strontium carbonate[3] in 6 ml. of benzene and the mixture stirred in a hydrogen atmosphere at room temperature until absorption of the gas ceased (11-ml. uptake). After removal of the catalyst filtration and the benzene by distillation at reduced pressure, the residue was boiled with 2 ml. of petroleum ether for 1 min., cooled, and filtered free of 15 mg. of a white, insoluble solid. After cooling the petroleum ether filtrate in the refrigerator overnight, there was deposited 85 mg. (73%) of the ester **34**, m.p. 89–90°, as thick, colorless prisms. The melting point of a mixture of this material and that (m.p. 89–90°) prepared (diazomethane) from the corresponding tricyclic keto acid supplied by Professor R. B. Woodward was also 89–90°. The infrared and ultraviolet spectra of the two samples were also identical.

Anal. Calcd. for $C_{17}H_{24}O_3$: C, 73.88; H, 8.75. Found: C, 74.00; H, 8.84.

Infrared: $\lambda_{max}^{Nujol\ mull}$ 5.78 μ (—$COOCH_3$); 6.0 μ (> C=O); 6.22 μ (> C=C <).

Ultraviolet: λ_{max}^{alc} 248 mμ (ϵ 15500).

11-Oxygenated Pregnenolones. II. Synthesis of 11α-Hydroxy-, 11β-Hydroxy-, and 11-Ketopregnenolones and 11-Keto-17α-pregnenolone

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6β-Hydroxy-3,5α-cyclopregnan-20-one has been hydroxylated microbially in high yield to 6β,11α-dihydroxy-3,5α-cyclopregnan-20-one. From this intermediate are synthesized 11α-hydroxy-, 11β-hydroxy-, and 11-ketopregnenolones and 11-keto-17α-pregnenolone. 6β,11β-Dihydroxy-3,5α-cyclopregnenolone has also been prepared by microbial hydroxylation of *i*-pregnenolone. The configuration of the side chain of steroid 20-ketals has been rigorously established.

While 3β-hydroxy-Δ^5-steroids such as pregnenolone and dehydroepiandrosterone occur widely in nature, their 11-hydroxy derivatives have only recently been described.[1,2] In a preliminary communication[2] we described the high yielding microbiological conversion of 6β-hydroxy-3,5α-cyclopregnan-20-one (I) to 6β,11α-dihydroxy-3,5α-cyclopregnan-23-one[1b,2] (II) and the subsequent conversion of this compound to 11α-hydroxypregnenolone[1-3] (III).

We now wish to report the experimental details of the microbial 11α- and 11β-hydroxylations of *i*-pregnenolone (I) as well as the synthesis of some simple 11-oxygenated pregnenolones. 6β-Hydroxy-3,5α-cyclopregnan-20-one[4] (I), prepared from pregnenolone (readily available from diosgenin) by the *i*-steroid rearrangement is hydroxylated by the mold *Rhizopus nigricans* (ATCC 6227b) in high yield to give the 6β,11α-diol (II).[5] The structure of this product was confirmed[6] by acid-catalyzed rearrangement to 11α-hydroxypregnenolone (IIIa) followed by Oppenauer oxidation of this diol to the microbial metabolite 11α-hydroxyprogesterone.[7] To synthesize the remaining 11-oxygenated pregnenolones, a method was required for differentiating the oxygen functions at C-11 and C-3 (or C-6). Three sequences, namely selective esterification, selective oxidation, and homoallylic displacement with chloride ion, were investigated in order to block chemically the C-3 oxygen function so that the C-11 oxygen might be both oxidized and reduced selectively.

Initially selective esterifications at C-3 were attempted. Reaction of the diol (II) with formic acid at low temperature resulted in the formation of the diformate (IIIb), which was hydrolyzed to the diol (IIIa). Treatment of the diol (II) with hot glacial acetic acid resulted in the isolation of a small amount of the desired 3-monoacetate (IIIc); however, the diacetate was the major product. The structure of the monoacetate (IIIc) was established by oxidation to 11-ketopregnenolone acetate (IXa, *vide infra*). Acetylation of the diol (IIIa) with a single mole of acetic anhydride was also unselective.

A second approach involved the Oppenauer oxidation of the 6,11-diol (II). This reaction was selective in producing in good yield the 11α-hydroxy-6,20-dione (V). However, the reactivities of the 6- and 23-ketonic functions are similar; consequently, this sequence offered little opportunity for the synthesis of the desired intermediates.

Lastly, treatment of the 6,11-diol (II) with concentrated hydrohalic acid under heterogeneous conditions provided the requisite protection of the "3-hydroxyl group" to allow further synthetic elaborations. Treatment with hydrochloric acid provided a mixture of chloro compounds (VIa) epimeric at C-17. The composition of this mixture was established by n.m.r.[8] which indicated approximately 97% of the 17β- and 3% of the 17α-isomer were present. Hydrobromic acid gave similar results.

Oxidation of the isomeric chlorides (VIa) gave a separable mixture of diones VIIa (17β) and VIII (17α). The configurations of the side chains of VIIa and VIII were established by the chemical shifts of the C-18 protons in their n.m.r. spectra.[6] Treatment of the

(1) (a) I. Chuma, Japanese Patent Specification 17231 (1960); (b) Y. Kurosawa, *J. Agr. Chem. Soc., Japan*, **32**, 515 (1958).

(2) W. J. Wechter and H. C. Murray, *Chem. Ind.* (London), 411 (1962).

(3) E. S. Rothman and M. S. Wall, *J. Am. Chem. Soc.*, **81**, 411 (1959), reported the synthesis of this compound from 3β-hydroxypregna-5,16-diene-11,20-dione (*via* synthetic 11-ketodiosgenin) by catalytic reduction. While the melting point of the material prepared by Y. Kurosawa[1b] and that prepared in our laboratory agree with that reported by Rothman and Wall, the specific rotation does not ($[\alpha]^{CHCL_3}D$ +70° *vs.* $[\alpha]^{CHCL_3}D$ +15°).

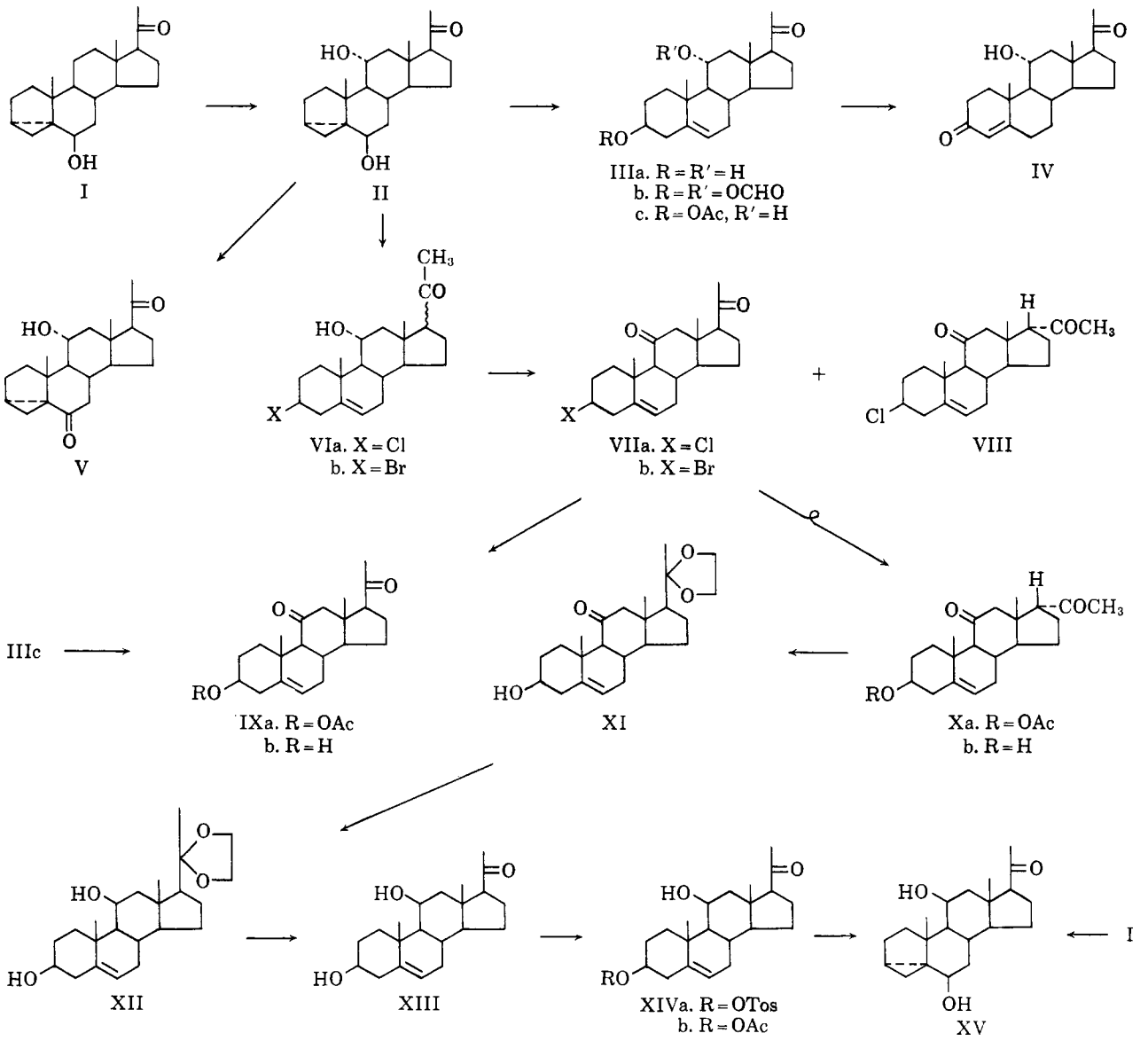
(4) V. Petrow, I. A. Stewart-Webb, and D. K. Patel, U. S. Patent 2,816,901 (1959).

(5) Earlier efforts to hydroxylate pregnenolone selectively at C-11 proceeded only in poor yield, if at all, owing to a competing facile oxidation at C-7 under aerobic conditions. *Cf.* A. Kramli and J. Horvath, *Nature*, **163**, 219 (1949) and D. H. Peterson, *Record Chem. Progr.*, **17**, 211 (1956).

(6) The configuration of the pregnane side chain was established in all cases by n.m.r. G. Slomp (private communication) has found by the examination of model compounds of known configuration (see ref. 12 for examples) that the C-18 proton absorption appears at about 36 to 40 c.p.s. (measured downfield from tetramethylsilane) in 17β isomers, while it is shifted downfield to 51 to 54 c.p.s. in the corresponding 17α isomers.

(7) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister, and H. M. Leigh, *J. Am. Chem. Soc.*, **74**, 1933 (1952).

(8) The quantitative constitution of the mixture was determined by integration of the area of the C-18 proton absorptions characteristic of the 17α and 17β compounds.



mixed chlorides VIIa and VIII (containing about 2 to 3% of the 17α- and 97 to 98% of the 17β-isomer) with zinc acetate in refluxing glacial acetic acid gave in high yield a mixture of isomeric acetates (IXa) and (Xa). Vapor phase chromatography (v.p.c.) employing 2% silicone gum as a liquid phase established that this mixture consisted of 15% of 17α- (Xa) and 85% of 17β-epimeric 11-ketopregnenolone acetate (IXa). The composition of this mixture was confirmed by its n.m.r. spectrum which indicated 14% of 17α- and 86% of 17β-isomer. Consequently, the zinc acetate–acetic acid treatment resulted in considerable isomerization of the pregnane side chain reminiscent of the equilibration of pregnenolone with base.[9] Hydrolysis of the mixed acetates (IXa) and (Xa) afforded quantitatively, approximately 83% of 11-ketopregnenolone (IXb) and 17% of 11-keto-17α-pregnenolone (Xb). The configurations of these epimeric 11-ketopregnenolones were established by n.m.r.

Ketalization with ethylene glycol of *either* of the epimeric 11-ketopregnenolones IXb or Xb, or a mixture of the two epimers gave the *same* 20-ketal (XI). Hydrolysis of this ketal (XI) with aqueous hydrochloric acid in acetone afforded a mixture estimated by v.p.c. to be 97.5% of the 17β- (IXb) and 2.5% of the 17α-isomer (Xb). Finally it was established that under the conditions of ketal hydrolysis the 17α-dione (Xb) is isomerized to a mixture of the isomeric ketones. Two alternative possibilities would explain this set of facts. Either ketalization and hydrolysis of the 17β-isomer (Xb) both proceed principally with retention or both proceed with inversion at C-17. The 17α-isomer (Xb), on the other hand, must proceed either by ketalization with inversion followed by hydrolysis with retention at C-17 or ketalization with retention followed by hydrolysis with inversion. From the time of the earliest reports[10] of the formation of 20-ketals, retention of configuration at C-17 has been assumed[11] in both ketalization and hydrolysis. To our knowledge, the C-17 configuration of unsubstituted 20-ketals (*i.e.*, C-17 substitution = H) has not been rigorously established.

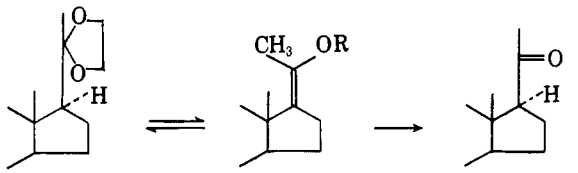
In order to determine the C-17 configuration of the ketal (XI), a sample was hydrolyzed in 67% acetic

(9) A. Butenandt and G. Fleischer, *Ber.*, **70**, 96 (1937) found that pregnenolone gave a mixture composed of 17β- (70%) and 17α- (30%) isomers with methanolic potassium hydroxide.

(10) R. Antonucci, S. Bernstein, R. Lenhard, K. J. Sax, and J. H. Williams, *J. Org. Chem.*, **17**, 1369 (1952).

(11) For example see: E. P. Oliveto, T. Clayton, and E. B. Hershberg, *J. Am. Chem. Soc.*, **75**, 486 (1953).

acid–H^2: 33% deuterium oxide. An excellent yield of the 17β-isomer (IXb) of the free ketone was isolated and the mother liquors from this reaction shown by thin layer chromatography (t.l.c.) not to contain detectable amounts of the epimeric 17α-isomer (Xb) or starting ketal XI. This material contained one labile atom of deuterium on the 3-hydroxyl which was readily exchanged by solution in ethanol. After exchange of the labile 3-hydroxy hydrogen, the compound IXb contained no deuterium. It is necessary that an intermediate enol (A) accompany inversion at C-17 and that such an enol must incorporate an atom of deuterium at C-17 upon ketonization or reketalization. It can be concluded therefore that (1) the configuration of the ketal (XI) is 17β; (2) the ketalization and hydrolysis reactions of the 17β-isomer involves retention of the C-17 configuration; (3) the 17α-isomer undergoes ketalization with inversion and hydrolysis with retention of the C-17 configuration.



XI A IXb

R = H, CH_2CH_2OH

Reduction of the ketal (XI) with sodium borohydride gave the 11β-hydroxy ketal (XII), which was in turn hydrolyzed to 11β-hydroxypregnenolone (XIII). The axial nature of the C-11 hydroxyl was established not only by its differences in physical properties, compared with those of the 11α-alcohol IIIa but also by the fact that both the monoacetyl (XIVb) and mono-*p*-toluenesulfonyl esters (XIVa) could be prepared quantitatively. The configuration of the side chain was established by both the n.m.r. and optical rotatory dispersion spectra. The latter spectrum exhibited a positive Cotton effect curve characteristic[12] of the 17β-configuration.

6β,11β - Dihydroxy - 3,5α - cyclopregnan - 20 - one (XV) was prepared by fermentation of i-pregnenolone (I) with the mold *Curvularia lunata* (ATCC 12017). The structure of this product was established by comparison with authentic material prepared by the i-steroid rearrangement of the tosylate (XIVa) in acetone–water with potassium acetate.

Thus, by biological hydroxylation of 6β-hydroxy-3,5α-cyclopregnan-20-one, all of the simple 11-oxygenated pregnenolones are readily available in high yield and these in turn serve as useful intermediates for the synthesis of the more complex cortical hormones.

Experimental[13,14]

6β,11α-Dihydroxy-3,5α-cyclopregnan-20-one (II).[1b]—A medium was prepared of 2.5 kg. of cornsteep liquor (60% solids) and 1250 g. of commercial dextrose diluted with tap water to 125 l. and adjusted to a pH of about 5.5; 250 ml. of lard oil was added for foam preventive. This sterilized medium (125 l.) was inoculated with a 72-hr. vegetative growth of *Rhizopus nigricans* (ATCC 6227*b*), a culture which is available from the American Type Culture Collection, Washington, D. C., and incubated for 72 hr. at a temperature of about 28° using a rate of aeration of 5 l. per min. at 300 r.p.m. After 72 hr. of agitation, at which time the pH of the beer was 7.2, a solution of 20 g. of 6β-hydroxy-3,5α-cyclopregnan-20-one in 300 ml. of *N*,*N*-dimethylformamide was added to the inoculated medium. Acetone (100 ml.) was used to wash the substrate into the tank. After an additional 24-hr. period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the wash water was added to the beer filtrate. The beer filtrate thus obtained was extracted four times with a volume of methylene chloride: ethyl acetate (2:1 by volume) equal to one fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude solid. The crude dry product was taken up in about 700 ml. of acetone and the solution treated with Darco G-60 activated carbon (7 g.) and filtered. The solution was distilled to a volume of about 150 ml. whereupon the product crystallized to give 14.26 g. of crystalline steroid, m.p. 211.5–217°. The mother liquors from this crystallization afforded two additional crops which were combined and recrystallized to give 2.22 g. of material, m.p. 211.5–217° (total yield 82%). Three recrystallizations from acetone afforded an analytical sample, m.p. 211.5–217.0°,[15] $[\alpha]_D$ +92° ($CHCl_3$, 1.0), homogeneous by paper chromatogram (4 systems), infrared ν_{max} 3500, 3430, and 1695 cm.$^{-1}$; n.m.r. 18-H, 43 c.p.s. (s), 19-H, 73 c.p.s. (s), 21-H, 129 c.p.s. (s), 6αH 195 c.p.s. (t), 11βH, 40 c.p.s. (m) (indicative of 17β-configuration).[6,11]

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.02; H, 10.00.

3β,11α-Dihydroxy-5-pregnen-20-one (IIIa).[1a]—The *i*-steroid II (5.0 g.) was dissolved in boiling acetone (250 ml.) which contained 0.1 *N* hydrochloric acid (50 ml.). The solution was heated to reflux for 45 min. after which the acid was neutralized by the addition of saturated sodium bicarbonate solution (10 ml.). The acetone was removed under reduced pressure to give a crystalline solid. After being washed with water the air dried solid was recrystallized from benzene to give 3.0 g. (60%) of IIIa, m.p. 168–174.5°. Recrystallization from ethyl acetate–petroleum ether (b.p. 30–60°) afforded an analytical sample, m.p. 179.0–181.0°,[16] infrared ν_{max} 4320, 3340, 3020, 3000, and 1680 cm.$^{-1}$, homogeneous by Bush B-3 paper chromatogram; $[\alpha]_D$ +36° (methanol C = 1.0), $[\alpha]_D$ +15° ($CHCl_3$, C = 1.0); n.m.r.[6] 21-H, 128 c.p.s., 19-H, 71 c.p.s., 18-H, 40 c.p.s. (indicative of the 17β-configuration).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.83; H, 9.38.

3β,11α-Dihydroxy-5-pregnen-20-one Diformate (IIIb).—The i-steroid II (500 mg.) was dissolved in 98% formic acid and allowed to stand at room temperature overnight. The formic acid was removed *in vacuo* and the product crystallized from ethanol giving 500 mg. of IIIb, m.p. 191.5–196.5°. Two recrystallizations afforded an analytical sample m.p. 195.5–198.0°, infrared ν_{max} 2990, 1715, 1700, and 1675 cm.$^{-1}$, homogeneous by Bush B-3 paper chromatogram.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.87; H, 8.40.

Hydrolysis of 3β,11α-Dihydroxy-5β-pregnen-20-one Diformate (IIIb).—A sample of the diester (IIIb) from above (250 mg.) was dissolved in methanol (10 ml.) containing 5% potassium hydroxide by weight and heated at reflux for 1 hr. The hot solution was diluted with water (50 ml.) and the product crystallized at 4° overnight. Filtration, water wash, and drying (*in vacuo* at 60°) afforded 145 mg. of a product, m.p. 168–175.5°, whose infrared and paper chromatographic (Bush B-3) mobility were identical with that of 11α-hydroxypregnenolone (IIIa).

3β,11α-Dihydroxy-5-pregnen-20-one 3-Acetate (IIIc).—A solution of the *i*-steroid II (250 mg.) in glacial acetic (50 ml.) was

(12) W. A. Struck and R. L. Houtman, *J. Org. Chem.*, **26**, 3883 (1961).

(13) Melting points were taken by capillary (except where noted) and are uncorrected. Infrared spectra were recorded as mineral oil mulls on a Perkin–Elmer Model 21 spectrophotometer. N.m.r. spectra were determined on solutions in deuteriochloroform at 60 Mc. with a Varian A-60 spectrometer, employing tetramethylsilane as an internal reference. Frequencies are reported in c.p.s. relative to tetramethylsilane as 0 c.p.s.

(14) (a) The column for gas chromatography was an 8-mm. Pyrex tube, 190 cm. long containing 2% by weight of General Electric SE-30 silicone on purified Chromosorb W (60–80 mesh).[14b] Helium was used as a carrier gas at approximately 11 p.s.i. and thermistors were employed for detection of the sample peaks. The composition of reaction mixtures was calculated using integrated areas from the chromatogram and no correction was made for slight differences in thermal conductivity of the components. (b) Chromosorb W supplied by the Wilkens Instrument and Research, Inc., Walnut Creek, Calif.

(15) Ref. 1b reports m.p. 212–218°, yield 16.7% with *Metarrhizium anisipliae*.

(16) Ref. 1b reports m.p. 182–183°.

distilled at atmospheric pressure to a volume of about 8 ml. during 3 hr. The resulting solution was diluted with water (50 ml.) and the product extracted into ether. The ether extracts were washed consecutively with water, 4% sodium bicarbonate solution, water, saturated sodium chloride solution, and finally dried (Na_2SO_4). The ether was distilled and the residue taken up in methylene chloride. This solution was adsorbed onto 25 g. of Florisil[17] and eluted with a gradient of from 5–15% acetone–petroleum ether over fifteen 50-ml. fractions. Fractions 8–12 contained 120 mg. of the crude monoacetate and were recrystallized from acetone–petroleum ether to give 70 mg. of colorless needles, m.p. 134.0–136.0°, infrared ν_{max} 3480, 3030, 2990, 1705, 1663, and 1260 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.30; H, 8.97.

11α-Hydroxyprogesterone (IV) from 3β,11α-Dihydroxy-5-pregnen-20-one (IIIa).—3β,11α-Dihydroxy-5-pregnen-20-one (500 mg.) dissolved in toluene (10 ml.) and cyclohexanone (1 ml.) was distilled to remove traces of water. Aluminum isopropoxide (500 mg.) was added and the mixture was heated at reflux for 2 hr. The hot solution was extracted with *M* hydrochloric acid (5 ml.) and diluted with benzene (10 ml.). The hydrocarbon solution was separated, washed consecutively with water, saturated sodium chloride solution, and dried (Na_2SO_4). The solvents were distilled to a volume of about 5 ml. under reduced pressure and the solution adsorbed onto Florisil (50 g.). The product was eluted during 25 fractions over a gradient of from 5 to 20% acetone in petroleum ether. Fractions 14–19 (255 mg.) were combined and recrystallized from methanol–water to give 144 mg. of 11α-hydroxyprogesterone, m.p. 162.5–163.0°. The infrared spectrum was identical with that of an authentic sample[18] and admixture with an authentic sample exhibited no depression in melting point.

11α-Hydroxy-3,5α-cyclopregnane-6,20-dione (V).—6β,11α-Dihydroxy-3,5α-cyclopregnan-20-one (II) (2 g.) was oxidized by the Oppenauer procedure as described above. Chromatography on 125 g. of Florisil by gradient elution (5–20% acetone) in petroleum ether over twenty fractions gave (fractions 9–12), after recrystallization from acetone–petroleum ether 0.75 g. of a crystalline solid, m.p. 166.5–170.0. Two recrystallizations afforded an analytical sample, m.p. 169.5–171.0°, infrared ν_{max} 3480 sh, 1700, 1680, and 1230 cm.$^{-1}$; λ_{max}^{EtOH} 281 mμ (ε 99).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.30; H, 9.35.

3β-Chloro-11α-hydroxy-17ξ-pregn-5-en-20-one (Va).—6β,11α-Dihydroxy-5-pregnen-20-one (10 g.) was suspended in a mixture of benzene (200 ml.) and concentrated hydrochloric acid (20 ml.) and stirred vigorously at room temperature under a cover of nitrogen for 2 hr. The acid layer was then discarded and the benzene solution washed consecutively with water (2 × 200 ml.), 4% sodium bicarbonate solution (100 ml.), saturated sodium chloride solution (50 ml.), and dried (Na_2SO_4). The benzene was removed under reduced pressure to give a solid which was recrystallized from acetone–petroleum ether to give in two crops 9.07 g. (86%), m.p. 146.0–148.0°. (A second crystalline polymorph has been isolated, m.p. 137.5–139.5°.) A sample for analysis was recrystallized three times from acetone–petroleum ether to m.p. 138.0–139.5°; infrared ν_{max} 3440, 3370, 3060, 3040, 1693, and 1653 cm.$^{-1}$; n.m.r. (crude product) 21-H, 128; 19-H, 72; 18-H, 40 (~95%) and 57 c.p.s. (~5%).

Anal. Calcd. for $C_{21}H_{31}O_2Cl$: C, 71.88; H, 8.91; Cl, 10.11. Found: C, 71.81; H, 8.82; Cl, 10.11.

3β-Chloro-5-pregnene-11,20-dione (VIIa) and 3β-Chloro-17α-pregn-5-ene-11,20-dione (VIII).—3β-Chloro-11α-hydroxy-pregnen-20-one (1.15 g., 3.27 mmoles) in acetone (15 ml.) was treated with 2.68 *M* Jones regent[19] (1.1 ml., 3 mmoles) and stirred vigorously for 10 min. followed by dilution with water (*ca.* 100 ml.) giving a white solid. The product was filtered, washed, dried at room temperature to give 1.05 g. (98.5%) of a crystalline white solid, m.p. 135.0–137.5°. Two recrystallizations from acetone–petroleum ether afforded an analytical sample, m.p. 138.0–139.0°, infrared ν_{max} 1710 and 1630 cm.$^{-1}$; n.m.r.[6] 18-H, 37 c.p.s.; 19-H, 74 c.p.s.; and 21-H, 127 c.p.s.; n.m.r.[6] crude contained about 3% 17% (by integration of 18-H at 52 c.p.s.)

Anal. Calcd. for $C_{21}H_{29}O_2Cl$: C, 72.28; H, 8.38; Cl, 10.17. Found: C, 72.26; H, 8.47; Cl, 10.21.

Chromatography of a sample of the above crude product on Florisil (400 mg. on 25 g.) over a gradient of from 5–8% acetone in petroleum ether (15 fractions) eluted in fractions 2 and 3 a new chloride (50 mg.), which, when crystallized from petroleum ether gave colorless bars, m.p. 126.0–127.0°, a sample of which was recrystallized for analysis, m.p. 126.5–128.0°, infrared ν_{max} 1697 and 1662 cm.$^{-1}$; n.m.r.[6] indicates that this material possesses the 17α-side chain, 21-H 128, 19-H, 73 c.p.s. and 18-H, 52 c.p.s.

Anal. Calcd. for $C_{21}H_{29}O_2Cl$: C, 72.28; H, 8.38; Cl, 10.17. Found: C, 72.23; H, 8.51; Cl, 10.44.

3β-Bromo-11α-hydroxy-5-pregnen-20-one (VIb).—6β,11α-Dihydroxy-3,5α-cyclopregnan-20-one (1.34 g.) suspended in benzene (50 ml.) was stirred with 48% hydrobromic acid (5 ml.) overnight. The reaction mixture was diluted with water, the benzene layer separated, and washed successively with water, 4% sodium bicarbonate solution, water, saturated sodium chloride solution, dried (Na_2SO_4), and taken to dryness *in vacuo*. The residue was adsorbed onto a short Florisil column in methylene chloride and eluted with 5% acetone in petroleum ether giving a white solid. Recrystallization from acetone–petroleum ether afforded 470 mg. of colorless plates, m.p. 142.0–144.0°. A sample was recrystallized for analysis, m.p. 144.5–145.5°, infrared ν_{max} 3400 and 1695 cm.$^{-1}$; n.m.r.[6] 21-H, 128 c.p.s.; 19-H, 72 c.p.s.; 18-H, 39 c.p.s., indicative of the 17β-configuration.

Anal. Calcd. for $C_{21}H_{33}O_2Br$: C, 63.47; H, 8.39; Br, 20.11. Found: C, 64.03; H, 7.80; Br, 20.40.

3β-Bromo-17ξ-pregn-5-ene-11,20-dione (VIIb).—The above bromide (VIb) (390 mg., 1 mmole) dissolved in acetone (2 ml.) was treated with 2.67 *M* Jones reagent[19] with stirring for 10 min., after which the reaction mixture was diluted with water (9 ml.) giving a white solid which was recovered by filtration, washed with water, and dried *in vacuo* at 60°. Recrystallization from acetone–petroleum ether afforded 320 mg. of colorless prisms, m.p. 148.0–149.0°, infrared ν_{max} 1710 and 1665 cm.$^{-1}$, n.m.r.[6] crude 21-H, 127 c.p.s.; 19-H, 74 c.p.s.; 18-H, 36 c.p.s., indicative of 17β configuration (2% 17α, 18-H peak 52 c.p.s.).

Anal. Calcd. for $C_{21}H_{31}O_2Br$: Br, 20.21. Found: Br, 20.04.

11-Keto-17β- and 17α-pregnenolone 3-Acetates (IXa and Xa).—3β-Chloro-5-pregnene-11,20-dione (6.54 g. of the 17β,α mixture) and zinc acetate dihydrate (13 g.) were suspended in glacial acetic acid (100 ml.) and heated at reflux for 5 hr. Acetic acid (55 ml.) was then distilled at atmospheric pressure followed by dilution of the residue with water (350 ml.) giving a crystalline solid that was recovered by filtration. This material was washed with water and dried (*in vacuo* at 60°), yield, 6.5 g. Recrystallization from ethanol–water gave 4.8 g. of crystalline acetates, m.p. 128–133°. A sample was recrystallized three times from ethanol for analysis, m.p. 133.5–167°; infrared ν_{max} 1725, 1700, 1667, and 1248 cm.$^{-1}$ v.p.c.$^{257°}$ crude indicated two materials[13] with retention times of 10.1 min. (15%, corresponding to the 17β-isomer) and 12.1 min. (85%, corresponding to the 17α-isomer), n.m.r.[6] (crude) 86% 17α, 14% 17β (by integration of 18-H, peaks).

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.02; H, 8.55.

11-Keto-17β- and 17α-pregnenolone (IXb and Xb).—The crystalline diacetate mixture IXa and Xa from above (250 mg.) was suspended in 5 ml. of 5% alcoholic potassium hydroxide and warmed on a steam bath for 1 hr. The solution was acidified with 3 *N* hydrochloric acid (2.5 ml.) and water added to turbidity. On cooling a white crystalline solid separated. The product was filtered, washed with water, and dried (*in vacuo* at 60°) giving 200 mg. (91%) of the diols. V.p.c.$^{255°}$ indicated[13] the presence of two materials with retention times of 7.3 min. (17%, corresponding to the 17β-isomer) and 8.8 min. (83%, corresponding to the 17α-isomer). The product dissolved in methylene chloride was adsorbed onto 10 g. of Florisil dissolved in methylene chloride and eluted by a gradient of from 5 to 15% acetone–petroleum ether during twenty 20-ml. fractions.

Fractions 8–12 (IXb) were combined and recrystallized from petroleum ether (acetone) to give colorless bars m.p. 166.2–167.0, 31 mg.; infrared ν_{max} 3340, 1705, and 1670 cm.$^{-1}$:

(17) A synthetic magnesium silicate manufactured by the Floridin Co., Warren, Pa.

(18) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister, and H. M. Leigh, *J. Am. Chem. Soc.*, **74**, 1933 (1952).

(19) Jones reagent consists of 26.72 g. of chromic acid and 23 ml. of sulfuric acid diluted to 100 ml. with water. K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

v.p.c.$^{255°}$ exhibited a single peak with a retention time of 7.5 min.; n.m.r.[6], 19-H, 72 c.p.s.; 21-H, 129 c.p.s., and 18-H, 53 c.p.s. indicative of the 17α-configuration.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.32; H, 9.16.

Fractions 15–20 (Xb) were combined and recrystallized from acetone–petroleum ether to give 81 mg., m.p. 169.8–171.9°. Recrystallization afforded an analytical sample m.p. 172.0–174.0°; infrared ν_{max} 3460, 3400, 1705, and 1670 cm.$^{-1}$; v.p.c.$^{255°}$ exhibited[12] a single peak with a retention time of 8.9 min.; n.m.r.[6], 19-H, 72 c.p.s.; 21-H, 127 c.p.s., and 18-C, 36 c.p.s. indicative of the 17β-configuration.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.46; H, 9.29.

3β-Hydroxy-5-pregnene-11,20-dione 20-Cyclic (Ethylene Acetal) (XI). A. From 11-Ketopregnenolone (IXb).—One gram of 11-ketopregnenolone, 25 ml. of benzene, 5 ml. of ethylene glycol, and 5 mg. of *p*-toluenesulfonic acid were heated to reflux overnight employing a Dean-Stark water trap. The hot solution was washed with 4% sodium bicarbonate solution, water, saturated sodium chloride, dried, and the solvent removed under reduced pressure to give a crystalline solid. Recrystallization from acetone afforded 660 mg. (64%), m.p. 150.5–154.0°. A second crop was isolated, 150 mg., m.p. 148.0–152.5°. A sample of the crop 1 material was recrystallized three times for analysis, m.p. 155.0–157.0°, infrared ν_{max} 3230, 1708, and 1670 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.05; H, 8.90.

B. From 11-Keto-17α-pregnenolone (Xb).—The steroid (Xb) (136 mg.) was treated as above in benzene (15 ml.), giving after isolation 66 mg., m.p. 153.0–156.0°. A second crop of 15 mg. (60% total), m.p. 147.0–151.5° was also isolated. The infrared spectrum of crop 1 was identical in all respects with the ketal from A above, mixed m.p. 155–157° with drop 1 from A above.

C. From a Mixture of 85% 11-Ketopregnenolone and 15% 17α-Isomer (IXb and Xb).—One gram of the mixture (85% IXb + 15% Xb) was treated as above (A) giving 660 mg. (64%) of product crystallized from acetone, m.p. 149.0–153°, infrared spectrum with products of procedures A and B above. Admixture with material from A exhibited no melting point depression.

Hydrolysis of 3β-Hydroxy-17α-pregn-5-ene-11,20-dione, 20-Cyclic (Ethylene Acetal).—The ketal XI (100 mg.) was dissolved with warming in acetone, 5 drops of 3 *M* hydrochloric acid added and the solution allowed to stand at room temperature overnight. The reaction mixture was then diluted with warming to about 10 ml. with water whereupon the product crystallized as needles. The crystalline solid was filtered, washed with water, and air dried giving 80.0 mg. (91%), m.p. 168.0–170.0°. Infrared spectrum identical with that of the 17β,20-ketone (IXb). Admixture with an authentic sample exhibited no melting point depression. V.p.c.$^{258°}$ indicated[13] 2.5% 17α-isomer, 97.5% 17β-isomer when compared with standard samples (IXa and Xb).

Isomerization of 11-Keto-17α-pregnenolone(Xb) in Dilute Acid–Acetone.—11-Keto-17α-pregnenolone (9.0 mg.) was dissolved in acetone (1 ml.) with warming on the steam bath, 3 drops of 3 *M* hydrochloric acid added, and the solution allowed to stand at room temperature overnight. Thin layer chromatography employing Silica-Gel G coated glass plates and development with cyclohexane–ethyl acetate (1:1) indicated approximately a 1:1 mixture of the two isomers (17α and 17β) when compared to synthetic mixtures.

Hydrolysis of 3β-Hydroxy-5-pregnene-11,20-dione, Cyclic-20 (Ethylene Acetal) in Deuterium Oxide Acetic Acid-H^2.—A 55.3-mg. sample of pure ketal was dissolved in 3.0 ml. of 67% acetic acid-H^2 in H_2^2O. The solution was allowed to remain at room temperature. After 2 hr. an aliquot was withdrawn and spotted on a Silica-Gel G plate for t.l.c. and the plate eluted with ethyl acetate–cyclohexane (1:1). At this time only a trace of the ketal remained. After 3 hr. the solution was diluted with 7.0 ml. of H_2^2O and the suspension refrigerated (4°) for 2 hr. The crystalline solid was filtered, washed with three 2-ml. portions of H_2^2O, and dried overnight at 0.3 mm. to give 34.4 mg. (71%), m.p. 170.5–172.0°. This material was demonstrated by t.l.c. to be the pure 11-ketopregnenolone-H^2.

Anal. Calcd. for $C_{21}H_{31}O_3H^2$: H^2, 3.12% (excess H^2 = /H^2 + H^1 × 100). Found: H^2, 2.92.

A 20-mg. sample of this material was taken up in ethanol and evaporated to dryness under reduced pressure. This dissolution sequence was repeated three times after which the crystalline residue was recrystallized from acetone–petroleum ether to give 16 mg. (80%), m.p. 169.5–171.5°, one spot by t.l.c. as above, infrared, no CD stretching, identical with authentic 11-keto-pregnenolone.

Anal. Calcd. for $C_{21}H_{32}O_3$: H^2, 0.00. Found: H^2, 0.00.

3β,11β-Dihydroxy-5-pregnen-20-one Cyclic (Ethylene Acetal) (XII).—11-Ketopregnenolone 20-cyclic (ethylene acetal) (XI) (600 mg.) was suspended in isopropyl alcohol (25 ml.) and allowed to react with sodium borohydride (600 mg.) in 0.1 *N* sodium hydroxide (2.5 ml.) at reflux overnight. The resulting solution was diluted with water (50 ml.) and the crystalline product filtered, washed with water, and dried *in vacuo* at 60° giving 510 mg., m.p. 213.5–218.5°. Two recrystallizations from acetone afforded an analytical sample, m.p. 210–215.5°, infrared ν_{max} 3540, 3460, 1670 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.27; H, 9.50.

11β-Hydroxypregnenolone (XIII).—11β-Hydroxypregnenolone cyclic (ethylene acetal) XII (400 mg.) was dissolved with warming in acetone (40 ml.) and treated with 3 *N* hydrochloric acid (5 ml.) at room temperature. After 18 hr. the solution was diluted with warm water (150 ml.) giving a white crystalline solid. The product was washed with aqueous acetone, water, and dried *in vacuo* at 60°, m.p. 187.0–189.5°. Recrystallization from acetone–petroleum ether afforded an analytical sample, m.p. 187.0–189.5°, infrared ν_{max} 3430 and 1690 cm.$^{-1}$; o.r.d. in dioxane (C = 1.053, 25°): $[M]_{589}$ 0.0°, $[M]_{440}$ + 31.0°, $[M]_{360}$ + 980°, $[M]_{317.5}$ + 5430°, $[M]_{285}$ −2595°, $[M]_{270}$ + 2055° (indicative of 17β-configuration).[12]

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.21; H, 9.90.

11β-Hydroxypregnenolone 3-Acetate XIVb.—11β-Hydroxypregnenolone (XIII) (1.0 g.) dissolved in pyridine (6 ml.) and acetic anhydride (1 ml.) was allowed to stand overnight at room temperature. The solution was then poured into 120 ml. of water and the crystalline product filtered, washed with water, and dried *in vacuo* at 60° giving 1.10 g. (98%) m.p. 175.5–177.5°. A sample was recrystallized once for analysis from acetone m.p. 179.0–180.0°, ν_{max} 3460, 1710, 1670, and 1265 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.91; H, 9.16.

6β,11β-Dihydroxy-3,5α-cyclopregnan-20-one (XV).—11β-Hydroxypregnenolone (XIII) (250 mg.) was dissolved in pyridine (7 ml.) and treated with *p*-toluenesulfonyl chloride (250 mg.) at room temperature overnight. The pyridine solution was poured into water (45 ml.) containing concentrated hydrochloric acid (2 ml.) and after 4 hr. the crystalline product was filtered, washed with water, and dried *in vacuo* at 60° giving 280 mg. of the tosylate, m.p. 142.0–143.0° (dec.). The tosylate was dissolved in acetone (50 ml.) and treated with potassium acetate (500 mg.) dissolved in water (25 ml.) at reflux overnight. The greater part of the acetone was distilled, precipitating 190 mg. of crude product, m.p. 187–225°. This material was recovered by filtration and adsorbed onto Florisil (25 g.) and eluted over a gradient of from 2 to 20% acetone–petroleum ether over twenty-nine, 50-ml. fractions. Fractions 15–22 were combined and recrystallized for analysis from acetone affording 37 mg., m.p. 239–243°, infrared ν_{max} 3500, 3440, and 1695 cm.$^{-1}$ (Polymorph II, m.p. 243–249°). Solution infrared spectrum identical with Polymorph I.

Anal. Calcd. for $C_{21}H_{32}O$: C, 75.86; H, 9.70. Found: C, 75.68; H, 9.71.

6β,11β-Dihydroxy-3,5α-cyclopregnan-20-one (XV) by Microbial Hydroxylation.—Ten liters of a medium was prepared of water, 100 g. of cornsteep liquor solids, and 100 g. of commercial dextrose. The pH of the medium was adjusted to 5.0 with sodium hydroxide and 10 ml. of lard oil added as an anti-foam. The sterile fermenter was inoculated aseptically with 500 ml. of a 72-hr. vegetative growth of *Curvularia lanata* (ATCC 12017). The stirred culture aerated with 1 l. of air per min. was allowed to grow for 48 hr. at which time the pH had risen to 7.0. A solution of 2 g. of 6β-hydroxy-3,5α-cyclopregnan-20-one was added in 20 ml. of *N*,*N*-dimethylformamide. The aeration and agitation were continued for 40 hr. The fermenter contents were then extracted three times with one-quarter volume of methylene chloride and the combined extracts were evaporated to dryness in a current of air at room temperature. This residue was taken up in ether–water (200 ml. each) and the ether layer separated

and washed consecutively with water (twice), saturated sodium chloride solution, dried (Na_2SO_4), treated with Darco G-60, and evaporated to dryness under reduced pressure giving a partially crystalline material. The residue was taken up in methylene chloride (10 ml.) and adsorbed onto a column of 75 g. of Florisil made up with petroleum ether. The column was eluted during 25 fractions with a gradient of from 6 to 20% acetone–petroleum ether in 100-ml. fractions. Fractions 9–11 containing 206 mg. of crystalline solid were combined and the material recrystallized from acetone to give 45 mg. of 11β-hydroxypregnenolone, m.p. 228–237°. Recrystallization gave pure (XV), m.p. 243.5–249.5°. The infrared spectrum of this material was identical with that of an authentic sample of XV and a mixed melting point exhibited no depression.

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C-6 Hydroxylated Steroids. V.[1] 6β-Hydroxytriamcinolone and Related Compounds

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The synthesis of the 6β-hydroxy derivatives of 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione (triamcinolone) and its corresponding 16α,17α-acetonide is described.

In continuation of our interest in C-6 hydroxylated steroids we wish here to report on the synthesis of 6β-hydroxytriamcinolone[2] and a number of related compounds which contain an oxygen function at C-16 and the fluorohydrin grouping at C-9,11.

A convenient starting material for the preparation of the titled compound was 9α-fluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione (I) or its 16,21-diacetate II.[3] When the latter reacted with trimethyl orthoformate in the presence of sulfuric acid it was smoothly converted into its $\Delta^{3,5}$-enol ether which could be purified by chromatography on Florisil[4] affording an analytical sample of the enol ether.[5] Reaction of the enol ether III with monoperphthalic acid[6] gave a 35% yield of 16α,21-diacetoxy-9α-fluoro-6β,11β,17α-trihydroxypregn-4-ene-3,20-dione (IV).[7] Aqueous potassium carbonate hydrolysis of IV gave 9α-fluoro-6β,11β,16α,17α,21 - pentahydroxypregn - 4 - ene - 3,20 - dione (V).

An alternate pathway for the preparation of a suitable 6-hydroxylated intermediate was based on 9α-fluoro-11β,21 - dihydroxy - 3 - methoxy - 16α,17α - methoxymethylenedioxypregna - 3,5 - dien - 20 - one (VI) and its 21-acetate VII.[8] Oxidation of an ethereal solution of VII[9] with monoperphthalic acid afforded 21-acetoxy-9α - fluoro - 6β,11β - dihydroxy - 16α,17α - methoxymethylenedioxypregn - 4 - ene - 3,20 - dione (VIII). Hydrolytic conditions required for removal of the ortho ester without isomerization of the 6β-hydroxy-Δ^4-3-one to the 3,6-dione[10] could be achieved by use of a hot aqueous acetic acid solution.[11] In this manner, the 21-monoacetate IX was prepared which was then hydrolyzed to the pentol V.

Introduction of the C-1,2-double bond was accomplished through the use of 2,3-dichloro-5,6-dicyanobenzoquinone.[12] To avoid the complications inherent in the use of the quinone reagent on the unprotected 6-hydroxyl function, the acetylation of IV afforded the triacetate X which could not be obtained crystalline. Subjecting this material to 1,2-dehydrogenation gave the $\Delta^{1,4}$-triacetate XI which also resisted crystallization. Aqueous potassium carbonate hydrolysis gave the desired 9α-fluoro-6β,11β,16α,17α,21-pentahydroxypregna-1,4-diene-3,20-dione (XII).[13] Whereas the reaction of XII with acetone in the presence of perchloric acid led to the formation of the 16α,17α-acetonide XIII,[2,14] an identical reaction on 9α-fluoro-6β,11β,16α,17α,21-pentahydroxypregn-4-ene-3,20-dione (V) yielded the 3,6-dione-16α,17α-acetonide XIV. In the latter, acetonide formation was accompanied by 6β-hydroxy-Δ^4-3-one rearrangement.

An alternate synthesis of the $\Delta^{1,4}$-acetonide XIII was accomplished from the starting material 21-acetoxy-9α-fluoro - 11β - hydroxy 16α,17α - isopropylidenedioxypregn-4-ene-3,20-dione (XV).[15] Oxidation of either the

(1) Paper IV, J. P. Dusza, J. P. Joseph, and S. Bernstein, *J. Org. Chem.*, **28**, 92 (1963).

(2) J. R. Florini, L. L. Smith, and D. A. Buyske, *J. Biol. Chem.*, **236**, 1038 (1961), have observed that triamcinolone is metabolized principally to 6β-hydroxytriamcinolone by the dog and, apparently, by man.

(3) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman, and R. H. Blank, *J. Am. Chem. Soc.*, **78**, 5693 (1956); **81**, 1689 (1959).

(4) A registered trademark of the Floridin Corporation for a synthetic magnesium silicate.

(5) Passage through the adsorbent not only removed unchanged Δ^4-3-one but also solvating molecules which tend to hydrolyze the enol ether. However, the purified enol ether is also rather easily hydrolyzed on exposure to atmospheric moisture.

(6) J. P. Dusza, J. P. Joseph, and S. Bernstein, *J. Org. Chem.*, **27**, 4046 (1962).

(7) Chromatographically pure enol ether was not essential in this preparation. An ethereal solution of the crude material could be oxidized in equally good yields.

(8) J. P. Dusza and S. Bernstein, *J. Org. Chem.*, **27**, 4677 (1962).

(9) As in the oxidation of III with monoperphthalic acid, a crude preparation of VII may be used with equal success. The acetates, although necessitating an additional hydrolysis step, have a more favorable solubility factor than the free alcohols and are apparently subject to fewer side reactions.

(10) P. T. Herzig and M. Ehrenstein, *J. Org. Chem.*, **16**, 1050 (1951).

(11) It has been observed that the hydrolysis of 16α,17α-ortho esters using brief mineral acid treatment leads to the formation of 16α-esters; ref. 8.

(12) D. Burn, D. N. Kirk, and V. Petrow, *Proc. Chem. Soc.*, 14 (1960).

(13) Poor yields are encountered with aqueous potassium carbonate hydrolyses (methanol) due to the solubility of the polyhydroxylated compounds in the reaction mixture. These compounds are also quite insoluble in the normal organic solvents employed in product isolation.

(14) C. Holmlund, L. I. Feldman, R. H. Evans, Jr., N. E. Rigler, B. Nielsen, and N. Barbacci, (unpublished work), have also prepared 6β-hydroxytriamcinolone 16α,17α-acetonide (XIII) and the corresponding Δ^4-compound XIX by a microbiological route.

(15) J. Fried, A. Borman, W. B. Kessler, P. Grabowich, and E. F. Sabo. *J. Am. Chem. Soc.*, **80**, 2338 (1958).