165–170° at 0.01 mm. pressure did not remove the water. Sublimation of the hydrate gave the anhydrous VIa.

B. The higher melting, second crop, was recrystallized twice to give a solid m.p. 221–224°; $[\alpha]_D^{20}$ +61.8° (*c*, 0.6509); $[M]_D$ +216°; ultraviolet: $\lambda_{max.}^{MeOH}$ none in the 220–240 mμ region; infrared: $\nu_{max.}^{KBr}$ 3540, 3480, 1710, 1048 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.46; H, 9.35.

Both alcohols, VIa, were acetylated in the usual manner to yield the same diacetate VIb m.p. 203–206°. Infrared: $\nu_{max.}^{KBr}$ 3600, 1750, 1718, 1245, 1030 cm.$^{-1}$

Paper chromatography of 3β,11β,17α,21-tetrahydroxyallopregnan-20-one (VIa).[18] The anhydrous and hemihydrate samples were chromatographed on paper in the chloroform-formamide system. Both samples showed identical mobilities and a mixture of the two could not be separated.

3β,11β,17α,21-Tetrahydroxypregnan-20-one (VIIa). *Chromatography 2.* Fractions 74–95 which were eluted with a mixture of ethyl acetate-benzene (1:1), gave 635 mg. of VIIa, m.p. 131–138°. A sample was recrystallized from ethyl acetate, m.p. 135–138°; $[\alpha]_D^{22}$ +54.5° (*c*, 0.5288); $[M]_D$ +205; ultraviolet: $\lambda_{max.}^{MeOH}$ no selective absorption in the 200–240 mμ region; infrared: $\nu_{max.}^{KBr}$ 3520, 1712, 1025 (axial hydroxyl).

Anal. Calcd. for $C_{21}H_{34}O_5 \cdot {}^1/_2H_2O$: C, 67.17; H, 9.40. Found: C, 66.85; H, 9.39.

3α,11β,17α,21-Tetrahydroxypregnan-20-one (Xa). *Chromatography 2.* Fractions 108–167 which were eluted with a mixture of benzene-ethyl acetate (1:1), gave 357 mg. of Xa. A sample was crystallized from ethyl acetate methanol, m.p. 207–209°. The infrared spectrum had bands at ν_{max}^{KBr} 3550, 1705, and 1040 cm.$^{-1}$ and was identical to that of authentic Xa.

3β,11β-Dihydroxyetiocholan-17-one (VIIIa). A. *Chromatography 2.* Fractions 58–66 which were eluted with a mixture of ethyl acetate-benzene (1:1), gave 22 mg. of VIIIa.

B. A solution of 50 mg. of 3β,11β,17α,20β,21-pentahydroxypregnane (IIIa) in 2 ml. of aqueous acetic acid (1:1) was shaken 16 hr. with 500 mg. of sodium bismuthate. The excess reagent was reduced with a 10% solution of sodium bisulfite, then diluted with water and extracted thoroughly with ether. The extract was washed with 1 *N* sodium hydroxide, then with a 25% saline solution and dried over sodium sulfate. The removal of the solvent left 43.3 mg. of crystalline VIIIa, m.p. 218–225°.

C. A portion of 3β,11β,17α,21-tetrahydroxypregnan-20-one (VIIa) was treated with sodium bismuthate as above to yield a crystalline residue of VIIIa, m.p. 251–253°. The infrared spectra of the three crystalline samples and of the mother liquor of "C" were identical.

A sample was recrystallized from methanol ethyl acetate, m.p. 259–261°; $[\alpha]_D^{22}$ +82.4° (*c*, 0.5181 in methanol); $[M]_D^{22}$ +252°; infrared: $\nu_{max.}^{KBr}$ 3600, 3550, 3400, 1728, 1026 (axial hydroxyl) cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.88. Found: C, 74.07; H, 9.90.

The alcohol VIIIa was acetylated in the usual manner to yield VIIIb. The crystalline structure of the acetate

changed at 204–209° and the substance melted at 212–215°; $[\alpha]_D^{18}$ +86.5° (*c*, 0.4108); $[M]_D$ +301°; infrared: $\nu_{max.}^{KBr}$ 3600, 1740, 1257, 1240, 1023 (axial acetoxy) cm.$^{-1}$.

Etiocholane-3,11,17-trione (IX). A. To a solution of 93 mg. of authentic 3α,11β,17α,21-tetrahydroxypregnan-20-one (X) in 3 ml. of glacial acetic acid a solution of 237 mg. of chromium trioxide in 0.4 ml. of glacial acetic acid and 0.1 ml. of water was added and the mixture was left for 16 hr. at room temperature. The reaction mixture was processed in the usual manner and the residue was dissolved in a mixture of ethyl acetate and ether. After two weeks in the refrigerator, 22 mg. of IX m.p. 128–131° separated.

B. A solution of 15 mg. of 3β,11β-dihydroxyetiocholan-17-one (VIIIa) in 0.5 ml. of pyridine was added to a suspension of 20 mg. of chromium trioxide in 0.2 ml. of pyridine and the mixture was left for 16 hr. at room temperature. The product was recovered in the usual manner and was crystallized as above to yield 2 mg. of IX, m.p. 129–131°, infrared: $\nu_{max.}^{KBr}$ 1745, 1705 cm.$^{-1}$.

3α,11β-Dihydroxyethicholan-17-one (XIa). A. The side chain of IIa was cleaved with sodium bismuthate and the steroid was recovered as previously described. The residue was crystallized from ethyl acetate to produce XIa.

B. Authentic 3α,11β,17α,21-tetrahydroxypregnan-20-one was oxidized as above to yield XIa.

The infrared spectra of both samples were identical. The recrystallized sample showed a m.p. 239–241°; infrared: $\nu_{max.}^{KBr}$ 3620, 1715, 1031 (equatorial hydroxyl).

3α,20β,21-Triacetoxy-17α-hydroxypregnan-11-one (XIIb). The sirupy triacetate IIb, 40 mg., was dissolved in pyridine, 0.5 ml., and oxidized for 16 hr. at room temperature with a suspension of 70 mg. of chromium trioxide in 0.7 ml. of pyridine. The reaction mixture was processed as previously described to yield 29 mg. of XIIb, m.p. 197–200°. Recrystallization from ethyl acetate-neohexane raised the m.p. to 202–203°; $[\alpha]_D^{20}$ +104° (*c*, 0.6561); $[M]_D$ +513°; infrared; $\nu_{max.}^{KBr}$ 3600, 1738, 1698, 1245, 1026 (equatorial acetoxy) cm.$^{-1}$.

3α,17α,20β,21-Tetrahydroxypregnan-11-one (XIIa). A solution of 10 mg. of potassium bicarbonate in 0.1 ml. of water was added to a solution of 10 mg. of XIIb in 0.5 ml. methanol. The air was replaced with nitrogen and the mixture was left for 16 hr. at room temperature. After the addition of a drop of acetic acid the volatile components were removed *in vacuo* and the residue dissolved in ethyl acetate. The ethyl acetate solution was washed with water and dried over sodium sulfate. On concentration the solution gave 4.5 mg. of XIIa, m.p. 240–245°; $[\alpha]_D^{21}$ +28.4° (*c*, 0.2047); $[M]_D$ +104°; infrared: $\nu_{max.}^{KBr}$ 3550, 1700, 1045 cm.$^{-1}$.

3β,11β-Dihydroxyandrostan-17-one (XIII). A. A solution of 30 mg. of IVa in 15 ml. of methanol was treated with 6 ml. of a stock solution of sodium metaperiodate[3i,1b] and was left for 120 min. in the dark at room temperature. The reaction mixture was processed as previously described[1b] to yield 20 mg. of XIII. The product was recrystallized from ethyl acetate, and showed a m.p. 225–228°.

B. A portion of VIa was oxidized with sodium bismuthate and the 17-ketosteroid was isolated in the usual manner.

The infrared spectra of both samples were identical with that of authentic XIII.[12]

Spectra of 3β,11β,17α,21-tetrahydroxyallopregnan-20-one (VIa), *of* VIa-*hemihydrate and of the diacetate* VIb *in sulfuric acid solution.* The solutions and the spectra were prepared as described by E. Caspi and M. M. Pechet.[19] The spectra were identical and changed in an identical manner with time.

Acknowledgment. The author is indebted to Drs. D. K. Fukushima, T. F. Gallagher, and A. H. Soloway of the Sloan-Kettering Institute for Cancer Research, New York, Drs. H. L. Herzog and E. B. Hershberg of the Schering Corporation, Bloomfield, N. J., and Dr. L. H. Sarret of Merck & Co., Inc., Rahway, N. J., for various samples of steroids. Thanks are due to Mrs. M. Rayner for technical assistance.

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[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY AND CHEMISTRY DEPARTMENT, CLARK UNIVERSITY]

Synthesis of Radioactive Dehydroepiandrosterone[1,2]

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Received November 7, 1958

A method for the synthesis of dehydroepiandrosterone-4-C^{14} using testosterone-4-C^{14} as starting material and 17-yl sulfite as an intermediate is described. Also described is specifically tritiated dehydroepiandrosterone, obtained by the reduction of 7α-bromodehydroepiandrosterone acetate.

Endocrinological studies on the metabolism of dehydroepiandrosterone prompted us to study the preparation of radioactive dehydroepiandrosterone whereby highest specific activity might be obtained. We are reporting here the synthesis of dehydroepiandrosterone-C^{14} labelled in position 4 and of dehydroepiandrosterone-H^3 labelled mostly in position 7.

The only previously published method for the preparation of isotopically labelled dehydroepiandrosterone (dehydroepiandrosterone-16-C^{13}) by E. B. Hershberg *et al.*[3] was rather involved, requiring the preparation and use of diazomethane C^{14}. Furthermore a few reports[4] on the bio-oxidation of ring D justified the elaboration of a ring A, B, or C labelled dehydroepiandrosterone.

(1) Presented in part at the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April 1958.

(2) This investigation was supported in part by research grants USPH C-231 and A-2672.

(3) E. B. Hershberg, E. Schwenk, and E. Stahl, *Arch. Biochem.* **19**, 300 (1948).

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