gonal prisms softening and sweating at 205° and subliming incompletely above 217° to little squares. A characteristic melting with transition within the melt to thick quadrilaterals remelting from 219–220° occurred. The analytical sample obtained by ether recrystallization showed a double m.p. 203–205, 209–220°, $[\alpha]^{25}D$ +13°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 64.63; H, 7.81. Found: C, 64.30; H, 7.55.

The hydroxyl group was not acetylatable under mild conditions (pyridine–acetic anhydride 16 hours, 25°). Attempts at saponification gave non-crystalline material soluble in aqueous alkali.

Acknowledgment.—The authors wish to express their thanks to H. C. Amsterdam for technical assistance, to C. R. Eddy, C. Fenske and M. A. Barnes for infrared curves, to C. L. Ogg and K. Zbinden for microanalyses, and to R. F. Mininger for optical rotation determinations.

PHILADELPHIA 18, PENNSYLVANIA

[CONTRIBUTION FROM THE CHEMICAL AND BIOLOGICAL RESEARCH SECTION, AMERICAN CYANAMID COMPANY, RESEARCH DIVISION, LEDERLE LABORATORIES]

Steroidal Cyclic Ketals. XIV.[1] The Preparation of Pregnane-5α,21-diol-3,20-dione and Pregnane-5α,11β,17α,21-tetrol-3,20-dione

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Pregnane-5α,21-diol-3,20-dione (VIIa) and pregnane-5α,11β,17α,21-tetrol-3,20-dione (XIa) have been prepared for evaluation of the biological influence of the substitution of a 3-keto-5α-hydroxyl group for the Δ⁴-3-ketone group in desoxycorticosterone (I) and hydrocortisone.

Androstane-5α-ol-3,17-dione, androstane-5α,17β-diol-3-one and 17α-methylandrostane-5α,17β-diol-3-one have recently been prepared for evaluation as possible metabolic precursors of their corresponding Δ⁴-3-ketonic hormones.[2] In all three cases, the substitution of the 3-ketone-5α-ol group for the usual Δ⁴-3-ketone group resulted in an appreciable decrease in androgenicity (subcutaneous injection-capon assay), and, consequently, this possible metabolic pathway was discounted.[2] Notwithstanding these results, it was of interest to us to extend this work to the pregnane series, and more particularly to the 5α-hydroxy analog of desoxycorticosterone and hydrocortisone.[3] We have succeeded in preparing pregnane-5α,21-diol-3,20-dione (VIIa) and pregnane-5α,11β,17α,21-tetrol-3,20-dione (XIa) by a pathway which capitalized on the fact that during the formation of an ethylene ketal of a Δ⁴-3-ketosteroid the double bond migrates from the C-4 to C-5 position.[4] The preparative details for these compounds (VIIa and XIa), and their biological activities form the basis of this paper.

Desoxycorticosterone (I, Flow Sheet A) on ketalization with ethylene glycol was converted into the expected Δ⁵-3,20-bis-ethylene ketal (IIa). Acetylation gave the Δ⁵-21-acetate 3,20-bis-ethylene ketal (IIb). Epoxidation of IIb in benzene with a solution of perbenzoic acid in ethyl acetate afforded a product which consisted of the α-oxide IIIa and presumably the β-oxide IIIb. On the basis of solubility in acetone–petroleum ether, the mixture was separated into two fractions, 1 and 2, the former being the more insoluble fraction.

Fraction 1 in tetrahydrofuran–ether on treatment with lithium aluminum hydride gave a product which proved difficult to purify by recrystallization. It therefore was hydrolyzed under acidic conditions for the removal of the ketal groups. This afforded what is probably pregnane-5α,6β,21-triol-3,20-dione (Va) in a practically pure state. Its 6,21-diacetate Vb was obtained analytically pure.[5]

Fraction 2 on similar reaction with lithium aluminum hydride, followed by acetylation, resulted in a complex mixture which was resolved in the following way. Chromatography on alumina gave three fractions in order of increasing polarity, called 2A, 2B and 2C.

Fraction 2A on saponification, chromatography and acid hydrolysis gave pregnane-5α,21-diol-3,20-dione (VIIa). Fraction 2B on purification by recrystallization gave pregnane-5α,21-diol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (VIb). Fraction 2C on purification also by recrystallization gave pregnane-5α,21-diol-3,20-dione 3,20-bis-ethylene ketal (VIa). The presence of this compound may be ascribed to incomplete acetylation or saponification during the chromatography on alumina.

Hydrolysis of the 5α,21-diol 3,20-bis-ethylene ketal (VIa) in methanol with sulfuric acid resulted in a mixture of pregnane-5α,21-diol-3,20-dione (VIIa) and desoxycorticosterone (I), which were separated by fractional recrystallization.[6] The

(1) Paper XIII, S. Bernstein and R. H. Lenhard, THIS JOURNAL, **77**, 2331 (1955).

(2) (a) S. A. Julia, Pl. A. Plattner and H. Heusser, *Helv. Chim. Acta*, **35**, 665 (1952); (b) S. A. Julia and H. Heusser, *ibid.*, **35**, 2080 (1952).

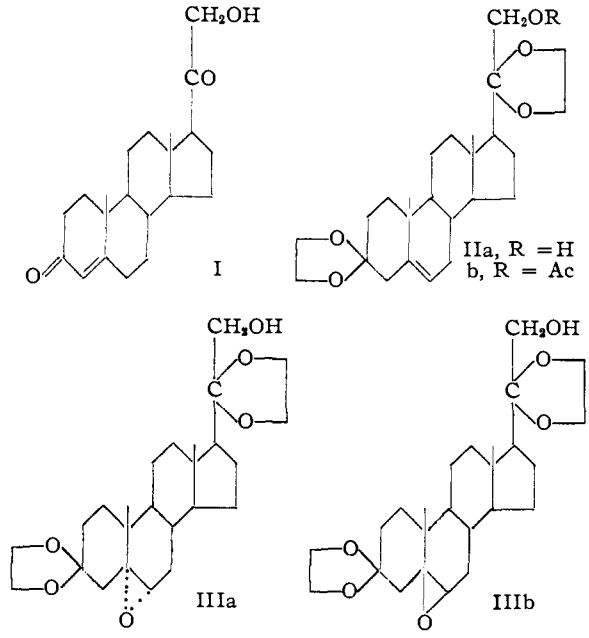
(3) The Swiss workers[2a] have indicated a forthcoming communication dealing with the 5α-oxy-compounds in the pregnane and 21-oxy-pregnane series; but this work has not appeared as yet.

(4) E. Fernholz and H. E. Stavely, Abstracts of the 102nd Meeting of the American Chemical Society, Atlantic City, N. J., September 8–12, 1941, p. M39; E. Fernholz, U. S. Patents 2,356,154 (August 22, 1944) and 2,378,918 (June 26, 1945); R. Antonucci, S. Bernstein, R. Littell, K. J. Sax and J. H. Williams, *J. Org. Chem.*, **17**, 1341 (1952); and G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

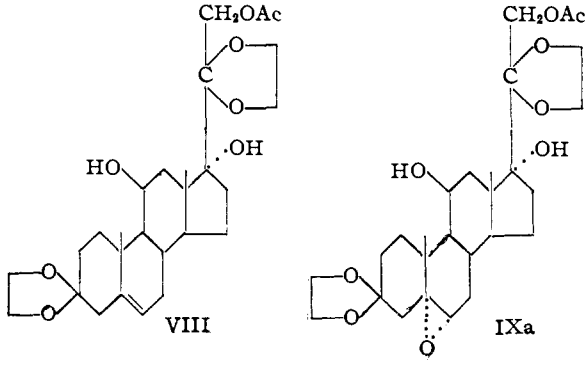
(5) The 5α,6β,21-triols appear to have been derived at some stage principally from the β-oxide IIIb. Theoretically, the β-oxide IIIb on treatment with lithium aluminum hydride would give rise predominantly to allopregnane-6β,21-diol-3,20-dione 3,20-bis-ethylene ketal (IV); none of this compound was isolated. In this connection, see Pl. A. Plattner, H. Heusser and M. Feurer, *Helv. Chim. Acta*, **32**, 587 (1949), for the definitive publication on the reduction of 5,6-oxides with lithium aluminum hydride.

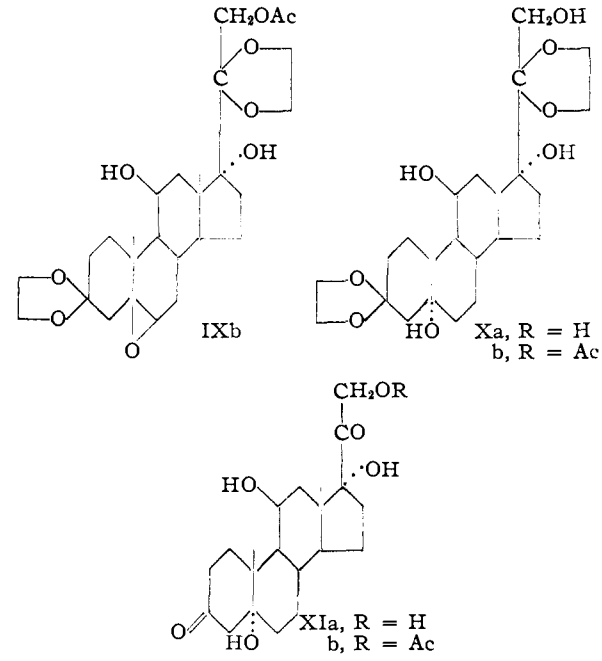
(6) See S. A. Julia, Pl. A. Plattner and H. Heusser (ref. 2a) and references cited therein which deal with the elimination of water from 3-keto-5α-oxysteroids to afford Δ⁴-3-ketosteroids.

FLOW SHEET A



FLOW SHEET B





structure of the 5α,21-diol VIIa was supported by the formation of its 21-acetate VIIb.[7]

With the intermediate pregnane-5α,21-diol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (VIb) at hand, it was of interest to study the stability of its 5α-hydroxyl group toward phosphorus oxychloride and thionyl chloride. Compound VIb in pyridine was recovered unchanged on treatment with phosphorus oxychloride at room temperature for 17 hours. Similar treatment at −5° with thionyl chloride resulted in the removal of the hydroxyl group. Although the reaction product was obtained in an impure state and in low yield its identification was definitely established by infrared absorption analysis as Δ^5-pregnene-21-ol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (IIb). These observations suggest certain possibilities in synthesis which we hope to exploit.

The above pathway for the introduction of a 5α-hydroxyl group was applied also to hydrocortisone. Hydrocortisone acetate 3,20-bis-ethylene ketal (VIII)[8] (Flow Sheet B) on peroxidation gave a mixture of the α- and β-oxides IXa and b, respectively, which were separated successfully by chromatography on alumina. The less polar β-oxide IXb was eluted first from the column, and this was understandable on steric considerations.

Treatment of the α-oxide IXa with lithium aluminum hydride in tetrahydrofuran gave the 5α,11β,17α,21-tetrol 3,20-bis-ethylene ketal (Xa), which on acetylation was converted into its 21-acetate (Xb).

The 5α,11β,17α,21-tetrol 21-acetate 3,20-bis-ethylene ketal (Xb) also was obtained in the following manner, which offered no great difficulty in contrast to the desoxycorticosterone example. The

(7) This procedure for the preparation of the 5α,21-diol VIIa and its acetate VIIb undoubtedly would have been simplified greatly if the α-oxide IIIa had been isolated in a pure state.

(8) S. Bernstein, R. Littell and J. H. Williams, THIS JOURNAL, 75, 4830 (1953); and W. S. Allen, S. Bernstein and R. Littell, *ibid.*, 76, 6116 (1954).

mixture of the oxides IXa and IXb was treated directly with lithium aluminum hydride. The reduction mixture was acetylated, and chromatographed on a silica gel column. The desired product Xb was eluted with 5% acetone–ether.

Saponification of the 21-acetate 3,20-bis-ethylene ketal (Xb) gave the 5α,11β,17α,21-tetrol 3,20-bis-ethylene ketal (Xa). Hydrolysis of the latter with aqueous sulfuric acid in methanol gave a mixture of pregnane-5α,11β,17α,21-tetrol-3,20-dione (XIa) and a Δ^4-3-ketosteroid which was presumably hydrocortisone. The desired product XIa was isolated pure by distribution of the mixture between 50% aqueous methanol and benzene, followed by evaporation of the aqueous methanol phase, and by recrystallization. Acetylation at room temperature gave the 21-acetate XIb.

Bioassays.[9]—In the electrolyte assay (K/Na ratio) on adrenalectomized rats, pregnane-5α,21-diol-3,20-dione (VIIa) and pregnane-5α,11β,17α,21-tetrol-3,20-dione (XIa) exhibited no activity at both the 6- and 25-μg. dose levels where desoxycorticosterone (I) gave significantly positive results.

In a preliminary thymus involution assay (adrenalectomized and ovariectomized mice), pregnane-5α,11β,17α,21-tetrol-3,20-dione (XIa) possessed an activity of the same order as cortisone acetate. More extensive assay work is planned for XIa.

Thus, it would appear that the biological influence of the substitution of a 3-keto-5α-hydroxyl group for the usual Δ^4-3-ketone group found in certain steroidal hormones is variable, and dependent on the hormone type.

Acknowledgment.—We are indebted to Messrs. Louis M. Brancone, Samuel S. Modes, Gerald P. McTernan and John G. Heider for the microanalytical data, and to Messrs. William Fulmor and George Morton and Miss Anne Callaghan for the optical rotation data and the infrared absorption spectra.

Experimental

Melting Points.—All melting points are uncorrected, and were determined with uncalibrated Anschütz thermometers.

Optical Rotations.—The sample was dissolved in chloroform (unless otherwise noted) to make a 2-ml. solution, and the rotation was determined in a 1-dm. semi-micro tube (unless otherwise noted) at wave length 5893 Å. (D).

Absorption Spectra.—The ultraviolet spectra were determined in absolute alcohol with a Beckman spectrophotometer (model DU). The infrared spectra (Nujol mull or pressed potassium bromide as noted) were determined with a Perkin–Elmer spectrophotometer (model 21).

Petroleum Ether.—The fraction used had a b.p. 60–70° (Skellysolve B).

All evaporations were carried out under reduced pressure.

Δ^5-Pregnene-21-ol-3,20-dione 3,20-Bis-ethylene Ketal (IIa). A.—A mixture of desoxycorticosterone (I, 10 g.), ethylene glycol (75 ml.), benzene (500 ml.) and *p*-toluenesulfonic acid monohydrate (300 mg.) was treated in the conventional manner (7 hours reflux).[10] The dried benzene extract was evaporated to afford a white solid. Crystallization from methanol gave 6.78 g. of IIa, m.p. 186.5–190.5° with previous softening.

B.—In another run,[11] the product obtained by essentially the above procedure was purified for characterization by crystallization from methanol, m.p. 190–193°; ultraviolet: $\lambda_{max.}$ none; infrared: λ_{max}^{Nujol} 3390, 1650 and 1113 cm.$^{-1}$; $[\alpha]^{23}D$ −19° (19.3 mg., αD −0.18°), $[M]D$ −80.

Anal. Calcd. for $C_{25}H_{38}O_5$ (418.55): C, 71.74; H, 9.15. Found: C, 71.77; H, 9.09.

Δ^5-Pregnene-21-ol-3,20-dione 21-Acetate 3,20-Bis-ethylene Ketal (IIb).—The bis-ethylene ketal (IIa, 1.33 g.) on acetylation gave 1.24 g. of pure IIb, m.p. 162–164° (from methanol); infrared: λ_{max}^{KBr} 1762, 1245 and 1118 cm.$^{-1}$; $[\alpha]^{24}D$ −19° (19.3 mg., αD −0.18°), $[M]D$ −88.

Anal. Calcd. for $C_{27}H_{40}O_6$ (460.59): C, 70.40; H, 8.75. Found: C, 70.09; H, 8.92.

Pregnane-5α,6β,21-triol-3,20-dione (Va).—The Δ^5-acetate bisketal (IIb, 6.0 g.) in benzene (35 ml.) was oxidized with perbenzoic acid (2.0 g.) in ethyl acetate (70 ml.) (116 hours at room temperature). Additional ethyl acetate was added, and the benzene–ethyl acetate solution was washed successively with 5% aqueous potassium hydroxide solution, saturated saline solution and with water. The dried extract was evaporated to afford a white solid (mixture of IIIa and b). Two crystallizations from acetone–petroleum ether gave 3.0 g., m.p. 170.5–172.5° (fraction 1). The mother liquors were combined, evaporated and treated separately (fraction 2) (*vide infra*).

Fraction 1 (3.0 g.) in tetrahydrofuran (100 ml.) and ether (25 ml.) was treated with lithium aluminum hydride (2.0 g.), and the mixture was refluxed for 4 hours (and was then allowed to stand at room temperature overnight). The excess hydride was decomposed with water; ethyl acetate was added, and the inorganic solid was removed by filtration. The solid was triturated several times with ether–ethyl acetate. The organic extracts were combined, and washed with a saline solution. The dried extract on evaporation gave an oil which was crystallized from acetone–petroleum ether. In this manner, there was obtained 1.55 g. of a crude product m.p. 156–160°, bubbles in melt (solvated?). Two crystallizations from acetone–petroleum ether gave 1.03 g. of solvated material with no improvement in melting point. After a 250-mg. portion was set aside, the remainder of the solid together with the evaporated mother liquor was dissolved in methanol (50 ml.), and was hydrolyzed by being refluxed for 0.5 hour with 8% (v./v.) sulfuric acid (5 ml.). Solid sodium bicarbonate was added; and the mixture was concentrated to a small volume, and was extracted with benzene, ethyl acetate and chloroform. The combined extracts were washed with a saline solution, dried and evaporated. Crystallization of the residue from acetone–petroleum ether gave 245 mg. of impure Va, m.p. 223–230°, with previous softening and decomposition. Four crystallizations from acetone (petroleum ether wash) gave 100 mg. of almost pure triol-dione Va, m.p. 239–243.5°, with previous softening and decomposition; ultraviolet: $\lambda_{max.}$ none (end absorption only); infrared: λ_{max}^{Nujol} 3390 and 1705 cm.$^{-1}$; $[\alpha]^{24}D$ +66° (21.8 mg., pyridine, αD +0.72°), $[M]D$ +240.

Anal. Calcd. for $C_{21}H_{32}O_6$ (364.47): C, 69.20; H, 8.85. Found: C, 69.88, 69.83; H, 9.02, 9.02.

Further recrystallization did not sharpen the m.p. nor improve the analysis.

Pregnane-5α,6β,21-triol-3,20-dione 6β,21-Diacetate (Vb).—The last three mother liquors from the recrystallization of Va were combined and evaporated. The residue (84 mg.) was dissolved in pyridine (5 ml.), and treated with acetic anhydride (2.5 ml.) (overnight at room temperature). The reaction mixture was diluted with water, and extracted with ethyl acetate. The extract was washed with saline, dried and evaporated. The oily residue was dissolved in 15 ml. of benzene–petroleum ether (1:1), and was adsorbed on a column of silica gel (5 g., previously washed with ether, and dried at 110°). The product was eluted with 225 ml. of ether–benzene (1:3). Two crystallizations from acetone–petroleum ether gave 35 mg. of pure Vb, m.p. 167–169°, with previous softening; infrared: λ_{max}^{Nujol} 3420, 1750, 1730, 1710 and 1235 cm.$^{-1}$; $[\alpha]^{24}D$ +19° (10.60 mg., αD +0.10°), $[M]D$ +85.

Anal. Calcd. for $C_{25}H_{36}O_7$ (448.54): C, 66.94; H, 8.09; OAc, 19.2. Found: C, 66.96, 67.23; H, 8.12, 8.39; OAc, 19.9.

Pregnane-5α,21-diol-3,20-dione 3,20-Bis-ethylene Ketal (VIa), Pregnane-5α,21-diol-3,20-dione 21-Acetate 3,20-

(9) The bioassays were carried out under the direction of Dr. Ralph I. Dorfman at the Worcester Foundation for Experimental Biology, Shrewsbury, Mass. It is a pleasure to acknowledge this collaboration on the biological aspects of the work.

(10) R. Antonucci, S. Bernstein, R. Littell, K. J. Sax and J. H. Williams, *J. Org. Chem.*, **17**, 1341 (1952).

(11) This preparation was carried out by William S. Allen.

Bis-ethylene Ketal (VIb) and Pregnane-5α,21-diol-3,20-dione (VIIa).—Fraction 2 (see above, preparation of Va) was reduced with lithium aluminum hydride in the same manner as above. The crystalline residue obtained was dissolved in pyridine (15 ml.) and treated with acetic anhydride (7.5 ml.) (4 days at room temperature). The reaction mixture was poured into ice-water, and the crystals were collected, 2.55 g., m.p. 149–156°, with previous softening. Seven crystallizations from acetone–petroleum ether gave 0.42 g. of a constant melting mixture, m.p. 167–168.5°, with previous softening; infrared: λ_{max}^{Nujol} 3540, 1730, 1250 and 1102 cm.$^{-1}$.

The latter fraction combined with the evaporated mother liquors was dissolved in 100 ml. of benzene–petroleum ether (1:1), and was adsorbed on alumina (125 g., Merck, acid washed). Elution with 2600 ml. of 50% ether–benzene, 400 ml. of 10% acetone–ether and 1500 ml. of 50% acetone–ether gave fractions 2A, 2B and 2C, respectively.

Recrystallization of fraction 2A from acetone–petroleum ether gave 0.37 g. of material which presumably was a mixture, m.p. 167–168°; infrared: λ_{max}^{Nujol} 3540, 1730, 1250 and 1102 cm.$^{-1}$.

Anal. Found: C, 68.49; H, 8.83.

The material (0.34 g.) was dissolved in 2.5% alcoholic potassium hydroxide (10 ml.) and was refluxed for 40 minutes. The reaction mixture was cooled, and water was added. The gelatinous precipitate so formed was collected (0.28 g.), and was recrystallized from acetone–petroleum ether. This gave 0.18 g. of a crystalline solid having a wide melting range, m.p. 162–190°, with previous softening. The latter fraction combined with its evaporated mother liquor was dissolved in 35 ml. of benzene–petroleum ether (1:1), and was adsorbed on silica gel (9.5 g.). Elution with 700 ml. of 50% ether–benzene, and subsequent crystallization from acetone–petroleum ether gave 0.11 g. of solid with a wider melting range (m.p. 166–215°). Since the material was difficult to purify, it was hydrolyzed by being refluxed for 40 minutes with 7 ml. of methanol and 0.07 ml. of 8% (v./v.) sulfuric acid. Water and salt were added to the cooled reaction mixture, and the product was extracted with ethyl acetate, benzene and ether. Evaporation of the dried extract gave 58 mg. of crude pregnane-5α,21-diol-3,20-dione (VIIa). Four crystallizations from acetone–petroleum ether gave 20 mg. of pure VIIa, m.p. 226.5–231°, with previous softening, yellowing and decomposition; infrared: λ_{max}^{KBr} 3485 and 1715 cm.$^{-1}$. Its infrared absorption spectrum was identical with that of the sample of VIIa described below.

Recrystallization of fraction 2B from ether, and acetone–petroleum ether gave 107 mg. of pure pregnane-5α,21-diol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (VIb), m.p. 151.5–153° with previous softening; infrared: λ_{max}^{Nujol} 3545, 1740, 1263 and 1108 cm.$^{-1}$; $[\alpha]^{24}D$ ±0° (10.20 mg., αD +0.01°), $[M]D$ ±0.

Anal. Calcd. for $C_{27}H_{42}O_7$ (478.61): C, 67.75; H, 8.85. Found: C, 67.83; H, 9.05.

Three crystallizations of fraction 2C from acetone–petroleum ether afforded 142 mg. of pure pregnane-5α,21-diol-3,20-dione 3,20-bis-ethylene ketal (VIa), m.p. 213–215° with previous softening; infrared: λ_{max}^{Nujol} 3480, 3390 and 1110 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{40}O_6$ (436.57): C, 68.77; H, 9.24. Found: C, 68.73; H, 9.43.

The acetate bis-ketal VIb was readily saponified by alcoholic potassium hydroxide to give the diol bis-ketal VIa, and, conversely, VIa was acetylated readily to give VIb, as confirmed by melting point, admixture melting point and infrared absorption data.

Pregnane-5α,21-diol-3,20-dione (VIIa).—The diol bis-ketal (VIa, 153 mg.) in methanol (10 ml.) and 8% (v./v.) sulfuric acid (1 ml.) was refluxed for 40 minutes. Addition of water to the cooled reaction mixture, and filtration gave 84 mg. of a mixture of the desired product VIIa and desoxycorticosterone (I), m.p. 190–197° with previous softening. Five crystallizations from acetone–petroleum ether gave 32 mg. of pure 5α,21-diol VIIa, identical in all respects with the sample of VIIa prepared above, m.p. 226.5–231.5° with previous softening, and decomposition; ultraviolet: $\lambda_{max.}$ none (end absorption only); infrared: λ_{max}^{KBr} 3520 and 1718 cm.$^{-1}$; $[\alpha]^{24}D$ +106° (7.9 mg., αD +0.42°), $[M]D$ +369.

Anal. Calcd. for $C_{21}H_{32}O_4$ (348.47): C, 72.38; H, 9.26. Found: C, 72.21; H, 9.60.

The initial acetone–petroleum ether mother liquor was evaporated to yield pure desoxycorticosterone (I), 22 mg., m.p. 142–143°; ultraviolet: $\lambda_{max.}$ 240 mμ (ε 16,900); infrared: λ_{max}^{KBr} 3508, 1718, 1680 and 1625 cm.$^{-1}$.

Pregnane-5α,21-diol-3,20-dione 21-Acetate (VIIb).—The diol-dione (VIIa, 15 mg.) in pyridine (0.5 ml.) was treated with acetic anhydride (0.5 ml.) (19 hours at room temperature). The addition of water to the cooled reaction mixture gave 16 mg. of crystals, m.p. 198–200° with previous softening. Two crystallizations from acetone–petroleum ether gave 11 mg. of pure monoacetate VIIb, m.p. 205.5–207.5° with previous softening; infrared: λ_{max}^{KBr} 3520, 1755, 1735, 1718 (shoulder) and 1238 cm.$^{-1}$; $[\alpha]^{24}D$ +109° (3.04 mg., in 0.4 ml. of absolute alcohol, 1-dm. micro-tube, αD +0.83°), $[M]D$ +425.

Anal. Calcd. for $C_{23}H_{34}O_5$ (390.50): C, 70.74; H, 8.78. Found: C, 70.44; H, 8.81.

Reaction of Phosphorus Oxychloride and Thionyl Chloride with Pregnane-5α,21-diol-3,20-dione 21-Acetate 3,20-Bis-ethylene Ketal (VIb).—Compound VIb (16 mg.) in pyridine (0.2 ml.) was treated with phosphorus oxychloride (0.02 ml.), and was allowed to stand at room temperature for 17 hours. The addition of water and cooling gave 13 mg. of crystals, m.p. 143.5–148.5°, with previous softening. Recrystallization from acetone–petroleum ether gave 6 mg. of crystals, m.p. 149–151.5° with previous softening. This material was practically pure VIb as shown by infrared absorption analysis: λ_{max}^{KBr} 3555, 1750, 1242 and 1108 cm.$^{-1}$. Compound VIb (15 mg.) was dissolved in pyridine (0.5 ml.) and was cooled in a methanol–ice-bath. Thionyl chloride (0.05 ml.) was added, and the mixture was allowed to stand at −5° for 17 hours. Addition of water, and cooling gave an orange paste which was collected, and washed with water. A solution of the paste in acetone was treated with Norite, dried and evaporated. This gave an oil which crystallized on the addition of a few drops of methanol with subsequent removal of the solvent. Infrared absorption analysis showed the crystals to be crude Δ^5-pregnene-21-ol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (IIb); λ_{max}^{KBr} 1760, 1247 and 1103 cm.$^{-1}$. Recrystallization from aqueous methanol gave 2 mg. of impure IIb, m.p. 148–155°.

Pregnane-11β,17α,21-triol-3,20-dione-5α,6α-oxide 21-Acetate 3,20-Bis-ethylene Ketal (IXa) and Pregnane-11β,-17α,21-triol-3,20-dione-5β,6β-oxide 21-Acetate 3,20-Bis-ethylene Ketal (IXb).[12]—Hydrocortisone acetate bisketal (VIII, 3.0 g.) was dissolved in chloroform (18 ml.), and perbenzoic acid (1.27 g.) in ethyl acetate (25 ml.) was added. The mixture was allowed to stand at room temperature for 6 days. It was then poured into saturated sodium carbonate solution (50 ml.) and the product was extracted with ethyl acetate. The extract was washed with water, dried and evaporated; this afforded 3.2 g. of a colorless glass.

The glass was dissolved in 25% chloroform–benzene (40 ml.) and was adsorbed on a column of alumina (200 g., Merck, untreated). Elution with 50% chloroform–benzene (200 ml.) gave 300 mg. of crystals (fraction 1), and elution with 60% chloroform–benzene (400 ml.) gave 1.6 g. of crystals (fraction 2).

Four crystallizations of fraction 1 from acetone–petroleum ether gave 138 mg. of pure 5β,6β-oxide IXb, m.p. 206–207°; infrared: λ_{max}^{KBr} 3545, 1750, 1235 and 1099 cm.$^{-1}$; $[\alpha]^{25}D$ +17° (20.40 mg., αD +0.17°), $[M]D$ +86.

Anal. Calcd. for $C_{27}H_{40}O_9$ (508.59): C, 63.76; H, 7.93. Found: C, 63.75; H, 7.96.

Four crystallizations of fraction 2 from acetone–petroleum ether gave 440 mg. of pure 5α,6α-oxide IXa, m.p. 259–261.5°; infrared: λ_{max}^{KBr} 3560, 1750, 1245 and 1096 cm.$^{-1}$; $[\alpha]^{25}D$ −39° (9.27 mg., αD −0.36°), $[M]D$ −198.

Anal. Found: C, 63.99; H, 7.98.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione 21-Acetate 3,20-Bis-ethylene Ketal (Xb). A.—Hydrocortisone acetate bis-ketal (VIII, 6.23 g.) in chloroform (30 ml.) was treated with perbenzoic acid (1.9 g.) in ethyl acetate (66 ml.) at room temperature for 5 days. Additional ethyl acetate was added, and the reaction mixture was washed several times with 5% aqueous potassium hydroxide followed by saturated

(12) This preparation was carried out by Ruddy Littell.

saline and water. The dried extract was evaporated to afford a white crystalline solid (mixture of IXa and b).

The mixture was dissolved in tetrahydrofuran (250 ml.) and ether (50 ml.). Lithium aluminum hydride (3.0 g.) was added, and the mixture was refluxed for 4 hours (and then allowed to stand at room temperature overnight). The excess hydride was decomposed cautiously with water, ethyl acetate was added, and the inorganic precipitate was removed by filtration. The solid was triturated several times with benzene–ethyl acetate. The extracts were combined, washed with saturated saline and water, dried and evaporated. This afforded a white crystalline solid which was dissolved in pyridine (15 ml.) and acetylated with acetic anhydride (7.5 ml.) (4 days at room temperature). The mixture was poured into ice-water, and was extracted with ethyl acetate. The extract was washed with saturated saline and water, and was dried. Evaporation gave a white crystalline solid which was recrystallized from acetone–petroleum ether; 2.35 g., m.p. 207–227° with previous softening. Further recrystallization did not appreciably alter the wide-range melting point, 209–227° with previous softening. The latter solid together with its evaporated mother liquor was dissolved in benzene (200 ml.), and adsorbed on a silica gel column (120 g., ether washed and re-dried at 110°). The product was eluted with 1 l. of 5% acetone–ether, and was crystallized from acetone–petroleum ether to give practically pure Xb; 0.97 g., m.p. 228–230° with previous softening. Three further crystallizations from acetone–petroleum ether gave pure Xb, m.p. 231–233° with previous softening; infrared: λ_{max}^{KBr} 3530, 1748 and 1100 cm.$^{-1}$; $[\alpha]^{24}D$ +4.1° (24.71 mg., αD +0.05°), $[M]D$ +21.

Anal. Calcd. for $C_{27}H_{42}O_6$ (510.61): C, 63.51; H, 8.29. Found: C, 63.66; H, 8.44.

B.[12]—The 5α,6α-oxide (IXa, about 0.7 g.) of hydrocortisone acetate bis-ketal was dissolved in tetrahydrofuran (90 ml.), and lithium aluminum hydride (1.25 g.) was added. The mixture was refluxed for 3 hours, cooled, and was treated cautiously with water. Ethyl acetate (*ca.* 100 ml.) was added, and the inorganic precipitate was removed by filtration. The product was worked up by extraction with ethyl acetate. Evaporation gave a white powder which was recrystallized from acetone–petroleum ether. This gave 410 mg. of the 5α,11β,17α,21-tetrol-bis-ketal Xa, m.p. 258–261°. Acetylation at room temperature (72 hours) with acetic anhydride (1.5 ml.) and pyridine (4 ml.) followed by the addition of water gave 390 mg. of the 21-acetate Xb, m.p. 227–230°. Its infrared absorption spectrum was practically identical with that of preparation A.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione 3,20-Bis-ethylene Ketal (Xa).—The 21-acetate bis-ketal (Xb, 0.50 g.) was saponified by being refluxed for 0.5 hour with 2.5% alcoholic potassium hydroxide (12 ml.). Water was added to the cooled solution and it was allowed to stand overnight at 5°. The crystals were collected and washed with water. In this manner there was obtained 0.40 g. of pure Xa, m.p. 261.5–264° with previous softening. Recrystallization from acetone–petroleum ether did not alter the m.p.; infrared: λ_{max}^{KBr} 3510 and 1100 cm.$^{-1}$; $[\alpha]^{24}D$ +6.5° (15.44 mg., αD +0.05°), $[M]D$ +32.

Anal. Calcd. for $C_{26}H_{40}O_6$ (468.57): C, 64.08; H, 8.60. Found: C, 64.01; H, 8.61.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione (XIa).—The 5α,11β,17α,21-tetrol bis-ketal (Xa, 0.42 g.) was dissolved in methanol (23 ml.), and was hydrolyzed by being refluxed for 10 minutes with 8.5% (v./v.) sulfuric acid (2.3 ml.). Water was added, the solution was neutralized with sodium bicarbonate and the mixture was saturated with salt. Crystals were formed on scratching the flask, and they were collected by filtration. In this manner there was obtained 0.12 g. of crude XIa, m.p. 251.5–255° with previous softening, browning and decomposition. Two crystallizations from acetone improved the m.p., but did not remove the small amount of Δ^4-3-ketone found present in the crude material. Consequently, the crystalline material, mother liquors and a benzene extract of the reaction mixture were combined and evaporated to dryness. The solid residue was dissolved in 50% aqueous methanol (100 ml.), and was extracted ten times with 100-ml. portions of benzene. The aqueous methanol phase was evaporated (the water was distilled azeotropically with benzene). Several crystallizations from acetone gave 59 mg. of pure XIa, m.p. 261–264° with previous softening, browning and decomposition; ultraviolet: $\lambda_{max.}$ none (end absorption only); infrared: λ_{max}^{KBr} 3530 and 1710 cm.$^{-1}$; positive Blue Tetrazolium test; $[\alpha]^{24}D$ +75° (9.30 mg., pyridine, αD +0.35°), $[M]D$ +285.

Anal. Calcd. for $C_{21}H_{32}O_6$ (380.47): C, 66.30; H, 8.48. Found: C, 66.29; H, 8.54.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione 21-Acetate (XIb).—The free steroid (XIa, 20 mg.) was dissolved in pyridine (0.5 ml.) and was treated with acetic anhydride (0.5 ml.) at room temperature for 65 hours. Addition of water to the cooled mixture gave 14 mg. of XIb, m.p. 241–244° with previous softening. Recrystallization from acetone–petroleum ether gave 12 mg., m.p. 241–244.5° with previous softening; ultraviolet: $\lambda_{max.}$ none (end absorption only); infrared: λ_{max}^{KBr} 3530, 3440, 1744, 1724 (shoulder) 1710 and 1245 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{34}O_7$ (422.50): C, 65.38; H, 8.11. Found: C, 65.10; H, 8.28.

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Characteristic Infrared Absorption Bands of Steroids with Reduced Ring A. I. Tetrahydro Compounds[1]

BY HARRIS ROSENKRANTZ AND PAUL SKOGSTROM

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A band for band analysis has been made of the infrared spectra of 50 3-hydroxy reduced ring A steroids and 57 related acetylated derivatives. The spacial isomers of the 3,5-centers of the free steroids could be differentiated by their absorption as follows: 3α,5α-structures by a band near 1005; 3β,5β- by one near 1035; 3β,5α- by absorption near 1044, 995, 978 and 956 and 3α,5β-arrangements by a band near 1041 cm.$^{-1}$. C-21 *cis* forms also gave rise to a band near 932 while the *trans* orientations absorbed nearer 941 cm.$^{-1}$. In the acetate spectra no relationship between the number of acetate groups and acetate absorption bands could be found. Some possibilities of characteristic acetate absorptions have been discussed. A combination of weak to medium weak bands near 1311, 1265, 1242, 1217 and 1127 cm.$^{-1}$ may aid eventually in the spectroscopic characterization of steroid substances.

A most thorough endeavor has been projected by several investigators in the search for steroid structure–infrared absorption correlations. Dobriner, Katzenellenbogen and Jones[2] have climaxed this

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(2) K. Dobriner, E. R. Katzenellenbogen and R. N. Jones, "Infrared Absorption Spectra of Steroids, An Atlas," Interscience Publishers, Inc., New York, N. Y., 1953.