(IX).—A suspension of 0.30 g. of 5% palladium on charcoal in 200 ml. of pyridine was shaken in an atmosphere of hydrogen for 45 min. There was then added 1.50 g. of the ethynyl compound VIII. Within 4 hr. the theoretical hydrogen uptake had been observed. The catalyst was removed by filtration and the solution concentrated to 5–10 ml. *in vacuo*. The solid which was obtained on dilution of the residue with water was recrystallized from aqueous methanol to yield 0.77 g. of product m.p. 154–156°; ν_{max} 3030, 2750, 1670, 1637, 1615 cm.$^{-1}$

Anal. Calcd. for $C_{23}H_{35}NO$: C, 80.88; H, 10.33; N, 4.10. Found: C, 81.14; H, 10.29; N, 4.18.

17β-*N*,*N*-Dimethylamino-17-cyano-3-methoxyestra-1,3,5-triene (XII).—Methylamine was bubbled through a melt of 5 g. of estrone methyl ether heated to 195° for 7 hr. The solid which was obtained on cooling was taken up in methylene chloride, and this solution washed with water and taken to dryness.

A solution of the crude product from above in 50 ml. of methylene chloride was allowed to stand for 2 hr. with 15 ml. of iodomethane. The solid which was obtained when the mixture was poured into ether (500 ml.) was dissolved in 120 ml. of acetonitrile and added to 50 ml. of 10% aqueous potassium cyanide. Following 1 hour standing the solution was diluted with water. The solid thus obtained (4.80 g.; m.p. 140–147°), was recrystallized from ethyl acetate–hexane to yield 4.15 g. of XII, m.p. 148–150°.

A sample was recrystallized once again from the same solvent; m.p. 148–150°.

Anal. Calcd. for $C_{22}H_{30}N_2O$: C, 78.06; H, 8.93; N, 8.28. Found: C, 78.27; H, 9.13; N, 8.26.

17β-*N*,*N*-Dimethyalmino-17-methyl-3-methoxyestra-1,3,5-triene (XIII). (A) **From XII.**—A solution of 1.50 g. of aminonitrile XII in 25 ml. of THF was added to 10 ml. of 3 *M* methylmagnesium bromide in ether. After 3 hr. of heating under reflux the reaction mixture was worked up in the same manner as XV to give 1.20 g. of product m.p. 104–108°. A single crystallization from aqueous methanol gave 1.10 g. of fine long needles of XIII, m.p. 110.5–112°.

The analytical sample, m.p. 110.5–111.5°, was obtained by crystallization from the same solvent.

Anal. Calcd. for $C_{22}H_{30}N_2O$: C, 80.68; H, 10.16; N, 4.68. Found: C, 80.77; H, 10.40; N, 4.52.

(B) **From XI.**—A solution of 0.021 mole of methylmagnesium bromide in 131 ml. of ether was added to a suspension of 0.88 g. of the salt XI in 30 ml. of THF. The resulting solution was heated at reflux for 2 hr. After cooling, the reaction mixture was worked up as above to give 0.30 g. of basic material, m.p. 96–110°. Two crystallizations from aqueous methanol gave 0.21 g. of XIV m.p. 104–108; mixture m.p. with XIV obtained from aminonitrile: 105–109°.

17β-Dimethylamino-17-ethynyl-3-methoxyestra-1,3,5-triene (XIV).—A solution of 1.5 g. of the intermediate XII in 25 ml. of THF was added to 0.03 mole of ethynylmagnesium bromide in 20 ml. of ether-free THF. The mixture was heated at reflux for 3 hr., allowed to cool, and worked up in the same manner as above. The crude products were recrystallized twice from chloroform hexane to afford 0.60 g. of XIV, m.p. 198–200°.

The analytical sample showed m.p. 199.5–201°.

Anal. Calcd. for $C_{23}H_{31}NO$: C, 81.85; H, 9.26; N, 4.15. Found: C, 81.94; H, 9.68; N, 4.61.

C-6 Hydroxylated Steroids. II. Preparation of 6α- and 6β-Hydroxyhydrocortisone and 6α-Hydroxyprednisolone[1]

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The preparation of 6α- and 6β-hydroxyhydrocortisone from the 5α,6α-epoxide of hydrocortisone bisethylene ketal *via* the intermediate, 5α,6β,11β,17α,21-pentahydroxypregnane-3,20-dione is described. 6α-Hydroxyprednisolone was obtained by selenium dioxide dehydrogenation of 6α-hydroxyhydrocortisone 6,21-diacetate followed by saponification.

The biochemical importance of C-6 oxygenated steroids, especially 6β-hydroxyhydrocortisone (IIIa), is now well established. Numerous publications have appeared on C-6 hydroxylation under *in vitro* conditions.[2] *Inter alia*, it has been demonstrated by incubation studies that the human adrenal contains a C-6 β-hydroxylase as this gland is capable of producing 6β-hydroxyhydrocortisone (IIIa).[2g]

6β-Hydroxyhydrocortisone (IIIa) has been isolated as an urinary metabolite in both the normal guinea pig[3] and normal human after the oral administration of hydrocortisone, and from late human pregnancy urine.[4,5] Moreover in the third trimester of normal pregnancy and in toxemia a rise in the excretion of 6β-hydroxyhydrocortisone (IIIa)[5] has been observed. This compound appears also to be the principal unconjugated urinary metabolite in the newborn human,[6] and a major urinary excretory product in human adrenal hyper-

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(6)(a) E. Colle and R. A. Ulstrom, *Am. J. Diseases Children*, **98**, 574 (1959); (b) R. A. Ulstrom, E. Colle, J. Burley, and R. Gunville, *J. Clin. Endocrinol. Metab.*, **20**, 1080 (1960).

function.[7,8] It has been reported that levels of 6β-hydroxyhydrocortisone (IIIa) in normal adult urine are higher than those of any other unconjugated hormone including hydrocortisone itself.[5,8]

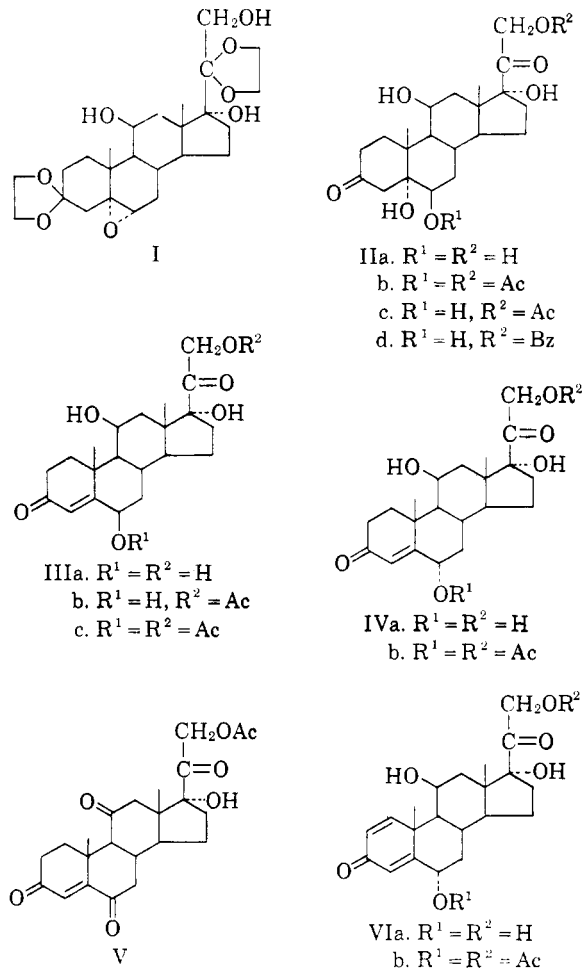
At present the source of the 6β-hydroxyhydrocortisone (IIIa) has not been established, although the increased level observed following administration of ACTH suggests an adrenal origin.[7] It has also been pointed out that it is necessary to consider that hydrocortisone metabolism has been altered leading to increased amounts of 6β-hydroxyhydrocortisone (IIIa).[7,9] The latter hypothesis is currently favored by the Columbia group.[5,10] In this connection, a study[11] based on the *in vivo* conversion of hydrocortisone by the human liver leads to the conclusion that this organ may be the site of formation of 6β-hydroxyhydrocortisone (IIIa), but that an adrenal source for some of the compound was not excluded.

Burstein and Dorfman[3] have speculated that 6α-hydroxyhydrocortisone (IVa) may also be a metabolite of hydrocortisone in the guinea pig. Frantz, Katz, and Jailer[5] have *tentatively* identified 6α-hydroxyhydrocortisone (IVa) as a metabolite in human urine in pregnancy and toxemia. More recently, the same workers[8] have isolated 6α-hydroxyhydrocortisone (IVa) from the urines of two normal men after hydrocortisone administration and from the urine of a patient with adrenal carcinoma.

6β-Hydroxyhydrocortisone (IIIa) was first synthesized *biochemically* by Hayano and Dorfman,[12] by incubation of 6β-hydroxy-substance S[13,14] with the 11β-hydroxylase system of beef adrenals. In this paper, we wish to report on the first *chemical* preparation of 6α- and 6β-hydroxyhydrocortisone which have been used as reference standard compounds in most of the above described biological work. In addition, the synthesis of 6α-hydroxyprednisolone (VIa) will be given.[15]

Treatment of the 5α,6α-epoxide (I) of hydrocortisone bisethylene ketal[16] with either perchloric or sulfuric acid in aqueous acetone resulted in the removal of both ketal groupings and in the opening of the epoxide grouping to give the water-soluble 5α,6β,11β,17α,21-pentahydroxypregnane-3,20-dione (IIa). Acetylation of the latter at room temperature overnight with excess acetic anhydride provided both the 21-monoacetate IIc and the 6β,21-diacetate IIb,[17] whereas acetylation under the same conditions with slightly less than one equivalent of acetic anhydride gave the 21-monoacetate IIc in 68% yield. Acetylation at 100° with excess acetic anhydride gave the diacetate IIb exclusively in contrast to benzoylation at 100° which resulted in esterification of only the primary hydroxyl group to give 21-benzoyloxy-5α,6β,11β,17α-tetrahydroxypregnane-3,20-dione (IId). These esterification studies thus estab-

Flow Sheet



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(17) The 6β,21-diacetate IIb usually proved difficult to obtain in a crystalline form, and generally was used as an oil in subsequent transformations. The oil was demonstrated by paper chromatographic analysis to be practically homogeneous.

TABLE I
PHYSICAL PROPERTIES OF C-6 OXYGENATED CORTICOIDS

Compound	M.p., °C.	λ_{max} mμ		$[\alpha]^{25}D$	Paper strip chromatography A(R_f)[a]	B(R_{epiF})[b]
6β-Hydroxyhydrocortisone 6,21-diacetate (IIIc)	148–150	235–236	12,100	+89° (methanol)	0.78	
6α-Hydroxyhydrocortisone 6,21-diacetate (IVb)	128–130	236	12,800	+113° (methanol)	.80	
6β-Hydroxyhydrocortisone 21-acetate (IIIb)	208–210	236	13,400	+107° (methanol)	.26	
6β-Hydroxyhydrocortisone (IIIa)	241–243	234–235	12,100	+90° (methanol)	.065	0.29
6α-Hydroxyhydrocortisone (IVa)	220–222	241	13,300	+122° (pyridine)	0.00–0.06	.22
6α-Hydroxyprednisolone 6,21-diacetate (VIb)	146–148	241–242	13,900	+80° (chloroform)	0.74	
6α-Hydroxyprednisolone (VIa)	248–250	242	13,800	+88° (methanol)	.03	.19
6-Ketocortisone acetate (V)	217–218	247–249	10,500	+119° (methanol)		

[a] System A was composed of benzene–acetic acid–*p*-dioxane–water (10:2.5:2.5:2) using 1-in. wide untreated Whatman No. 1 paper. [b] System B was composed of benzene–acetic acid–petroleum ether (b.p. 90–100°)–water (6.5:8:3.5:2).

lished, as anticipated, the hindered character of the 6β-hydroxyl group.

Dehydration of the 5α-hydroxyl group of the 6β,21-diacetate IIb was accomplished with hydrogen chloride in dry methylene chloride at 0°. The stereochemical course of this reaction, however, was not as easily controlled as has been observed with a steroid containing a C-11 carbonyl or C-11 methylene group.[13,18] Thus, when the 5α-hydroxy 6β,21-diacetate IIb was treated under anhydrous conditions with hydrogen chloride a mixture of 6β-acetoxy- and 6α-acetoxyhydrocortisone acetate (IIIc and IVb, respectively) was obtained. When the dehydration reaction mixture contained a few drops of alcohol, only the thermodynamically more stable 6α,21-diacetoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione (IVb) was obtained. Dehydration of the 5α-hydroxy group was also accomplished without acetylation of the 6β-hydroxy substituent by heating 21-acetoxy-5α,6β,-17α-trihydroxypregnane-3,20-dione (IIc) with aqueous acetic acid to produce 6β-hydroxyhydrocortisone 21-acetate IIIb after suitable chromatography. Acetylation of IIIb gave the 6β,21-diacetate IIIc.

Saponification of the 6α,21-diacetate IVb with potassium hydroxide, or, in higher yield, with potassium carbonate in methanol gave 6α-hydroxyhydrocortisone (IVa) as a solvate. Saponification of the *impure* 6β,21-diacetate IIIc with potassium carbonate in methanol followed by partition chromatography on diatomaceous earth provided 6β-hydroxyhydrocortisone (IIIa). The latter was reacetylated to give the pure 6β,21-diacetate IIIc. Both 6β-hydroxyhydrocortisone (IIIa) and its diacetate IIIc were shown by infrared analysis to be identical with the biochemically obtained samples.[19]

The structure of 6α-hydroxyhydrocortisone (IIIa) was established in a conventional manner. Monoacetylation at C-21 followed by oxidation with excess chromic acid gave the known 6-ketocortisone acetate (V),[18] thus confirming the presence of an oxygen function at the C-6 position. Saponification of the diacetate IVb (λ_{max} 236 mμ) produced the free steroid IVa (λ_{max} 241 mμ) which on reacetylation gave back the original diacetate IVb (λ_{max} 236 mμ). This bathochromic shift in the ultraviolet absorption spectrum observed in the transformation from a 6-acetoxy to a 6-hydroxy group is characteristic only of the 6α-series and not of the 6β-series[18] (Table I). Finally the relationship of the paper chromatographic mobility of 6α-hydroxyhydrocortisone (IVa) to that of 6β-hydroxyhydrocortisone (IIIa) is in conformity with the expected greater polarity of a 6α(equatorial)-hydroxy group than that of a 6β(axial)-hydroxyl group.[20] It will be noted in Table I, however, that the axial 6β-acetoxyl group is chromatographically more polar than the equatorial 6α-acetoxyl group. The same observation has been made with certain C-6-methyl compounds.[21]

Treatment of 6α-hydroxyhydrocortisone 6,21-diacetate (IVb) with selenium dioxide in *t*-butyl alcohol and acetic acid according to published procedures[22,23] gave after partition chromatography 6α,21-diacetoxy-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione (6α-hydroxyprednisolone 6,21-diacetate)(VIb). Saponification with potassium carbonate in methanol yielded 6α-hydroxyprednisolone (VIa). The structures of VIa and VIb were established by the method of synthesis, infrared spectral analyses, and their paper chromatographic behavior. Since increases in polarity of the C-3 carbonyl group induced by the extent of conjugation with unsaturated bonds are reflected in the mobility of the keto steroid, it will be seen from Table I that 6α-hydroxyprednisolone (VIa) and its 6,21-diacetate VIb show higher polarity (and therefore a greater degree of unsaturation) than 6α-hydroxyhydrocortisone (IVa) and its 6,21-diacetate IVb, respectively. It should also be observed in Table I that the ultraviolet absorption spectra of the 6α-hydroxy- and 6α-acetoxy-

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(23) C. Meystre, H. Frey, W. Voser, and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956); and T. Posthumus, M. DeWinter, and D. Van Dorp, *Rec. trav. chim.*, **75**, 475 (1956).

$\Delta^{1,4}$-3-one compound (IVa and IVb, respectively) are practically identical in contrast to the bathochromic shift observed with the corresponding Δ^{4}-3-one compounds (*vide supra*).

In Table I is given a summary of the physical properties of the C-6 oxygenated corticoids prepared herein.

Experimental

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

Absorption Spectra.—The ultraviolet absorption spectra were determined in methanol solution. The infrared absorption spectra were determined in a pressed potassium bromide disk.

Petroleum Ether.—The fraction used had a b.p. 60–70°.

5α,6β,11β,17α,21-Pentahydroxypregnane-3,20-dione (IIa).—A. To a suspension 1.1 g. of 5α,6α-epoxido-3,20-bisethylenedioxypregnane-11β,17α,21-triol (I) in 40 ml. of acetone was added 3 ml. of 1.5 *N* perchloric acid, and the resulting clear solution was allowed to stand at room temperature for 17 hr. After concentration under reduced pressure the reaction mixture was extracted with ethyl acetate, and the extract was washed with saturated ammonium chloride, saturated sodium bicarbonate, and finally with saturated saline. After drying and evaporation, 500 mg. of an oil was obtained. Several crystallizations from acetone–petroleum ether gave the pure pentol IIa, m.p. 273–274° dec., λ_{max} none; $[\alpha]^{25}D$ +35° (pyridine); ν_{max} 3650 and 1710 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{32}O_7$ (396.47): C, 63.61; H, 8.14. Found: C, 63.44; H, 8.46.

B. To a suspension of 10 g. of I in 400 ml. of acetone was added 2 ml. of sulfuric acid in 20 ml. of water, and the solution was stirred at room temperature for 4 hr. The solution was then neutralized with 10% potassium hydroxide in methanol and the inorganic precipitate was removed by filtration. The filtrate, on evaporation under reduced pressure, provided a residue which was leached several times with ethyl acetate. Evaporation of the extract and crystallization from acetone gave 1.0 g. of pentol IIa, m.p. 272–274°.

The above residue, after being leached with ethyl acetate, was dissolved in hot acetone, filtered through a column of magnesium silicate adsorbent and the column was washed with acetone. Evaporation and crystallization of the residue from acetone–petroleum ether gave 1.28 g. of IIa, m.p. 279–281°. Concentration of the mother liquor gave an additional 2.0 g., m.p. 267–269° for a total yield of 4.28 g. (49%).

21-Acetoxy-5α,6β,11β,17α-tetrahydroxypregnane-3,20-dione (IIc) and 6β,21-Diacetoxy-5α,11β,17α-trihydroxypregnane-3,20-dione (IIb).—A. A solution of 425 mg. of 5α,6β,11β,17α,21-pentahydroxypregnane-3,20-dione (IIa) in 4 ml. of pyridine and 0.4 ml. of acetic anhydride was allowed to stand at room temperature for 16 hr. The mixture was poured into water and extracted with ethyl acetate. The extract was washed with saturated saline, dried, and evaporated to give a white glass. Crystallization from acetone–petroleum ether gave 125 mg. of white crystals, m.p. 252–256°. Three recrystallizations from acetone–petroleum ether gave the pure 21-monoacetate IIc, m.p. 280–282°; $[\alpha]^{25}D$ +35° (pyridine), ν_{max} 3410, 1728, 1698, and 1242 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{34}O_8$ (438.50): C, 62.99; H, 7.82; OAc, 9.8. Found: C, 62.62; H, 8.00; OAc, 9.2.

The mother liquor from the first crystallization above was evaporated and the residue was crystallized from benzene to give 135 mg. of the diacetate IIb, m.p. 145–155°. Recrystallization from acetone–benzene did not change the melting point; $[\alpha]^{25}D$ +6° (pyridine); ν_{max} 3480, 1726, and 1242 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{36}O_9$ (480.54): C, 62.48; H, 7.55. Found: C, 62.46; H, 7.81.

B. A solution of 940 mg. of the pentol IIa in 5 ml. of pyridine and 3 ml. of acetic anhydride was allowed to stand at 100° for 1 hr. The mixture was cooled and water was added. This gave an oil which, after cooling, was separated by decanting the supernatant liquid and was then dissolved in ethyl acetate. The solution was washed with saturated saline, dried, and evaporated to give 1.1 g. of the diacetate IIb as an oil, λ_{max} none. Paper strip chromatographic analysis indicated that the product contained only trace impurities and its mobility was identical to the diacetate IIb prepared above.

C. A solution of 4.7 g. (11.9 mmoles) of the pentol IIa in 25 ml. of pyridine and 1.2 ml. (11.8 mmoles) of acetic anhydride was allowed to stand at room temperature for 24 hr. The mixture was then extracted with ethyl acetate, and the extract was washed twice with saturated saline, dried, and evaporated. Ether was added to the solid residue which was collected by filtration to give 3.5 g. of the 21-monoacetate IIc, m.p. 277–279°. The infrared spectrum was identical to that of the sample obtained in A.

21-Benzoyloxy-5α,6β,11β,17α-tetrahydroxypregnane-3,20-dione (IId).—A mixture of 300 mg. (0.76 mmole) of 5α,6β,11β,17α,21-pentahydroxypregnane-3,20-dione (IIa), 8 ml. of pyridine, and 240 mg. (1.7 mmoles) of benzoyl chloride was heated at 100° for 1.5 hr. After being cooled, the solution was poured into water and 380 mg. of white powder, m.p. 235–237°, was collected by filtration. Two recrystallizations from acetone gave the pure 21-monobenzoate IId, m.p. 296–298°; λ_{max} 228 mμ (ε 15,100); $[\alpha]^{25}D$ +80° (pyridine); ν_{max} 3420, 1709, 1602, 1582, 1450, and 1275 cm.$^{-1}$.

Anal. Calcd. for $C_{28}H_{36}O_8$ (500.57): C, 67.18; H, 7.25. Found: C, 67.07; H, 7.39.

6α,21-Diacetoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione (6α-Hydroxyhydrocortisone 6,21-Diacetate) (IVb).—A. A stream of dry hydrogen chloride gas was passed through a solution of 900 mg. of noncrystalline 6β,21-diacetoxy-5α,11β,17α-trihydroxypregnane-3,20-dione (IIb) in 100 ml. of dry methylene chloride at 0° for 1 hr. After being washed with ice water and with cold 1 *N* sodium bicarbonate the solution was dried and evaporated. The resulting oil was shown by paper strip chromatographic analysis to contain a mixture of 6α- and 6β-epimers. Chromatography on 80 g. of magnesium silicate adsorbent gave, from 8% acetone in benzene, 300 mg. of a white solid which was crystallized from acetone–benzene to give 160 mg., m.p. 126–129°. Recrystallization from acetone–benzene resulted in the pure 6α,21-diacetate IVb, m.p. 128–130°; λ_{max} 236 mμ (ε 12,800); $[\alpha]^{25}D$ +107° (chloroform); ν_{max} 3480, 1750, 1730, 1668, 1650, and 1232 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{34}O_8$ (462.52): C, 64.92; H, 7.41. Found: C, 64.72; H, 7.69.

The 6β-epimer could not be isolated in pure form from this reaction.

B. A stream of dry hydrogen chloride gas was passed through a solution of 900 mg. of noncrystalline IIb in 60 ml. of methylene chloride containing 4 drops of ethanol. The reaction was allowed to proceed at 0° for 1 hr. after which the reaction mixture was washed with cold, saturated sodium carbonate and once with saturated saline. Evaporation of the dried extract gave a crude product which was crystallized once from acetone–benzene, 490 mg. of IVb, m.p. 126–130°; λ_{max} 236 mμ (ε 13,500); $[\alpha]^{25}D$ +113° (methanol).

C. A solution of 30 mg. of 6α-hydroxyhydrocortisone (IVa) in 1.0 ml. of pyridine and 0.5 ml. of acetic anhydride was allowed to stand at room temperature for 18 hr. Evaporation of the solvents followed by crystallization of the crude residue from acetone–benzene gave 24 mg. of the diacetate IVb, m.p. 126–128°; λ_{max} 235–236 mμ (ε 12,600).

6α,11β,17α,21-Tetrahydroxypregn-4-ene-3,20-dione (6α-Hydroxyhydrocortisone) (IVa).—A mixture of 200 mg. of 6α-hydroxyhydrocortisone 6,21-diacetate (IVb), 30 ml. of methanol, and 0.65 ml. of 10% aqueous potassium carbonate solution was agitated at room temperature with a stream of nitrogen for 30 min., when 0.3 ml. of acetic acid was added. Concentration under reduced pressure and addition of water gave 118 mg. of white crystals, m.p. 216–217°. Recrystallization from acetone gave 84 mg. of the tetrol IVa, m.p. 220–222°; λ_{max} 241 mμ (ε 13,300).

In another run with 720 mg. of noncrystalline diacetate IVb, 50 ml. of methanol, and 6.6 ml. of 0.51 *N* potassium hydroxide, the crude product obtained as above, was crystallized from acetone to give 185 mg. of IVa, m.p. 217–220°. The sample for analysis was obtained by two crystallizations from acetone and was solvated, m.p. 220–222°; $[\alpha]^{25}D$ +122° (pyridine); ν_{max} 3460, 3320 (shoulder) 1722, and 1645 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{30}O_6$ (378.45): C, 66.64; H, 7.99. Found: C, 64.42; H, 7.96.

6β,11β,17α,21-Tetrahydroxypregn-4-ene-3,20-dione (6β-Hydroxyhydrocortisone) (IIIa).—A solution of 1.2 g. of crude 6β-hydroxyhydrocortisone 6,21-diacetate (IIIc) (obtained from the dehydration of IIb) in 100 ml. of methanol and 4.0 ml. of 10% aqueous potassium carbonate was agitated at room temperature with a stream of nitrogen for 30 min., when 2.0 ml. of acetic acid was added. The reaction mixture was extracted twice with ethyl acetate and five times with chloroform. The combined extracts were dried and evaporated to afford a yellow glass which was subjected to partition chromatography on 400 g. of diatomaceous earth with an ethyl acetate–ethylene glycol solvent system. Concentration of the third hold-back volume gave, after the addition of water, 78 mg. of white crystals, m.p. 239–242°; λ_{max} 234–235 mμ (ε 12,000). Crystallization from acetone gave 43 mg. of 6β-hydroxyhydrocortisone (IIIa), m.p. 241–243°; $[\alpha]^{25}D$ +90° (methanol); ν_{max} 3395, 1712, and 1665 cm.$^{-1}$. Infrared spectral analysis showed this compound to be identical with a reference sample.[14]

21-Acetoxy-17α-hydroxypregn-4-ene-3,6,11,20-tetrone (6-Ketocortisone Acetate) (V).—To a solution of 116 mg. (0.305 mmole) of 6α,11β,17α,21-tetrahydroxy-4-pregnene-3,20-dione (IVa) [m.p. 213–215°, λ_{max} 241 mμ (abs. alc.)] in 2 ml. of dry pyridine at −10° was added 0.9 ml. (0.325 mmole of acetic anhydride) of a stock solution of acetic anhydride (37 mg.) in pyridine (1 ml.), and the mixture was allowed to stand at −5° for 17 hr. The reaction mixture was extracted with ethyl acetate, and the extract was washed with dilute sulfuric acid, dilute sodium bicarbonate solution, and finally with water. After being dried the extract was evaporated to afford 128 mg. of a white glass. The latter was then treated with 4 ml. of acetic acid containing 50 mg. of chromic anhydride and 3 drops of water. The mixture was extracted with ethyl acetate, and the extract was washed with cold sodium bicarbonate solution and water, dried, and evaporated. The residue was subjected to partition chromatography on diatomaceous earth with the system, toluene (6 parts)–petroleum ether (4 parts)–methanol (6.5 parts)–water (3.5 parts), to give 15 mg. of a white solid, m.p. 214–215°. Two recrystallizations from acetone–petroleum ether gave 7 mg. of the tetrone V, m.p. 217–218°; λ_{max} 247–249 mμ (ε 10,500); $[\alpha]^{25}D$ +119° (chloroform); ν_{max} 3420, 1742 (shoulder), 1708, 1322, and 1235 cm.$^{-1}$ [lit.,[13] m.p. 210–212°; λ_{max} 245 mμ (ε 11,000); $[\alpha]^{20}D$ +115° (chloroform)].

6β,21-Diacetoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione (IIIc).—To the mother liquor residues from the crystallization of 6β-hydroxyhydrocortisone (IIIa) was added 3 ml. of pyridine and 2 ml. of acetic anhydride. After standing at room temperature for 18 hr., the solution was dissolved in ethyl acetate, washed with dilute sodium carbonate solution, dilute hydrochloric acid solution, and finally with saturated saline. The extract was concentrated to a small volume and poured into water. The resulting solid, 30 mg., m.p. 138–140°, was crystallized from ethanol–water to give 26 mg. of IIIc, m.p. 148–150°; λ_{max} 235–236 mμ (ε 12,100), $[\alpha]^{25}D$ +89° (methanol), ν_{max} 3400, 1742 (shoulder), 1725, 1660, and 1230 cm.$^{-1}$. Infrared spectral analysis showed this compound to be identical with a reference sample.[14]

21-Acetoxy-6β,11β,17α-trihydroxypregn-4-ene-3,20-dione (6β-Hydroxyhydrocortisone 21-Acetate) (IIIb).—A solution of 3 g. of impure 21-acetoxy-5α,6β,11β,17α,21-pentahydroxypregnane-3,20-dione (IIc) in 15 ml. of acetic acid containing 1.5 ml. of water was heated on a steam bath for 1.5 hr. The mixture was then extracted with ethyl acetate, and the extract was washed with saturated sodium bicarbonate solution and saturated saline, dried, and evaporated. The crude product, 2 g. of a yellow oil, λ_{max} 235 mμ (ε 5800), so obtained was subjected to partition chromatography on diatomaceous earth with the solvent system, methylene chloride (8 parts)–ethyl acetate (2 parts)–ethylene glycol (1 part). The second hold-back volume gave, after crystallization from acetone–petroleum ether, 135 mg. of 6β-hydroxyhydrocortisone 21-acetate (IIIb), m.p. 206°; λ_{max} 234 mμ (ε 14,000). Two recrystallizations from the same solvent pair gave solvated IIIb, m.p. 208–210° (with solidification and remelt at 220°); λ_{max} 236 mμ (ε 13,400); $[\alpha]^{25}D$ +107° (methanol); ν_{max} 3510, 1740, 1672, and 1235 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{32}O_7$ (420.49): C, 65.59; H, 7.67. Found: C, 64.99; H, 7.75.

A small sample of IIIb was acetylated to give 6β-hydroxyhydrocortisone 6,21-diacetate (IIIc), m.p. 149–150°.

6α,21-Diacetoxy-11β,17α-dihydroxypregna-1,4-diene-3,20-dione (6α-Hydroxyprednisolone 6,21-Diacetate) (VIb).—To a solution of 265 mg. of 6α-hydroxyhydrocortisone 6,21-diacetate (IVb) in 30 ml. of *t*-butyl alcohol and 1.5 ml. of acetic acid was added 200 mg. of selenium dioxide, and the mixture was heated at reflux for 18 hr. The cooled mixture was stirred for 0.5 hr. with deactivated Raney nickel catalyst, filtered, and evaporated. The residue was dissolved in ethyl acetate and the extract was washed successively with water, cold saturated sodium carbonate and finally with saturated saline, dried, and evaporated. The resulting 215 mg. of yellow glass, λ_{max} 241 mμ (ε 12,400), was subjected to partition chromatography on diatomaceous earth with the solvent system, *n*-heptane (3 parts)–ethyl acetate (3 parts)–methanol (3 parts)–water (2 parts). The fifth hold-back volume gave, after crystallization from acetone–benzene, 120 mg. of 6α-hydroxyprednisolone 6,21-diacetate (VIIb), m.p. 144–146°. Two further crystallizations from the same solvent pair gave the sure sample of VIb; m.p. 146–148°; λ_{max} 241–242 mμ (ε 13,900); $[\alpha]^{25}D$ +80° (chloroform); ν_{max} 3380, 1738, 1720, 1654, 1609, 1590, and 1230 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{32}O_8$ (460.51): C, 65.20; H, 7.00. Found: C, 65.08; H, 7.48.

6α,11β,17α,21-Tetrahydroxypregna-1,4-diene-3,20-dione (6α-Hydroxyprednisolone) (VIIa).—A mixture of 290 mg. of 6α-hydroxyprednisolone 6,21-diacetate VIb in 45 ml. of methanol and 1.0 ml. of 10% aqueous potassium carbonate was agitated with a stream of nitrogen for 0.5 hr. Addition of 0.5 ml. of acetic acid and several ml. of water followed by concentration under reduced pressure gave 150 mg. of white crystals, m.p. 239–241°. Two crystallizations from acetone–benzene gave the constant melting sample of 6α-hydroxyprednisolone (VIa) in a solvated state, m.p. 248–250°; λ_{max} 242 mμ (ε 13,800); $[\alpha]^{25}D$ +88° (methanol); ν_{max} 3420, 1712, 1656, and 1602 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{28}O_6$ (376.44): C, 67.00; H, 7.50. Found: C, 66.28; H, 7.97.

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11-Alkylated Steroids. IV.[1] Synthesis and Reactions of Olefins Derived from 11β-Hydroxy-11-methyl-5β-pregnane-3,20-dione

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The dehydration of 11β-hydroxy-1-methyl-5β-pregnane-3,20-dione yielded both the $\Delta^{9(11)}$- and Δ^{11}-11-methyl steroids. Employing these olefinic intermediates, the chemistry of C-ring altered steroids has been investigated. The structures of many of these products were elucidated by their NMR spectra and an interesting unshielding effect of allylic systems was observed.

Among recent reports on the reaction of methyllithium with 11-keto steroids[2a,2b,3] the preparation of 11β-hydroxy-11-methyl-5β-pregnane-3,20-dione (I), a potent central nervous system depressant,[4] was described. Prompted by the marked biological properties of this compound, the present work was undertaken as part of the continuing investigation of 11-alkylated steroids.

By the method of Drake, Fonken, and Howard[5] (*N*-bromoacetamide–pyridine–sulfur dioxide) 11β-hydroxy-11-methyl-5β-pregnane-3,20-dione (I) was dehydrated giving a mixture of 11-methyl $\Delta^{9(11)}$-5β-pregnene-3,20-dione (II) and 11-methyl-Δ^{11}-5β-pregnene-3,20-dione (III). The composition of this mixture was established by NMR spectroscopy as roughly 85% of the $\Delta^{9(11)}$ (II) and 15% of the Δ^{11}-pregnene (III).

Ozonolysis of the above mixture of the olefins yielded 46% of a neutral tetrone (IV) in addition to 3% of a polyketo acid (IVa) which was characterized only by its infrared spectrum. The 9,11-secotetrone (IV) gave upon treatment with base the expected aldol products (V and VI) characterized by a variety of chemical and physical methods including NMR, ultraviolet, infrared, and X-ray unit cell determination. While the isomeric triones V and VI differed in their physical constants and NMR spectra, they could not be distinguished. The other somewhat more remote possibilities for the structure of aldol products [structures (VII) and (VIII)] were incompatible with the ultraviolet spectrum of the α,β-unsaturated ketone system [$\lambda_{max}^{C_2H_5OH}$ 239 mμ (log ε 4.08)]. Construction of a Courtaud Model[6] of the isomeric C-homo steroid (IX) indicated that the unsaturated ketone system of this molecule could not become co-planar and since the ultraviolet and infrared spectra (γ_{max} 1715, 1650, and 1625 cm.$^{-1}$) were normal this possibility was unlikely. Further the C-homo possibility (IX) appeared highly unlikely since the 9,11-secobisketal (XXI) did not undergo aldol condensation. The NMR spectra of the aldol products, in addition to the two angular methyl absorptions[7] at 9.07 and 8.60 and at 9.12 and 8.62 τ,[8] showed methyls on double bonds as close doublets at 8.03 and 8.02 τ, respectively. The magnitude of the coupling constants (both 1.5 c.p.s.) was indicative of splitting through a double bond by an adjacent vinyl hydrogen which was present as a multiplet in the two condensation products at 4.10 or 4.19 τ, respectively. Structures VII, VIII, and IX lacked this configuration and were thus clearly eliminated.

In addition to the tetrone IV and keto acid IVa the 9α,11α-epoxide (X) derived from the original olefin was isolated from the ozonization mixture. The structure of this compound was established by three independent syntheses from the 11-methyl-$\Delta^{9(11)}$-olefin (II) employing separately trifluoroperacetic, chromic acid, and potassium permanganate as epoxidizing agents, all of which would be expected to attack the relatively unhindered backside (α-attack) of the molecule. Further evidence for this structural assignment was the inactivity of the epoxide towards perchloric acid in acetone. (9β,-11β-Epoxides are known to open hydrolytically

(1) This paper was presented at the Michigan–Toledo–South Bend Meeting-in-Miniature of the American Chemical Society, February 27, 1960. Since the original presentation of this paper, the chemistry of some 5α-11-methyl steroids has appeared [J. Elks, *J. Chem. Soc.*, 3333 (1960)].

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(7) For Basic Steroid NMR assignments see J. N. Schoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958).

(8) These spectra were measured in a Varian A-60 spectrometer in $CDCl_3$ solution.