alumina. Benzene eluted 29.4 mg. of α-cholestanol, which gave, after one recrystallization from aqueous acetone, 29 mg. (67%), m.p. 184–185°, $[\alpha]_D$ +31°.

Elution with ether yielded no β-cholestanol, which, if present, should be obtained at this point.[5]

Kinetic Studies

β-Cholestanyl Tosylate in Methanol.—The weighed sample of β-cholestanyl tosylate in a Pyrex capsule was dropped into the boiling methanol, and the resulting solution heated under reflux. Periodically 5-ml. aliquots were withdrawn and titrated with 0.02 *N* sodium ethoxide in ethanol, using phenolphthalein indicator. During the first 12 hours of reaction samples were taken every hour and then every 5 or 6 hours. The results are shown in Table II.

For the reactions with sodium methoxide in methanol, solutions of sodium methoxide in methanol were standardized just before use. The procedure was similar to the one above except that the aliquots were pipeted into known amounts of standard solutions of potassium acid phthalate in ethanol and the resulting solutions back titrated with standard sodium ethoxide in ethanol. The results are shown in Table II.

Reaction of β-Cholestanyl Tosylate with Sodium *t*-Butoxide in *t*-Butyl Alcohol.—The procedure was similar to the one above for sodium methoxide in methanol. However, the end-points in the titrations were not sharp and were masked by the opalescence of the solutions which developed during the titrations. It was also difficult to get reproducible results in the several runs studied, and for these reasons the values given in Table II must be considered as only approximate.

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(5) F. Galinovsky and O. Vogl, *Monatsh.*, **79**, 325 (1948).

[CONTRIBUTION FROM THE MEDICINAL CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Steroidal Cyclic Ketals. XX.[1] 16-Hydroxylated Steroids. III.[2] The Preparation of 16α-Hydroxyhydrocortisone and Related Compounds

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Hydroxylation of 16-dehydroprogesterone (I) with osmium tetroxide yielded 16α,17α-dihydroxy-progesterone (IIa). In a similar manner by hydroxylation of the corresponding Δ^{16}-compounds (free steroid or 21-acetate derivatives), there were obtained 16α-hydroxy-Reichstein's substance S (VII), 16α-hydroxycortisone (XIa) and 16α-hydroxyhydrocortisone (XIIIa). Various acetate derivatives of these 16-hydroxylated compounds were also characterized. An alternative pathway to compounds VII, XIa and XIIIa proceeded *via* $\Delta^{5,16}$-bis-ethylene ketal intermediates.

Various investigations[3] on the corticosteroids present in the adrenal venous blood of a variety of species have indicated, by paper chromatographic techniques, the presence of substances more polar than hydrocortisone. The determination of the exact nature of these substances presents a difficult problem. Furthermore, in this connection, certain workers[4] have elicited interest in "corticoids" possessing additional hydroxyl groups at the 6- and/or 16-positions. Concurrent to these developments, we initiated a project on the chemical preparation of a number of such hydroxylated "corticoids." Our efforts were first directed to the synthesis of compounds in which a 16α-hydroxyl group was present and, in particular, to the synthesis of 16α-hydroxyhydrocortisone (XIIIa).[5] A discussion of the preparation of the latter and other related compounds forms the basis of this paper.

In a recent paper[6] from this Laboratory, a novel pathway was described for the preparation of a number of $\Delta^{4,16}$-3,20-diketosteroids and their corresponding 3,20-bis-ethylene ketal derivatives. These two types of compounds served in this investigation as key intermediates for the desired 16α-hydroxylated steroids.

16-Dehydroprogesterone ($\Delta^{4,16}$-pregnadiene-3,20-dione) (I) (Flowsheet A) was selected as a model substance for this work. Thus, compound I dissolved in benzene (containing pyridine), on treatment with osmium tetroxide was converted into 16α,17α-dihydroxy-progesterone (α^4-pregnene-16α,-17α-diol-3,20-dione) (IIa) (47% yield).[7,8] Acetylation under mild conditions afforded the 16α-acetate IIb. Considerations bearing on the structure of IIa and the other 16α-hydroxylated steroids will be presented collectively later in the paper.

The diol IIa on ketalization with ethylene glycol in the conventional manner gave in poor yield Δ^5-

(1) Paper XIX, R. H. Lenhard and S. Bernstein, THIS JOURNAL, **78**, 989 (1956).

(2) Paper II, S. Bernstein, M. Heller and S. M. Stolar, *ibid.*, **77**, 5327 (1955).

(3) See O. Hechter and G. Pincus, *Physiol. Revs.*, **34**, 459 (1954), and references cited therein.

(4) R. I. Dorfman and F. Ungar, "Metabolism of Steroid Hormones," Burgess Publishing Co., Minneapolis, Minn., 1953, p. 13, and references cited therein; see also, M. Hayano and R. I. Dorfman, *Arch. Biochem.*, **50**, 218 (1954).

(5) In a previous paper [S. Bernstein, M. Heller and S. M. Stolar, THIS JOURNAL, **76**, 5674 (1954)], there was reported the *chemical* preparation of Δ^5-pregnene-3β,16α-diol-20-one and 16α-hydroxyprogesterone.

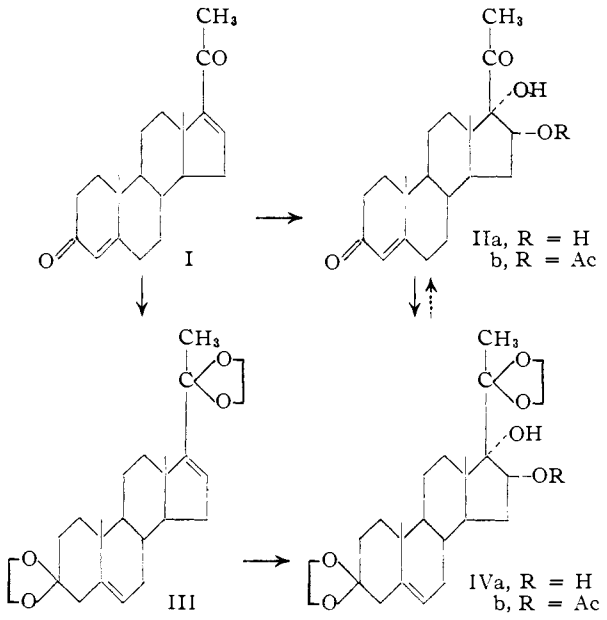
(6) W. S. Allen and S. Bernstein, *ibid.*, **77**, 1028 (1955).

(7) Examples of hydroxylation of a Δ^{16}-double bond with osmium tetroxide have been described by A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **24**, 1127 (1941), and by V. Prelog, L. Ruzicka and P. Wieland, *ibid.*, **28**, 250 (1945). Also the preparation of IIa from 16-dehydroprogesterone (I) by selective hydroxylation with osmium tetroxide has been reported by H. H. Inhoffen, F. Blomeyer and K. Brückner, *Chem. Ber.*, **87**, 593 (1954). We wish to state here that our preparation of IIa, and, in fact, of all the 16α-hydroxylated steroids described herein was completed prior to the appearance of the publication of the German workers.

(8) No difficulty was encountered in effecting the selective hydroxylation of the Δ^{16}-double bond in a $\Delta^{4,16}$-3,20-diketosteroid (four examples) (30–72% yield). A 9–48% excess of osmium tetroxide was employed, and the reactions were allowed to take place for the arbitrary time of 1–5 days. The hydroxylation generally appeared to proceed rapidly. However, opportunity was not available for a definitive time study of the reaction.

Moreover, the Δ^{16}-double bond in a $\Delta^{5,16}$-3,20-bis-ethylene ketal (three examples) may also be selectively hydroxylated (45–94% yield, 3–20% excess of osmium tetroxide, 3–7 days). Here, also, a time study of the reaction was not carried out.

FLOW SHEET A



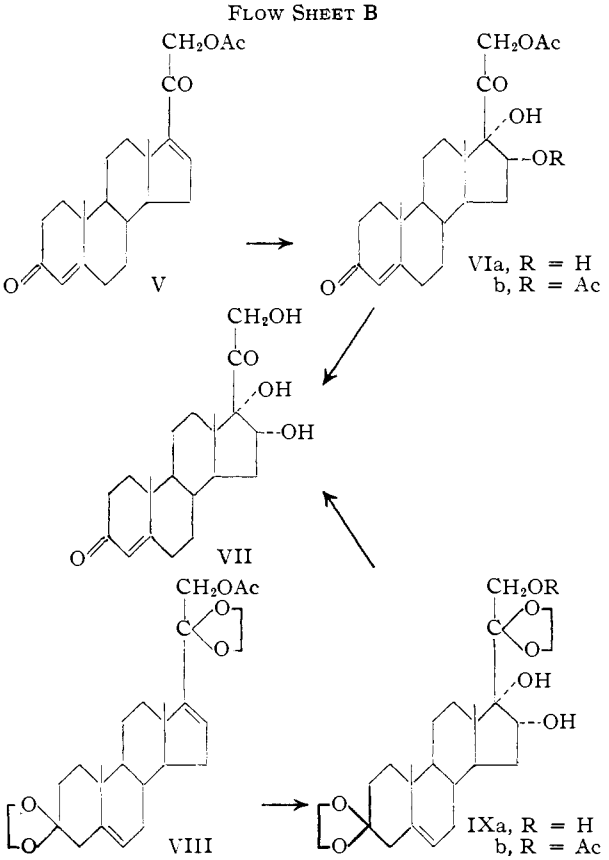
pregnene-16α,17α-diol-3,20-dione 3,20-bis-ethylene ketal (IVa) which was identical with the product obtained by hydroxylation of $\Delta^{5,16}$-pregnadiene-3,20-dione 3,20-bis-ethylene ketal (III).[2]

In an attempt to prepare the free steroid IIa from the bis-ketal IVa by hydrolysis (aqueous sulfuric acid–methanol) an inconclusive result was obtained. This transformation was not investigated further in view of the successful execution of this type of reaction in the other series (*vide infra*)

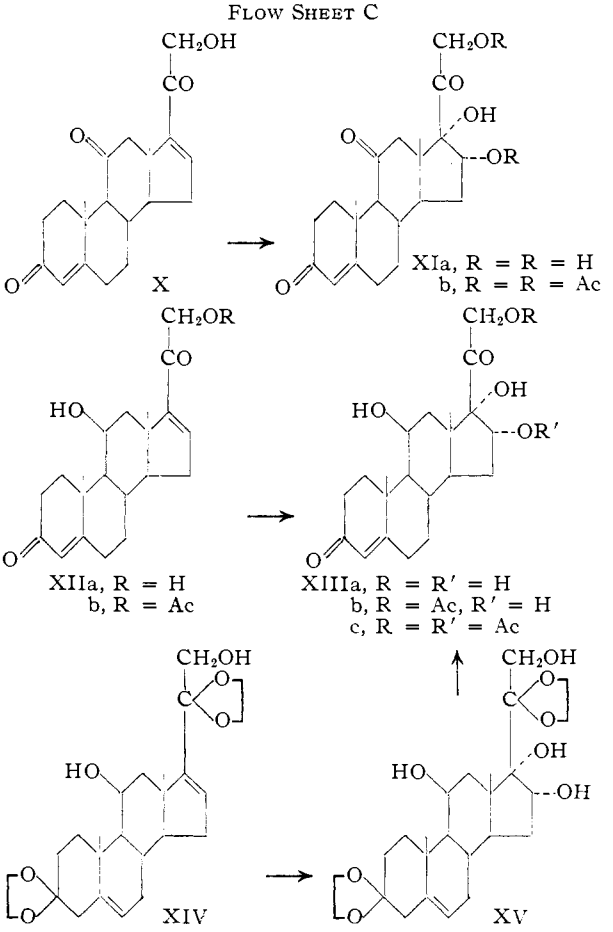
The 16α-hydroxylated analogs of Reichstein's substance S, cortisone and hydrocortisone were similarly prepared. $\Delta^{4,16}$-Pregnadiene-21-ol-3,20-dione 21-acetate (V)[6] (Flow Sheet B) on hydroxylation afforded the 16α,17α,21-triol 21-acetate VIa (72% yield). Saponification of VIa under ester exchange conditions with sodium methoxide produced the free steroid, Δ^4-pregnene-16α,17α,21-triol-3,20-dione (VII). Acetylation of VIa gave the 16α,17α,21-triol 16,21-diacetate VIb. The free steroid VII was also prepared from $\Delta^{5,16}$-pregnadiene-21-ol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (VIII). Hydroxylation of the latter gave the Δ^5-16α,17α,21-triol 21-acetate IXb (65% yield). Saponification afforded IXa, which on hydrolysis with aqueous sulfuric-acid–methanol was converted in 81% yield into the desired free steroid VII.

16α-Hydroxycortisone (Δ^4-pregnene-16α,17α,-21-triol-3,11,20-trione) (XIa) (Flow Sheet C) was prepared directly by hydroxylation of $\Delta^{4,16}$-pregnadiene-21-ol-3,11,20-trione (X)[6] (50% yield). Its 16,21-diacetate (XIb) was obtained on acetylation under mild conditions.

FLOW SHEET B



FLOW SHEET C



Finally, 16α-hydroxyhydrocortisone (Δ^4-pregnene-11β,16α,17α,21-tetrol-3,20-dione) (XIIIa) was prepared as follows. Hydroxylation of $\Delta^{4,16}$-pregnadiene-11β,21-diol-3,20-dione 21-acetate

(XIIb)[6] produced the 11β,16α,17α,21-tetrol 21-acetate XIIIb (30% yield). In another run, inadvertently, during the reaction or work-up (probably the latter) the 21-acetate group was removed, and the reaction product was the free steroid, 16α-hydroxyhydrocortisone (XIIIa) (44% yield). Acetylation of the 21-acetate XIIIb gave the 16,21-diacetate (XIIIc).

16α-Hydroxyhydrocortisone (XIIIa) was also prepared from the $\Delta^{5,16}$-bis-ethylene ketal (XIV).[6] Hydroxylation of the latter gave the Δ^{5}-11β,16α,-17α,21-tetrol bis-ethylene ketal XV (94% yield), which on hydrolysis with aqueous sulfuric acid–methanol was converted into the free steroid XIIIa (88% yield). The 16,21-diacetate XIIIc obtained on acetylation was identical with that prepared above.

The structures of the various 16α-hydroxylated steroids described have been assigned on the basis of the following considerations, other than elemental analyses:

A.—*cis*-Hydroxylation of the Δ^{16}-double bond of a Δ^{16}-20-ketosteroid or Δ^{16}-20-ketal[9] with osmium tetroxide most probably proceeds by rear attack, and thereby a 16α,17α-diol would be produced. The approach from the rear side appears valid on the basis of steric considerations, and finds precedents in the rear-attack mechanism of epoxidation[10] and the addition of alcohols[11] to the Δ^{16}-double bond.[12]

B.—16α-Hydroxy-Reichstein's substance S (VII), 16α-hydroxycortisone (Xa) and 16α-hydroxyhydrocortisone (XIIIa), which contain a 17-ketol grouping, gave a positive Blue Tetrazolium test, diagnostic for such a grouping. It was interesting to observe that these compounds also gave a positive Porter–Silber test.[13] Thus, in regard to the latter, it was indicated that at least qualitatively the 16α-hydroxyl group does not alter this color test, which is diagnostic for the dihydroxyacetone side-chain[13] or a Δ^{16}-17-ketol grouping.[6,14]

C.—Ultraviolet absorption analysis substantiated the structures of the 16α-hydroxylated compounds. All of the ten new Δ^{4}-3-ketosteroids (free steroids and acetate derivatives) exhibited the characteristic selective absorption of such a grouping, *i.e.*, in the range 237–242 mμ, with a molecular extinction coefficient of 14,900–17,500.[15] It is interesting to observe that substitution of a 16α-hydroxyl or acetoxy group has no apparent influence on this chromophore in the manner that, *e.g.*, substitution at C-6 or 11 does.[15,16] It is also pertinent to remark that 16α-substitution does not influence the generalization that 11-keto-Δ^{4}-3-ketosteroids generally exhibit a maximum at 237–238 mμ, whereas 11-(α or β)-hydroxy-Δ^{4}-3-ketosteroids exhibit one at 240–242.5 mμ.[15,16] This may be illustrated by 16α-hydroxycortisone (Xa) and 16α-hydroxyhydrocortisone (XIIIa) which show selective absorption at 237–238 and 241–243 mμ, respectively.

D.—The described transformations were readily followed by infrared absorption analysis which further supported the assigned structures. Infrared absorption data are reported in the Experimental. Besides the hydroxyl and carbonyl bands we have also indicated one of the principal "C–O" stretching bands of the ketal function at approximately 1100 cm.$^{-1}$.

Bioassays.[17]—Preliminary results have indicated that 16α-hydroxyhydrocortisone (XIIIa) possesses appreciable activity in a number of assays (glycogen, thymus involution, anti-inflammatory and Karnofsky egg embryo tests), and, moreover, has a very low, if any, electrolyte activity.

A detailed report on the biological activity of 16α-hydroxyhydrocortisone (XIIIa) will be forthcoming.

ADDED IN PROOF.—Since submission of this manuscript V. Petrow and coworkers [*J. Chem. Soc.*, 4373, 4377, 4383 (1955)] have published a series of papers on the hydroxylation of Δ^{16}-pregnene-20-ones with potassium permanganate and osmium tetroxide. The preparation of compounds IIa, b, VIa, b, and XIb, among others, are described. The English workers demonstrated that the Inhoffen[7] method of preparation of IIa led to D-homo rearrangement. It should be pointed out that our method of preparation and work-up does indeed lead to the desired IIa.

Acknowledgment.—We are indebted to Messrs. Louis M. Brancone and Samuel S. Modes for the microanalytical data, and to Messrs. William Fulmor, George Morton and Miss Anne Callaghan for the optical rotation data and the infrared 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 at wave length 5893 Å.(D).

Absorption Spectra.—The ultraviolet spectra were determined in absolute alcohol (unless otherwise noted) with

(9) S. Bernstein, M. Heller and S. M. Stolar (ref. 5) have presented evidence which proved that condensation of a Δ^{16}-20-ketosteroid with ethylene glycol to form the corresponding 20-ketal does *not* result in rearrangement of the Δ^{16}-double bond. This was in contrast to the now well known coincident rearrangement of the double bond of a Δ^{4}-3-ketone during ketal formation. The preparation of 16α-hydroxy-Reichstein's substance S (VII) and 16α-hydroxyhydrocortisone (XIIIa) as well as Δ^{5}-pregnene-16α,17α-diol-3,20-dione 3,20-bis-ethylene ketal (IVa) by the two pathways described provide further chemical evidence for the non-rearrangement of the Δ^{16}-double bond.

(10) Pl. A. Plattner, H. Heusser and M. Feurer, *Helv. Chim. Acta*, **31**, 2210 (1948); P. L. Julian, E. W. Meyer and I. Ryden, THIS JOURNAL, **71**, 756 (1949); **72**, 367 (1950); and P. L. Julian, E. W. Meyer, W. J. Karpel and I. Ryden, *ibid.*, **71**, 3574 (1949).

(11) D. K. Fukushima and T. F. Gallagher, *ibid.*, **73**, 197 (1951).

(12) Further evidence in support of rear attack was sought unsuccessfully in a molecular rotation analysis. It has been demonstrated that introduction of a hydroxyl (or acetoxy) group at the C-16 position has a decided influence on the molecular rotation which is dependent on the configuration of the substituent [H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **184**, 259 (1950); D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **73**, 196 (1951); and D. Perlman, E. Titus and J. Fried, *ibid.*, **74**, 2126 (1952)]. Application of the rotational data obtained herein and, in addition, those on 16β-substitution recently published by K. Heusler and A. Wettstein, *Chem. Ber.*, **87**, 1301 (1954), to such an analysis led to anomalous rotation differences, undoubtedly ascribable to the vicinal action of the 17α-hydroxyl group on the C-16 substituent.

(13) C. C. Porter and R. H. Silber, *J. Biol. Chem.*, **185**, 201 (1950).

(14) V. R. Mattox, H. L. Mason, A. Albert and C. F. Code, THIS JOURNAL, **75**, 4869 (1953).

(15) L. Dorfman, *Chem. Revs.*, **53**, 47 (1953).

(16) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell and J. H. Williams, *J. Org. Chem.*, **18**, 70 (1953).

(17) The Karnofsky egg embryo assay was carried out under the direction of Dr. Jackson S. Kiser (Biological Research Section). The remainder of the assays were carried out under the direction of Dr. Ralph I. Dorfman at the Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

a Beckman spectrophotometer (model DU). The infrared spectra (Nujol mull or pressed potassium bromide as indicated) 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^4-Pregnene-16α,17α-diol-3,20-dione (IIa).—$\Delta^{4,16}$-Pregnadiene-3,20-dione [I, 1.2 g. (0.00347 mole)] was dissolved in dry benzene (25 ml.) containing pyridine (1.2 ml.), and osmium tetroxide (1 g., 0.00394 mole) was added. The mixture was allowed to stand at room temperature for 95 hours. The osmate ester was then decomposed by the addition of water (68 ml.), benzene (25 ml.), methanol (46 ml.), sodium sulfite (7.1 g.) and potassium bicarbonate (7.1 g.), the mixture being stirred for 4 hours. Chloroform (100 ml.) was added, and the precipitate was collected by filtration, and was washed with hot chloroform (300 ml.). The combined filtrates were washed twice with saturated saline solution, dried and evaporated. Acetone was added to the residue, and the crystalline material so obtained was collected; 0.52 g. (47%), m.p. 221–226°. Recrystallization from methanol–acetone changed the melting point to 219–223°; λ_{max} 241–242 mμ (ϵ 15,200); ν_{max}^{Nujol} 3390, 1711, 1652, 1612 and 1081 cm.$^{-1}$; $[\alpha]^{24}D$ +97° (32.51 mg., αD +1.57°), [M]D +336; literature[7]: m.p. 218–219.5°, λ_{max} 240 mμ (ϵ 15800) (solvent?).

Anal. Calcd. for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73. Found: C, 72.99; H, 8.96.

Δ^4-Pregnene-16α,17α-diol-3,20-dione 16-Acetate (IIb).—The mother liquor from the crystallization of Δ^4-pregnene-16α,17α-diol-3,20-dione (IIa) was evaporated, and the residue in pyridine (20 ml.) was treated with acetic anhydride (10 ml.) overnight at room temperature. The reagents were evaporated, and the residue was crystallized with ether. The yield was 90 mg., m.p. 176–177°. Recrystallization from acetone–petroleum ether raised the m.p. to 178–179°; λ_{max} 240 mμ (ϵ 14,900); ν_{max}^{Nujol} 3560, 1740, 1718, 1672, 1620, 1250 and 1075 cm.$^{-1}$; $[\alpha]^{26}D$ +45° (9.10 mg., αD + 0.41°), [M]D +175.

Anal. Calcd. for $C_{23}H_{32}O_5$ (388.49): C, 71.10; H, 8.30. Found: C, 70.94; H, 8.64.

Δ^5-Pregnene-16α,17α-diol-3,20-dione 3,20-Bis-ethylene Ketal (IVa). **A.**—A mixture of Δ^4-pregnene-16α,17α-diol-3,20-dione (IIa, 0.5 g.), ethylene glycol (4 ml.), benzene (40 ml.) and *p*-toluenesulfonic acid monohydrate (20 mg.) was treated in the conventional manner (6 hours reflux).[18] The dried benzene–chloroform extract was evaporated to afford a clear glass (0.63 g.) which resisted all attempts at crystallization. It was dissolved in benzene (20 ml.) and was adsorbed on alumina (20 g., acid washed). Chloroform eluted the desired product, 86 mg. (14%), m.p. 215–230°. Recrystallization from acetone gave pure IVa, m.p. 264–267°; λ_{max} none; ν_{max}^{KBr} 3532 and 1096 cm.$^{-1}$; $[\alpha]^{25}D$ −45° (9.82 mg., αD −0.22°), [M]D −195.

Anal. Calcd. for $C_{25}H_{38}O_6$ (434.55): C, 69.09; H, 8.81. Found: C, 69.34; H, 8.98.

B.—$\Delta^{5,16}$-Pregnadiene-3,20-dione 3,20-bis-ethylene ketal [III, 1.42 g. (0.00328 mole)] was treated in the manner described above with osmium tetroxide (1 g., 0.00394 mole) for 3 days. The dried chloroform extract was evaporated, and the residue was slurried with cold acetone to give 695 mg. (45% yield) of IVa, m.p. 254–257°. Recrystallization from acetone raised the m.p. to 262–266°. Admixture m.p. determination with preparation A showed no depression; $[\alpha]^{25}D$ −41° (11.86 mg., αD −0.24°). Its infrared absorption spectrum was identical with that of preparation A.

Δ^5-Pregnene-16α,17α-diol-3,20-dione 16-Acetate 3,20-Bis-ethylene Ketal (IVb). **A.**—A mixture of the 3,20-dione 16-acetate (IIb, 0.86 g.), ethylene glycol (7 ml.), benzene (70 ml.) and *p*-toluenesulfonic acid monohydrate (30 mg.) was treated in the usual manner (7 hours reflux). The benzene–chloroform extract was evaporated. The residue was dissolved in benzene (90 ml.) and was adsorbed on alumina (30 g., acid washed). The desired product was eluted with 30% ether–benzene, and was recrystallized from acetone–petroleum ether; 98 mg., m.p. 262–265°. An additional 127 mg., m.p. 252–255°, was isolated from the mother liquor (21% total yield). Recrystallization of the first fraction from acetone–petroleum ether sharpened the m.p. to 262–264°; λ_{max} none; ν_{max}^{KBr} 3716, 1734, 1248, 1100 and 1038 cm.$^{-1}$; $[\alpha]^{25}D$ −81° (21.21 mg., αD −0.86°), [M]D −386.

Anal. Calcd. for $C_{27}H_{40}O_7$ (476.59): C, 68.04; H, 8.46. Found: C, 68.02; H, 8.31.

B.—The Δ^5-16α,17α-diol bis-ketal IVa was acetylated in the usual manner to give IVb, m.p. 262–265° (from acetone–petroleum ether), $[\alpha]^{24}D$ −89° (16.15 mg., αD −0.72°), [M]D −424. Its infrared absorption spectrum was identical with that of preparation A.

Δ^4-Pregnene-16α,17α,21-triol-3,20-dione 21-Acetate (VIa).—$\Delta^{4,16}$-Pregnadiene-21-ol-3,20-dione 21-acetate [V, 0.6 g. (0.00149 mole)] was treated in the manner described above with osmium tetroxide (0.45 g., 0.00175 mole) for 5 days. The dried benzene–chloroform extract on evaporation gave a solid residue which was recrystallized from acetone–petroleum ether to yield 472 mg. (72%) of VIa, m.p. 201–204°. Recrystallization from acetone–ether raised the m.p. to 206–210°; λ_{max} 240.5 mμ (ϵ 17,500); ν_{max}^{KBr} 3400, 1760, 1736, 1668, 1622, 1220 and 1066 cm.$^{-1}$; $[\alpha]^{26}D$ +105° (13.96 mg., αD +0.73°), [M]D +424.

Anal. Calcd. for $C_{23}H_{32}O_6$ (404.49): C, 68.29; H, 7.97. Found: C, 68.56; H, 8.08.

Δ^5-Pregnene-16α,17α,21-triol-3,20-dione 21-Acetate 3,20-Bis-ethylene Ketal (IXb).—$\Delta^{5,16}$-Pregnadiene-21-ol-3,20-dione 21-acetate 3,20-bis-ethylene ketal [VIII, 1.75 g. (0.00356 mole)] was treated in the manner described above with osmium tetroxide (1 g., 0.00394 mole) for 7 days. The residue obtained on evaporation of a benzene–chloroform extract was slurried with warm ether, and filtered. This gave 1.23 g. (65%) of IXb, m.p. 228–229°. The compound solvated with acetone and methanol, and was therefore purified by slurrying with warm ether; m.p. 231–233°; λ_{max} none; ν_{max}^{KBr} 3554, 3500, 1748, 1252, 1108 and 1042 cm.$^{-1}$; $[\alpha]^{25}D$ −66° (14.32 mg., αD −0.47°), [M]D −325.

Anal. Calcd. for $C_{27}H_{40}O_8$ (492.59): C, 65.83; H, 8.19. Found: C, 66.02; H, 8.23.

Δ^5-Pregnene-16α,17α,21-triol-3,20-dione 3,20-Bis-ethylene Ketal (IXa).—The ether mother liquor from the purification of the 21-acetate 3,20-bis-ethylene ketal IXb was evaporated, and the residue in 2% alcoholic potassium hydroxide (20 ml.) was refluxed for 0.5 hour. Addition of water to the cooled mixture gave crystals which were collected, 450 mg., m.p. 254–257°. Recrystallization from acetone gave pure IXa, m.p. 271–273° (m.p. was dependent on the rate of heating); λ_{max} none; ν_{max}^{KBr} 3500, 3360, 1100 and 1052 cm.$^{-1}$; $[\alpha]^{25}D$ −34° (17.88 mg., pyridine, αD −0.30°), [M]D −153.

Anal. Calcd. for $C_{25}H_{38}O_7$ (450.55): C, 66.64; H, 8.50. Found: C, 66.39; H, 8.64.

Δ^4-Pregnene-16α,17α,21-triol-3,20-dione 16,21-Diacetate (VIb).—The mother liquors from the crystallization of Δ^4-pregnene-16α,17α,21-triol-3,20-dione 21-acetate (VIa) were evaporated, and the residue was acetylated in the usual manner with acetic anhydride and pyridine at room temperature. This gave the diacetate VIb, m.p. 205–206° (from acetone–petroleum ether); λ_{max} 240 mμ (ϵ 16,800); ν_{max}^{KBr} 3512, 1750, 1732 (shoulder), 1678, 1622, 1236 and 1062 cm.$^{-1}$; $[\alpha]^{25}D$ +51° (7.89 mg., αD +0.20°), [M]D +227.

Anal. Calcd. for $C_{25}H_{34}O_7$ (446.52): C, 67.24; H, 7.68. Found: C, 67.35; H, 7.92.

Δ^4-Pregnene-16α,17α,21-triol-3,20-dione (VII). **A.**—Δ^4-Pregnene-16α,17α,21-triol-3,20-dione 21-acetate (VIa, 100 mg.) was dissolved in anhydrous methanol (20 ml.), and was agitated for 10 minutes at room temperature under a nitrogen atmosphere with a solution of sodium (7 mg.) in methanol (10 ml.). The solution was then neutralized with glacial acetic acid (0.1 ml.), and most of the methanol was evaporated. Hot water was added, and the solution was cooled. The crystals so obtained were collected and washed with water. This gave 54 mg. (60%) of VII, m.p. 225–227°. Recrystallization from methanol-ether gave pure VII, m.p. 238–240°, positive Blue Tetrazolium and Porter–Silber tests; λ_{max} 240 mμ (ϵ 17,100); ν_{max}^{KBr} 3450, 1722, 1680, 1622 and 1080 cm.$^{-1}$; $[\alpha]^{24}D$ +93° (5.62 mg., 5 ml. of methanol, αD +0.21°), [M]D +337.

Anal. Calcd. for $C_{21}H_{30}O_5$ (362.45): C, 69.58; H, 8.34. Found: C, 69.50; H, 8.62.

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

B.—Δ^5-Pregnene-16α,17α,21-triol-3,20-dione 3,20-bis-ethylene ketal (IXa, 260 mg.) was dissolved in methanol (25 ml.) containing 8% (v./v.) sulfuric acid (2 ml.), and was refluxed for 40 minutes. The mixture was cooled, neutralized with sodium bicarbonate and water was added. The crystalline material which separated was collected and washed with water; 0.17 g. (81%) of VII, m.p. 229–231°. Recrystallization from methanol–ether gave pure VII, m.p. 238–240°. Its infrared absorption spectrum was identical with that of preparation A.

Δ^4-Pregnene-16α,17α,21-triol-3,11,20-trione (XIa).—[$\Delta^{4,15}$-Pregnadiene-21-ol-3,11,20-trione [X, 0.91 g. (0.00266 mole)] was treated in the manner described above with 1 g. (0.00394 mole) of osmium tetroxide (5 days). The residue obtained on evaporation of the benzene–chloroform extract was crystallized from acetone to afford 0.5 g. (50%) of Xa, m.p. 233–234°. Recrystallization from methanol–acetone gave pure XIa, m.p. 238–240°; positive Blue Tetrazolium and Porter–Silber tests; λ_{max} 237–238 mμ (ε 16,500); ν_{max}^{KBr} 3380, 1736, 1720, 1657, 1620, 1108, 1074 and 1062 cm.$^{-1}$; $[\alpha]^{24}D$ +153° (10.82 mg., 5 ml. of methanol, αD +0.33°), [M]D +575.

Anal. Calcd. for $C_{21}H_{28}O_6$ (376.44): C, 67.00; H, 7.50. Found: C, 67.37, 67.18; H, 7.55, 7.45.

Δ^4-Pregnene-16α,17α,21-triol-3,11,20-trione 16,21-Diacetate (XIb).—A mixture of XIa (100 mg.), pyridine (5 ml.) and acetic anhydride (2 ml.) was allowed to stand at room temperature for 16 hours when it was poured into ice-water. The mixture was extracted with chloroform (250 ml.), and the extract was washed with saturated saline, dried and evaporated. The resultant crystalline material solvated badly with polar solvents. Purification was accomplished by precipitation of the crystals from an acetone solution with petroleum ether. This gave 70 mg. (57%) of XIb, m.p. 224–227°, unchanged by further precipitation; λ_{max} 237–238 mμ (ε 16,400); ν_{max}^{KBr} 3450, 1752, 1730, 1714, 1670, 1630, 1245 and 1076 cm.$^{-1}$; $[\alpha]^{24}D$ +129° (21.40 mg., αD +1.38°), [M]D +593.

Anal. Calcd. for $C_{25}H_{32}O_8$ (460.51): C, 65.20; H, 7.00. Found: C, 64.99; H, 7.17.

Δ^4-Pregnene-11β,16α,17α,21-tetrol-3,20-dione 21-Acetate (XIIIb).—[$\Delta^{4,16}$-Pregnadiene-11β,21-diol-3,20-dione 21-acetate [XIIb, 0.6 g. (0.00155 mole)] was treated with osmium tetroxide (0.5 g., 0.00197 mole) in the manner described above for 3 days. The residue obtained from a benzene–chloroform extract was crystallized with acetone–petroleum ether. This gave 194 mg. (30%) of XIIIb, m.p. 211–214°. Recrystallization from acetone–petroleum ether did not alter the m.p.; λ_{max} 241 mμ (ε 15,400); ν_{max}^{KBr} 3510, 1756 (shoulder), 1734, 1672, 1628 (shoulder), 1220 and 1064 cm.$^{-1}$; $[\alpha]^{24}D$ +124° (10.14 mg., αD +0.63°), [M]D +521.

Anal. Calcd. for $C_{23}H_{32}O_7$ (420.49): C, 65.69; H, 7.67. Found: C, 65.94; H, 7.97.

Δ^4-Pregnene-11β,16α,17α,21-tetrol-3,20-dione (XIIIa). A.—[$\Delta^{4,16}$-Pregnadiene-11β,21-diol-3,20-dione 21-acetate [XIIb, 0.7 g. (0.00181 mole)] was dissolved in benzene (25 ml.) containing pyridine (0.7 ml.), and osmium tetroxide (0.5 g., 0.00197 mole) was added. The mixture was allowed to stand at room temperature for 1 day when the osmate ester was decomposed by being stirred for 8 hours with a mixture of water (34 ml.), benzene (20 ml.), methanol (20 ml.), sodium sulfite (3.6 g.) and potassium bicarbonate (3.6 g.). Chloroform (100 ml.) was added, and the brown precipitate was collected, and washed with hot chloroform (3 l.). The combined benzene–chloroform extracts were washed with saturated saline, dried and evaporated. The residue was crystallized from acetone–petroleum ether to give 304 mg. (44%) of XIIIa, m.p. 228–230°. Recrystallization from acetone raised the m.p. to 235–238°, positive Blue Tetrazolium and Porter–Silber tests; λ_{max} 241–242 mμ (ε 15,000); ν_{max}^{KBr} 3460, 1734, 1674, 1622 and 1050 cm.$^{-1}$; $[\alpha]^{25}D$ +119° (9.87 mg., 5 ml. of methanol, αD +0.47°), [M]D +450.

Anal. Calcd. for $C_{21}H_{30}O_6$ (378.45): C, 66.64; H, 7.99. Found: C, 66.74; H, 8.18.

B.—Δ^5-Pregnene-11β,16α,17α,21-tetrol-3,20-dione 3,20-bis-ethylene ketal (XV, 0.45 g.) was dissolved in methanol (40 ml.) containing 8% (v./v.) sulfuric acid (4 ml.), and was refluxed for 40 minutes. The mixture was cooled, neutralized with sodium bicarbonate solution, and the methanol was evaporated. The mixture was cooled, and crystals separated; the yield was 200 mg. (55%) m.p. 235–239°. Recrystallization from acetone sharpened the m.p. to 236–238°, $[\alpha]^{25}D$ +121° (10.32 mg., 5 ml. of methanol, αD +0.25°). Its infrared absorption spectrum was identical to that of preparation A.

In another run from XV (2.95 g.) there was obtained 1.37 g., m.p. 239–241°, and 0.72 g., m.p. 237–239° (88% yield).

Δ^4-Pregnene-11β,16α,17α,21-tetrol-3,20-dione 16,21-Diacetate (XIIIc). A.—The mother liquors from the crystallization of Δ^4-pregnene-11β,16α,17α,21-tetrol-3,20-dione 21-acetate (XIIIb) were evaporated, and the residue was acetylated in the usual manner with acetic anhydride and pyridine; m.p. 219–221° (from acetone–petroleum ether–ether); λ_{max} 240.5 mμ (ε 15,000); ν_{max}^{KBr} 3546, 1748, 1732 (shoulder), 1672, 1626, 1234 and 1055 cm.$^{-1}$; $[\alpha]^{24}D$ +75° (3.20 mg., 1 ml. of chloroform, αD +0.24°), [M]D +347.

Anal. Calcd. for $C_{25}H_{34}O_8$ (462.52): C, 64.92; H, 7.41. Found: C, 65.24; H, 7.61.

B.—Acetylation of Δ^4-pregnene-11β,16α,17α,21-tetrol-3,20-dione (XIIIa, 20 mg.) gave pure XIIIc (from ether), 13 mg. (53%), m.p. 221–224°. Its infrared absorption spectrum was identical to that of preparation A.

Δ^5-Pregnene-11β,16α,17α,21-tetrol-3,20-dione 3,20-Bis-ethylene Ketal (XV). A.—[$\Delta^{5,16}$-Pregnadiene-11β,21-diol-3,20-dione 3,20-bis-ethylene ketal [XIV, 0.84 g. (0.00194 mole)] was treated in the manner described above with osmium tetroxide (0.5 g., 0.00197 mole) for 5 days. The residue obtained on evaporation of a benzene chloroform extract was crystallized from acetone–petroleum ether. This gave 554 mg. (61%) of XV, m.p. 239–241°. Recrystallization from acetone–petroleum ether sharpened the m.p. to 238.5–239.5°; λ_{max} none; ν_{max}^{KBr} 3512, 3290, 1100 and 1044 cm.$^{-1}$; $[\alpha]^{25}D$ −24° (15.80 mg., methanol, αD −0.19°), [M]D −112.

Anal. Calcd. for $C_{25}H_{38}O_8$ (466.55): C, 64.36; H, 8.21. Found: C, 64.25; H, 8.51.

B.—In another run (3 days) with XIV (0.84 g.) there was obtained 850 mg. (94%) of XV, m.p. 230–237°.

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