Recrystallization from aqueous isopropyl alcohol gave 0.108 g. of product melting at 196–197°. A mixed melting point with an authentic sample m.p. 196–197° of the Dimedon derivative of formaldehyde showed no depression. The infrared spectra of the unknown and the Dimedon derivative of formaldehyde were identical.

Hydrolysis of 3β,17-Dibenzoxy-13,17-seco-18-norandrostan-13-one (XIV).—A solution of 1.48 g. (0.00293 mole) of the glassy product obtained from the ozonization of XVII was dissolved in a solution containing 50 ml. of 3A ethanol, 5 ml. of water and 2.0 g. of potassium hydroxide, warmed to 45° and allowed to stand overnight. The solvent was then removed by distillation under reduced pressure and the residue slurried with 30 ml. of water, filtered and dried overnight at 50° under reduced pressure, weight 0.79 g. (theory 0.84 g.), m.p. 150–165°. Recrystallization from acetone gave 0.57 g. (68%) of material melting at 174–176°.

An analytical sample was prepared by recrystallization from acetone; m.p. 174–176°, $[\alpha]^{23}D$ +2° (95% ethanol).

Anal. Calcd. for $C_{18}H_{30}O_3$: C, 73.43; H, 10.27. Found: C, 73.30; H, 10.33.

Since the infrared spectrum of this material showed strong hydroxyl but no carbonyl absorption, the probable structure is that of the hemiketal XVIII.

3β,13α,17,18-Tetrahydroxy-13,17-secoandrostane (XV).—To a slurry of 1.60 g. (0.0032 mole) of 3β,17-dibenzoxy-13,-17-seco-13,18-androstene (XVI) in 70 ml. of anhydrous diethyl ether was added, with stirring, 1.0 g. of osmic acid and 1.0 ml. of dry pyridine. The mixture was stirred an additional 3 hours and allowed to stand overnight. The ether was removed by distillation, 46 ml. of ethanol and a solution of 7.2 g. of sodium sulfite in 31 ml. of water was added, and the mixture was refluxed 3.5 hours with stirring. The reaction mixture was cooled and filtered and the solids were extracted several times with small portions of ethanol. The extracts were combined and the solvent was removed by distillation under reduced pressure. The residue was washed well with water and then redissolved in 30 ml. of ethanol. After adding 1.0 g. of potassium hydroxide dissolved in 6.0 ml. of water, the solution was heated on the steam-bath for 45 minutes. After diluting with 75 ml. of water and cooling in an ice-bath, the product was filtered, washed with water and dried at 60° *in vacuo;* weight 0.90 g. (86%), m.p. 224–227°.

An analytical sample was prepared by recrystallization from isopropyl alcohol; m.p. 229–230°, $[\alpha]^{23}D$ +23° (95% ethanol).

Anal. Calcd. for $C_{19}H_{34}O_4$: C, 69.90; H, 10.50. Found: C, 69.96; H, 10.41.

3-Keto-13α-hydroxy-13,17-secoandrostane-17-oic Acid, Lactone[10] (XVI).—A solution of 0.310 g. (0.001 mole) of X in 20 ml. of glacial acetic acid was treated with a solution of 0.351 g. (5.25 milliequivalents) of chromium trioxide in 2 ml. of water and 10 ml. of glacial acetic acid and allowed to stand 15 hours at room temperature. It was then diluted with 20 ml. of methanol and concentrated to room temperature in a stream of air. After dilution with 50 ml. of water the steroid was extracted with three 50-ml. portions of chloroform. The combined chloroform extracts were washed five times with 20-ml. portions of 5% sodium hydroxide and water, dried over sodium sulfate, and concentrated to give 0.204 g. of neutral residue. Recrystallization from acetone–Skellysolve B gave 0.070 g. product melting at 165–168°. Three additional recrystallizations gave 0.047 g. of material melting at 169–171° (Levy and Jacobsen[6] reported a m.p. of 166–167.7° for this compound).

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 75.00; H, 9.27.

The infrared spectrum also supported the structure for 3-keto-13α-hydroxy-13,17-secoandrostan-17-oic acid, lactone (XVI).

KALAMAZOO, MICHIGAN

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

Steroidal Cyclic Ketals. XVIII.[1] The Preparation of 9α-Hydroxyhydrocortisone

BY RUDDY LITTELL AND SEYMOUR BERNSTEIN

RECEIVED SEPTEMBER 2, 1955

9α-Hydroxyhydrocortisone (VIIIa) has been prepared by two different pathways for evaluation of the influence of a 9α-hydroxyl group on the biological activities of hydrocortisone.

The recent Communications by Fried and Sabo[2] on the preparation and biological activities of 9α-fluorohydrocortisone and related compounds have stimulated efforts in the direction of obtaining other variants of hydrocortisone which may also have an increased activity.[3] This work immediately suggested to us that the unknown 9α-hydroxy derivative of hydrocortisone would be a highly desirable compound for evaluation in this direction. We wish to report here that this compound has now been synthesized by two different pathways.

The synthesis to which most of our efforts were directed was based essentially on the successful hydroxylation with osmium tetroxide of a $\Delta^{9(11)}$-5α-hydroxy-steroid. The structure of the previously unknown 9α-hydroxyhydrocortisone (VIIIa) was established unequivocally by a synthesis which involved a hydrolytic fission of the oxide ring in Δ^4-pregnene-17α,21-diol-3,20-dione-9β,11β-oxide (IX), one of the key intermediates described by the Squibb workers[2] in their preparation of 9α-halogenated hydrocortisones. We wish to state that this second synthesis *via* IX was executed solely for corroborative purposes, and was not studied in detail.

The starting material for our projected synthesis was pregnane-5α,11β,17α,21-tetrol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (IIIb) recently prepared in this Laboratory.[4] However, a modified synthesis from the bis-ethylene ketal I of hydrocortisone was employed to circumvent a difficult chromatographic separation of oxides. This improved process involved the epoxidation of I in chloroform solution with peroxybenzoic acid in ethyl acetate solution to give a product which crystallized directly from the reaction mixture and consisted principally of the 5α,6α-oxide II (52% yield). Pure 5α,6α-oxide II was readily obtained

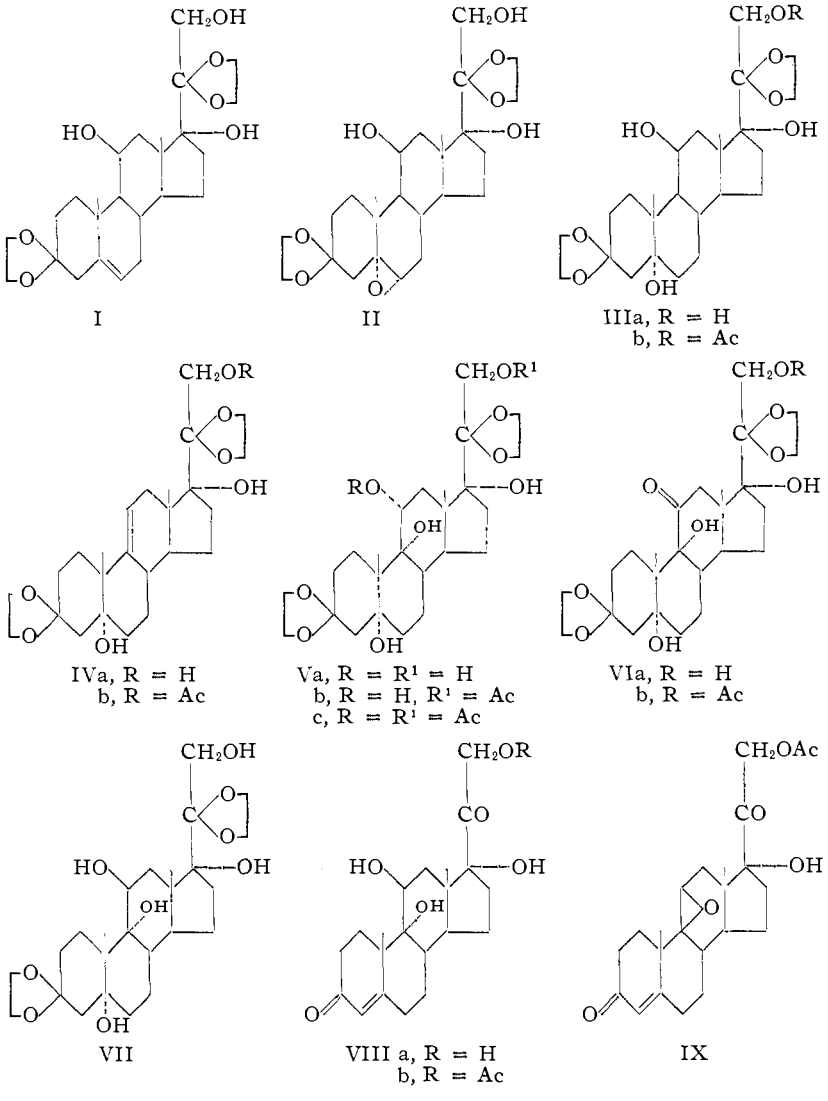
(1) Paper XVII, W. S. Allen, C. E. Linden and J. Clemente, THIS JOURNAL, **77**, 6612 (1955).

(2) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953); **76**, 1455 (1954); see also, J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer and P. Numerof, *ibid.*, **77**, 1068 (1955).

(3) In this connection, see H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg and P. L. Perlman, *Science*, **121**, 176 (1955), for a report on two compounds which are more "active" than cortisone or hydrocortisone, namely, metacortandracin ($\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione) and metacortandralone ($\Delta^{1,4}$-pregnadiene-11β,17α,21-triol-3,20-dione).

(4) S. Bernstein and R. H. Lenhard, THIS JOURNAL, **77**, 2233 (1955).

FLOW SHEET



by recrystallization.[5] Reduction of II in tetrahydrofuran with lithium aluminum hydride furnished in 97% yield the known[4] pregnane-5α,11β,17α,21-tetrol-3,20-dione 3,20-bis-ethylene ketal (IIa). Acetylation gave the 21-acetate bis-ketal IIIb (97% yield).

A pyridine solution of the 21-acetate bis-ketal IIIb was treated with phosphorus oxychloride at room temperature, and selective dehydration of the 11β-hydroxyl group took place.[6] In this manner $\Delta^{9(11)}$-pregnene-5α,17α,21-triol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (IVb) was obtained in 74% yield. Saponification furnished the $\Delta^{9(11)}$-5α,17α,21-triol bis-ketal IVa (68% yield). The successful preparation of the latter and its 21-acetate IVb afforded us the opportunity to study the influence of a 5α-hydroxyl group on the hydroxylation of a $\Delta^{9(11)}$-double bond with osmium tetroxide.[7]

It would appear that hydroxylation of a 5α-hydroxy-$\Delta^{9(11)}$-steroid (*e.g.*, IVb) can take place at room temperature (10 days), but the product was a mixture which resisted separation into its components. (However, subsequent experience with separating mixtures of this type has been obtained; *vide infra*. Unfortunately, opportunity was not available to reinvestigate room temperature experiments.) From a preparative viewpoint the following procedures were employed. The Δ^{9}-21-acetate bis-ketal IVb (benzene–pyridine solution) was treated with osmium tetroxide, and the mixture was heated at 37° for 25 days, followed by 17 days at 63°. The product so obtained consisted of mixed crystals as shown by sulfuric acid analysis.[8] Saponification and recrystallization furnished pregnane-5α,9α,11α,17α,21-pentol-3,20-dione 3,20-bis-ethylene ketal (Va) free of Δ^{9}-compound.[9]

In another run the Δ^{9}-21-acetate bis-ketal IVb on osmylation (18 days at 63°) gave an oily mixture which was separated by partition chro-

(5) Undoubtedly, the 5β,6β-oxide was formed in this reaction but its isolation in a pure state was set aside for future work.

(6) For the elimination of an 11β-hydroxyl group under these conditions; see, S. Bernstein, R. Littell and J. H. Williams, THIS JOURNAL, **75**, 4830 (1953); S. Bernstein, R. H. Lenhard and J. H. Williams, *J. Org. Chem.*, **19**, 41 (1954), and references cited therein. Both 5α- and 17α-hydroxyl groups have been found to be stable under these conditions; see P. Bladon, H. B. Henbest, E. R. H. Jones, B. J. Lovell and G. F. Woods, *J. Chem. Soc.*, 125 (1954), S. Bernstein, R. Littell and J. H. Williams, above, and S. Bernstein and E. H. Lenhard.[4] It should be noted all three types of hydroxyl groups undergo elimination with thionyl chloride in pyridine; see, S. Bernstein and R. H. Lenhard,[4] and W. S. Allen and S. Bernstein, THIS JOURNAL, **77**, 1028 (1955).

(7) This reaction was of special interest to us in view of the reports in the literature on other C_6-substituents. The non-reactivity toward osmium tetroxide by a $\Delta^{9(11)}$-double bond in a steroid containing rings A and B in the *cis* relationship has been noted by L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **73**, 118 (1951); see also, L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publ. Corp., New York, N. Y., 1949, p. 227. However, if rings A and B are in the *trans* relationship, hydroxylation was relatively facile (R. Hirschmann, C. S. Snoddy, Jr., and N. L. Wendler, THIS JOURNAL, **75**, 3252 (1953)). Moreover, H. B. Henbest, E. R. H. Jones, G. W. Wood and G. F. Woods, *J. Chem. Soc.*, 4894 (1952), have observed that a 5α,8α-epidioxido-$\Delta^{9(11)}$-steroid does not react with osmium tetroxide even after several weeks at 20°. On the other hand, R. B. Clayton, A. Crawshaw, H. B. Henbest, E. R. H. Jones, B. J. Lovell and G. W. Wood, *J. Chem. Soc.*, 2009 (1953), have found that a 5α,8α-epoxido-$\Delta^{9(11)}$-steroid reacts readily with osmium tetroxide.

(8) Sulfuric acid analysis (a) A. Zaffaroni, THIS JOURNAL, **72**, 3828 (1950); (b) S. Bernstein and R. H. Lenhard, *J. Org. Chem.*, **18**, 1146 (1953), played an important role in the evaluation of the hydroxylation product. The $\Delta^{9(11)}$-5α-hydroxy-bis-ketal (IVa or b) on treatment with sulfuric acid gave immediately a pink-red solution, whereas the 5α,9α,11α,17α,21-pentol bis-ketal (Va or b) gave, after a brief colorless induction period, a solution which was fluorescent green by reflected light (similar to 11-epi-hydrocortisone) and violet by transmitted light. In this connection, the 5α,9α,17α,21-tetrol-11-one bis-ketal (VIa or b) gave a pale yellow solution after a brief colorless induction period.

(9) Generally, this procedure was employed for the separation of any Δ^{9}-compound from the hydroxylation product.

matography on silica gel (alcohol as the stationary phase, and 1.5% ethanol–methylene chloride as the mobile phase) into starting material IVb (47%) and the desired 11α-pentol 21-acetate bis-ketal Vb (34%, based on the amount of IVb consumed).

The 11α-pentol 21-acetate bis-ketal Vb on acetylation afforded the 11,21-diacetate Vc (67% yield), an experimental result which corroborated the rear-attack mechanism of hydroxylation by osmium tetroxide on the $\Delta^{9(11)}$-double bond. Saponification of the 11,21-diacetate Vc gave in 71% yield pregnane-5α,9α,11α,17α,21-pentol-3,20-dione 3,20-bis-ethylene ketal Va (*vide supra*).

The next synthetic problem was the conversion of the 9α,11α-diol grouping into a 9α,11β-diol grouping without the removal of the ketal groups. For this purpose, our experience with a related conversion, namely, that of 11-epi-hydrocortisone bis-ethylene ketal into hydrocortisone bis-ethylene ketal was utilized.[10] The 11α-pentol bis-ketal Va in pyridine was oxidized with chromium trioxide–pyridine complex,[11] and pure 11-one bis-ketal VIa was obtained in fair yield (36%).[12] However, oxidation of the 11α-pentol 21-acetate bis-ketal Vb under the same conditions gave a 75% yield of the 11-one 21-acetate bis-ketal VIb.[13,14]

The 11-one 21-acetate bis-ketal VIb was also prepared in the following manner, albeit in poor yield. Treatment of the Δ^9-21-acetate bis-ketal IVb in benzene–pyridine solution with osmium tetroxide (14 days at 37°) resulted in a product which was principally the 11α-pentol 21-acetate bis-ketal Vb contaminated with some starting material.[8] The mixture was oxidized with chromium trioxide–pyridine complex and this product appeared difficult to purify. It was therefore treated with acetic anhydride and pyridine and the acetylation mixture gave after fractional recrystallization pure VIb.

The 11-one bis-ketal VIa in tetrahydrofuran was reduced with lithium aluminum hydride to give pregnane-5α,9α,11β,17α,21-pentol-3,20-dione 3,20-bis-ethylene ketal (VII) (68% "crude" yield).[15] It was of some interest to us that the absorption spectra in concd. sulfuric acid of the 11α-pentol 3,20-bis-ketal Va and that of the 11β-pentol 3,20-bis-ketal VII were different and could be easily distinguished from each other. The maxima and minima were approximately at the same wave lengths but possessed different extinction coefficients. This observation was in line with our previous experience with such epimeric pairs.[8b,15a,16]

Hydrolysis of the 11β-pentol bis-ketal VII in ethanol with 8% (v./v.) sulfuric acid gave in poor yield after an extensive partition chromatography the desired 9α-hydroxy-hydrocortisone (VIIIa) in the form of a hydrate. The compound gave a positive Blue Tetrazolium test and exhibited the characteristic selective absorption of a Δ^4-3-ketone in the ultraviolet spectrum. Acetylation furnished 9α-hydroxyhydrocortisone acetate (VIIIb).[17] The structure of VIIIb was established unequivocally by its synthesis from 9β,11β-oxido-Reichstein's substance S acetate (IX) with 3 *N* aqueous perchloric acid.

Bioassays.[18,18a]—A thymus involution assay (adrenalectomized and ovariectomized mice) of 9α-hydroxyhydrocortisone (VIIIa) indicated its activity to be $^1/_3$–$^1/_2$ that of hydrocortisone.

In an electrolyte assay (K/Na ratio) on adrenalectomized rats, 9α-hydroxyhydrocortisone (VIIIa) was ineffective at a 3-μg. dose level, whereas at a 50-μg. dose level there was a significant increased excretion of sodium which indicated that this compound possessed an activity of the nature of hydrocortisone in this type of assay.

Acknowledgment.—We are indebted to Messrs. Louis M. Brancone, Samuel S. Modes, Gerald P. McTernan and John G. Heider for the microanalytical data, and to Messrs. William Fulmor and George Morton, and Miss Anne Callaghan for the optical rotation data and the infrared absorption spectra. We also wish to thank Mr. Charles Pidacks for his assistance in performing the partition chromatographic separations.

Experimental

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

Optical Rotations.—The rotations are for chloroform solutions (unless otherwise noted), and were determined at 24–25° in a 1-dm. micro or semi-micro tube at wave length 5893 Å. (D).

(10) W. S. Allen, S. Bernstein and R. Littell, THIS JOURNAL, **76**, 6116 (1954).

(11) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953).

(12) Examples of the oxidation (chromium trioxide–acetic acid) of a 9α,11α-diol grouping to a 9α-hydroxy-11-one grouping have been reported by R. B. Clayton, A. Crawshaw, H. B. Henbest, E. R. H. Jones, B. J. Lovell and G. W. Wood, *J. Chem. Soc.*, 2009 (1953), and by R. Hirschmann, C. S. Snoddy, Jr., and N. L. Wendler, THIS JOURNAL, **75**, 3252 (1953).

(13) The course and completion of the oxidation was conveniently followed by sulfuric acid analysis.[8]

(14) It should not be inferred from this increased yield with the 11α-pentol 21-acetate bis-ketal Vb that the poor yield with the 11α-pentol bis-ketal Va may be ascribed to the oxidation of the C-21-hydroxyl group. This would appear to be contrary to our experience with the oxidation of 11-epi-hydrocortisone bis-ketal.[10] A more thorough investigation of the oxidation of the 11α-pentol bis-ketal Va will be required before any conclusions in this direction can be drawn.

(15) Unfortunately, we are not in a position to discuss the influence of a 9α-hydroxyl group on the known stereochemical course of the reduction of the C-11-ketone group. It may only be stated that the 11β-hydroxy-compound was obtained. In a related instance, C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.*, **16**, 1278 (1951), have reported in a sapogenin example, that a 12β-hydroxyl group appears not to effect the stereochemical reduction at C-11. It will be recalled that the latter work was completed some time prior to the discovery in this Laboratory (subsequently confirmed by others) that the 11α-epimer was simultaneously elaborated in low yield in the reduction of a C-11-ketone by metal hydrides; see (a) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell and J. H. Williams, *J. Org. Chem.*, **18**, 70 (1953); (b) R. Levin, B. Magerlein, A. McIntosh, A. Hanze, G. Fonken, J. Thompson, A. Searcy, M. Scheri and E. Gutsell, THIS JOURNAL, **76**, 546 (1954); (c) E. P. Oliveto, C. Gerold and E. B. Hershberg, *ibid.*, **76**, 6111 (1954), and (d) W. S. Allen, S. Bernstein and R. Littell, *ibid.*, **76**, 6116 (1954).

(16) S. Bernstein, R. Lenhard and J. H. Williams, *J. Org. Chem.*, **18**, 1166 (1953); see also, A. Zaffaroni, "Recent Progress in Hormone Research, The Proceedings of the Laurentian Hormone Conference," Academic Press, Inc., New York, N. Y., 1953, Vol. VIII, p. 51.

(17) The preparation of a monoacetate, *i.e.*, at C-21, corroborated the β-configuration for the C-11-hydroxyl group.

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

(18a) The glucocorticoid and sodium-retaining activities of 9α-hydroxyhydrocortisone acetate have been reported by Fried, *et al.*, *Recent Progress in Hormone Research*, **11**, 149 (1955), and *Ann. N. Y. Acad. Sciences*, **61**, 573 (1955).

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

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

All distillations and evaporations were carried out under reduced pressure.

Pregnane-11β,17α,21-triol-3,20-dione-5α,6α-oxide 3,20-Bis-ethylene Ketal (II).—Hydrocortisone bis-ketal (I, 4 g.) in chloroform (40 ml.) was treated with a solution of peroxybenzoic acid in ethyl acetate (1.88 g. in 75 ml.), and the mixture was allowed to stand at room temperature for 5.5 days. After 1 day, crystals separated. The mixture was cooled, and the crystals were collected; 1.75 g. (42% yield), m.p. 274–278°. A 250-mg. portion was crystallized twice from acetone to give 75 mg. of pure II, m.p. 283–286°, $[\alpha]_D$ −38° (*c* 0.881, pyridine).

In another run with I (6.88 g.) and peroxybenzoic acid (2.55 g.) there was obtained 3.7 g. (52% yield) of crude α-oxide II, m.p. 277–280°. A recrystallization from acetone–petroleum ether gave 2.93 g., m.p. 280–283°. A 200-mg. portion was crystallized 3 times from acetone–petroleum ether, and finally once from acetone to afford 10 mg., m.p. 281–283°; ν_{max} 3497, 1650 (weak, ?), 1098, 1075 and 1039 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{38}O_8$ (466.55): C, 64.36; H, 8.21. Found: C, 64.01; H, 8.40.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione 3,20-Bis-ethylene Ketal (IIIa).—A solution of the 5α,6α-oxide (2.75 g.) in tetrahydrofuran (250 ml.) was treated with lithium aluminum hydride (1.2 g.), and was refluxed for 5 hours. The excess hydride was discharged with water, and the inorganic precipitate was collected by filtration, and leached with chloroform (150 ml.). The filtrates were combined and evaporated. The solid residue was dissolved in chloroform, and the solution was washed with saturated saline and water. The dried extract on evaporation gave 2.65 g. (97% yield) of IIIa, m.p. 256–259°. Admixture melting point determination with an authentic sample gave no depression.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione 21-Acetate 3,-20-Bis-ethylene Ketal (IIIb).—Acetylation of the 5α,11β,17α,21-tetrol 3,20-bis-ketal IIIa (2.65 g.) was accomplished in the usual manner at room temperature with acetic anhydride (5 ml.) and pyridine (26 ml.). This gave 2.8 g. (97% yield) of the 21-acetate IIIb, m.p. 229–231°, identical with an authentic sample as shown by admixture m.p. determination and infrared absorption analysis.

$\Delta^{9(11)}$-Pregnene-5α,17α,21-triol-3,20-dione 21-Acetate 3,-20-Bis-ethylene Ketal (IVb).—The 5α,11β,17α,21-tetrol 21-acetate bis-ketal IIIb (0.12 g.) in pyridine (1.1 ml.) was treated (ice-cooling) with phosphorus oxychloride (0.095 ml.). The mixture was allowed to stand at room temperature for 46 hours and poured into water. The resulting crude IVb was collected by filtration, 86 mg. (74% yield), m.p. 202–205°. Five crystallizations from acetone–petroleum ether gave 40 mg. of pure IVb, m.p. 221–221.5°; ν_{max} 3571, 1764, 1242, 1224, 1211, 1196, 1093 and 1046 cm.$^{-1}$; $[\alpha]_D$ −24° (*c* 1.091).

Anal. Calcd. for $C_{27}H_{40}O_8$ (492.50): C, 65.83; H, 8.19. Found: C, 65.68; H, 8.27.

$\Delta^{9(11)}$-Pregnene-5α,17α,21-triol-3,20-dione 3,20-Bis-ethylene Ketal (IVa).—A solution of almost pure $\Delta^{9(11)}$-21-acetate bis-ketal IVb (250 mg.) in 2.5% alcoholic potassium hydroxide was refluxed for 0.5 hour. It was then concentrated under reduced pressure until crystals separated. Water was added, and the crystals were separated by centrifugation. The product so obtained was dissolved in chloroform, and the solution was dried and evaporated. The residue was recrystallized from acetone–petroleum ether to give 155 mg. (68% yield), m.p. 220–222°. Three further crystallizations from the same solvent pair gave 65 mg. of pure IVa, m.p. 222–223.5°; ν_{max} 3484, 3390, 1096 and 1058 cm.$^{-1}$; $[\alpha]_D$ −15° (*c* 1.501).

Anal. Calcd. for $C_{25}H_{38}O_7$ (450.55): C, 66.64; H, 8.50. Found: C, 66.69; H, 8.79.

Pregnane-5α,9α,11α,17α,21-pentol-3,20-dione 3,20-Bis-ethylene Ketal (Va). A.—To a solution of the Δ^9-21-acetate bis-ketal IVb (1.1 g.) in benzene (90 ml.) and pyridine (0.95 ml.) was added osmic acid (1 g.) and the mixture was allowed to stand at 37° for 25 days, and at 63° for 17 days (closed system). It was cooled to room temperature, and treated with methanol (60 ml.), water (90 ml.), sodium sulfite (11 g.) and potassium bicarbonate (11 g.). The mixture was stirred for 5 hours, treated with chloroform (200 ml.), and filtered. The brown solid was slurried with hot chloroform (150 ml.) and re-filtered. The filtrates were combined and concentrated almost to dryness. The residual mixture was extracted with ethyl acetate. The extract was washed with saline, water, dried and treated with Norite. Evaporation gave a glass which on being slurried with acetone–petroleum ether afforded mixed crystals which melted at 188–192°. A sulfuric acid color test showed the presence of starting material. The solid was combined with its evaporated mother liquor, and was treated with 2.5% alcoholic potassium hydroxide (25 ml., 1 hr. reflux). Water was added, and the alcohol was removed by distillation under reduced pressure. This gave crystals which were collected by filtration, 0.75 g., m.p. 238–243°; a sulfuric acid color test showed the presence of a trace of starting material. Two crystallizations of this material from acetone–petroleum ether gave 400 mg. (38% yield) of white crystals, m.p. 252–255°, which were free of starting material.

A 100-mg. portion was crystallized four times further from acetone–petroleum ether to afford 30 mg. of pure pentol bis-ketal Va, m.p. 258–260°, ν_{max} 3472, 3390, 1088 and 1046 cm.$^{-1}$; $[\alpha]_D$ ±0° (*c* 1.045).

Anal. Calcd. for $C_{25}H_{40}O_9$ (484.57): C, 61.96; H, 8.32. Found: C, 61.83; H, 8.59.

B.—A solution of the 11,21-diacetate bis-ketal Vc (0.19 g.) in ethanol (6 ml.) and 5% alcoholic potassium hydroxide (4 ml.) was refluxed for 1 hour. Water was added, and the alcohol was removed by distillation under reduced pressure. This gave 85 mg. of white crystals, m.p. 256–258°. An additional 15 mg., m.p. 254–256°, was obtained by allowing the aqueous mother liquor to stand.

The aqueous mother liquor from this second filtration was extracted with chloroform to afford a further quantity (no wt. taken) of product, m.p. 242–244°. Two crystallizations from acetone–petroleum ether gave 15 mg. of Va, m.p. 255–256°. Its infrared absorption spectrum was identical with that of A. The total weight of Va was 115 mg. (71% yield).

Anal. Found: C, 62.12; H, 8.44.

Pregnane-5α,9α,11α,17α,21-pentol-3,20-dione 21-Acetate 3,20-Bis-ethylene Ketal (Vb).—To a solution of the Δ^9-21-acetate bis-ketal IVb (2.1 g.) in benzene (100 ml.) and pyridine (1.8 ml.) was added osmic acid (2 g.), and the mixture was heated at 63° for 18 days (closed system). It was then cooled to room temperature, and treated with methanol (90 ml.), water (120 ml.), sodium sulfite (14 g.) and potassium bicarbonate (14 g.). This mixture was stirred for 6 hours, treated with chloroform (200 ml.) and filtered. The brown solid was slurried with hot chloroform (200 ml.) and re-filtered. The combined filtrates were concentrated to a small volume, and the product was extracted with ethyl acetate. The extract was washed with saturated saline, dried, treated with Norite, and evaporated. This afforded 2.2 g. of an oil which was chromatographed on silica gel (100 g.) containing 40 ml. of ethanol (stationary phase) with 1.5% ethanol–methylene chloride (mobile phase).[19] After elution of 1.05 g. (47%) of starting material IVb, crude pentol Vb, m.p. 198–201°, was obtained. A single recrystallization from acetone–petroleum ether afforded 380 mg. (17% yield based on unrecovered starting material), m.p. 208–210°. A portion was crystallized twice more from the same solvent pair; m.p. 208–209°; ν_{max} 3472, 1745, 1261, 1086 and 1050 cm.$^{-1}$; $[\alpha]_D$ +8° (*c* 1.715).

Anal. Calcd. for $C_{27}H_{42}O_{10}$ (526.61): C, 61.58; H, 8.04. Found: C, 61.34; H, 8.21.

Pregnane-5α,9α,11α,17α,21-pentol-3,20-dione 11,21-Diacetate 3,20-Bis-ethylene Ketal (Vc).—To a solution of the 21-acetate bis-ketal Vb (55 mg., practically pure mother liquor material) in pyridine (1 ml.) was added acetic anhydride (0.5 ml.), and the mixture was allowed to stand at room temperature overnight. Addition of water gave 40 mg. (67% yield) of crude material, m.p. 212–215°. Four crys-

(19) A. H. Soloway, A. S. Deutsch and T. F. Gallagher, THIS JOURNAL, **75**, 2356 (1953).

tallizations from acetone–petroleum ether gave 17 mg. of pure 11,21-diacetate bis-ketal Vc, m.p. 247–248°.

Anal. Calcd. for $C_{39}H_{44}O_{11}$ (568.64): C, 61.25; H, 7.80; OAc, 15.15. Found: C, 61.05; H, 7.88; OAc, 16.15.

Pregnane-5α,9α,17α,21-tetrol-3,11,20-trione 3,20-Bis-ethylene Ketal (VIa).—A cooled solution of the 11α-pentol bis-ketal Va (0.58 g.) in pyridine (35 ml.) was added to a mixture of chromium trioxide (0.36 g.) in cold pyridine (5 ml.). The reaction mixture was stirred for 18 hours at 25–30°. After the addition of sodium bicarbonate (0.45 g.), the pyridine was removed by steam distillation. The residual mixture was extracted with ethyl acetate (200 ml.), and the extract was washed with saline and water, dried and evaporated. This furnished a white powder, m.p. 252–258°. One recrystallization from acetone–petroleum ether gave 205 mg. (36%), m.p. 279–282°. Six further crystallizations gave the analytical sample, m.p. 291–293°; ν_{max} 3448, 1708, 1087, 1074 and 1042 cm.$^{-1}$; $[\alpha]_D$ +21° (*c* 0.518, pyridine).

Anal. Calcd. for $C_{25}H_{38}O_9$ (482.55): C, 62.22; H, 7.94. Found: C, 62.04; H, 8.02.

Pregnane-5α,9α,17α,21-tetrol-3,11,20-trione 21-Acetate 3,20-Bis-ethylene Ketal (VIb). A.—A cooled solution of the 11α-pentol 21-acetate bis-ketal Vb (80 mg.) in pyridine (3 ml.) was added to a cooled mixture of chromium trioxide (44 mg.) in pyridine (1 ml.). The reaction mixture was allowed to stand at 26° for 19 hours. It was then poured into a saturated saline solution, and the product was extracted with chloroform. The extract was washed with water, dried and treated with Norite. Evaporation afforded an oil which gave crystals from acetone–petroleum ether; 60 mg. (75% yield) of tan powder, m.p. 237–241°. Two further crystallizations from the same solvent pair gave 43 mg. of VIb as white crystals, m.p. 239–241°.

B.—To a solution of the Δ^9-21-acetate bis-ketal IVb (0.47 g.) in benzene (27 ml.) and pyridine (0.92 ml.) was added osmic acid (0.8 g.), and the mixture was allowed to stand at 37° for 14 days (closed system). The product was worked up in the manner described above, and this gave 350 mg., m.p. 195–196°. Sulfuric acid analysis indicated the material to be principally the desired product with some starting material.

The mixture was dissolved in pyridine (9 ml.) and was treated with chromium trioxide–pyridine complex (0.2 g. in 3 ml.) (20 hours at 24–31°). The crude oxidation product was a tan powder which was recrystallized from acetone–petroleum ether (Norite treatment) to afford 185 mg. of m.p. 215–218°. Sulfuric acid analysis gave a pale yellow solution. Recrystallization from acetone–petroleum ether indicated that purification would be difficult. Therefore the material was dissolved in pyridine (1.5 ml.) and treated with acetic anhydride (0.3 ml.) (overnight at room temperature). The mixture was evaporated with mild heating, and the residue was slurried with cold ether to afford after filtration 145 mg., m.p. 234–238°. Two crystallizations from acetone–petroleum ether gave 66 mg. of lower m.p., 221–223°. From the mother liquor of the second crystallization there was obtained 55 mg., m.p. 234–237°. Three crystallizations of this material from acetone–petroleum ether gave pure 11-one 21-acetate bis-ketal VIb, 33 mg., m.p. 238–240°; ν_{max} 3448, 1764, 1725, 1233, 1064, 1052 and 1096 cm.$^{-1}$; $[\alpha]_D$ +28° (*c* 0.955).

Anal. Calcd. for $C_{27}H_{40}O_{10}$ (524.59): C, 61.81; H, 7.69. Found: C, 62.14; H, 8.01.

Pregnane-5α,9α,11β,17α,21-pentol-3,20-dione 3,20-Bis-ethylene Ketal (VII).—The 11-one bis-ketal VIa (148 mg.) in tetrahydrofuran (10 ml.) was treated with lithium aluminum hydride (0.1 g.), and the mixture was refluxed for 3 hours. Water was added cautiously to the cooled mixture to decompose the excess hydride. Chloroform was added, and the mixture was warmed. Inorganic material was separated by filtration, and the filtrate was evaporated. The residual oil was slurried in cold ether to afford 100 mg. (68% yield) of white crystals, m.p. 260–265°. Three crystallizations from acetone (with ether washings) gave 21 mg. of VII, m.p. 284.5–286.5°; ν_{max} 3472 (shoulder), 3413, 1087 and 1046 cm.$^{-1}$; $[\alpha]_D$ +4° (*c* 1.006).

Anal. Calcd. for $C_{25}H_{40}O_9$ (484.57): C, 61.96; H, 8.32. Found: C, 62.12; H, 8.56.

Δ^4-Pregnene-9α,11β,17α,21-tetrol-3,20-dione (9α-Hydroxyhydrocortisone) (VIIIa).—To a suspension of the 11β-pentol bis-ketal (VII, 175 mg.) in ethanol (20 ml.) was added 8% (v./v.) sulfuric acid (2 ml.), and the mixture was refluxed for 45 minutes. Water (10 ml.) was added, and the alcohol was removed by distillation. The residual mixture was extracted with ethyl acetate, and the extract was washed with saturated saline solution, dried and evaporated. This afforded a glass which on trituration with ether crystallized, and 80 mg. of a low melting solvate was obtained. This solid was subjected to partition chromatography on Celite (150 g.)[20] in the following manner. The mobile phase consisted of 3 parts ethyl acetate and 2 parts petroleum ether (b.p. 90–100°), whereas the stationary phase consisted of 3 parts methanol and 2 parts water. Both phases were equilibrated with each other. The solid was dissolved in the stationary phase (3.5 ml.) and slurried with Celite (7 g.), and the mixture was added to the column. Chromatography was then initiated with the mobile phase, and 185 fractions of 20-ml. volume each were collected. Finally the column was washed with methanol. Fractions 155–180 (inclusive) on paper chromatographic analysis[21] (24 hours) gave only a single spot at 4 cm. The methanol wash gave a spot at 2 cm. and a sizable one at 4 cm.

Fractions 155–180 (inclusive) were combined and evaporated. Two crystallizations of the residue from acetone–petroleum ether afforded 10 mg. of almost pure VIIIa, m.p. 223–224°.

All fractions and mother liquors (with the exception of the above solid) were combined and evaporated. The residue was rechromatographed as above. Fractions 150–176 (inclusive) gave a single spot at 2.5–3.0 cm. (16 hours). Fractions 225–245 (inclusive) gave two spots at 1 and 2 cm. Fractions 150–176 (inclusive) were combined and evaporated, and the residue was triturated with ether to afford 20 mg. of VIIIa, m.p. 220–227°. Two crystallizations from acetone–petroleum ether gave 11 mg., m.p. 223–224.5°. This material was combined with the above 10 mg. of crystals, and crystallization from acetone–petroleum ether afforded 10 mg. of pure VIIIa, m.p. 223.5–225°, λ_{max} 242.5 mμ (ε 16,200); ν_{max} 3521, 1724, 1639, 1054 and 1044 cm.$^{-1}$; $[\alpha]_D$ +148° (*c* 0.460, pyridine).

Anal. Calcd. for $C_{21}H_{30}O_6 \cdot {}^1/_2H_2O$ (387.46): C, 65.10; H, 8.07. Found: C, 65.24; H, 8.00.

Δ^4-Pregnene-9α,11β,17α,21-tetrol-3,20-dione 21-Acetate (9α-Hydroxyhydrocortisone Acetate) (VIIIb). A.—The mother liquors from the above recrystallization of 9α-hydroxyhydrocortisone (VIIIa) were combined and evaporated to afford 35 mg. of an oil which was acetylated with pyridine (2 ml.) and acetic anhydride (0.5 ml.) (17 hours at room temperature). Addition of methanol and evaporation gave an oil. It was dissolved in ethyl acetate, and the solution was washed with a saturated sodium bicarbonate solution, and with a saturated saline solution, dried, treated with Norite and evaporated. The white solid so obtained appeared to be solvated and melted at about 195–200°. Paper chromatographic analysis (3.2 hours) revealed a single spot at 11.5 cm. (more polar than cortisone (free alcohol) and less polar than hydrocortisone (free alcohol)). Three crystallizations from acetone–petroleum ether gave 7 mg. (19% yield) of pure 21-acetate VIIIb, m.p. 216.5–217.5°, λ_{max} 242–243 mμ (ε 15,500); ν_{max} 3509, 1727, 1639, 1272, 1244 and 1047 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{32}O_7$ (420.49): C, 65.69; H, 7.67. Found: C, 65.46; H, 7.44.

B.—To Δ^4-pregnene-17α,21-diol-3,20-dione-9β,11β-oxide 21-acetate (9β,11β-oxido-Reichstein's substance S acetate) [IX, m.p. 222–223.5°, λ_{max} 242 mμ (ε 13,800), 82 mg.][22] in tetrahydrofuran (3 ml.) was added 3 *N* aqueous perchloric acid (1 ml.), and the mixture was allowed to stand at room temperature for 5 hours. It was poured into a saturated sodium bicarbonate solution, and the product was extracted

(20) The adsorbent was specially treated Celite "545" which was slurried in 6 *N* hydrochloric acid and allowed to stand overnight. It was then filtered and was washed with water, followed by 3A alcohol and/or acetone. Finally it was dried at 100°.

(21) The method was essentially that of I. E. Bush (*Biochem. J.*, **50**, 370 (1952)). The chromatography was carried out on untreated Whatman No. 1 paper with a benzene–water–methanol (2:1:1) solvent system; and was allowed to run at 37° for the designated time. The steroids were detected with an alkaline Blue Tetrazolium spray.

(22) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953), report m.p. 210–212°, $\lambda_{max}^{alc.}$ 243 mμ (ε 15,500).

with ethyl acetate. The extract was washed with saline and water, dried, treated with Norite and evaporated. This afforded an oil which on paper chromatographic analysis (4 hours) showed the presence of 6 distinct compounds. The mixture was then acetylated with acetic anhydride (0.5 ml.) and pyridine (2 ml.) (overnight at room temperature) to afford 88 mg. of an oil which on paper chromatographic analysis (3 hours) consisted of about equal amounts of 9α-hydroxyhydrocortisone acetate (VIIIb) (at 11 cm.) and material of the same polarity as starting material (at 18 cm.). There were also very minor traces of two other compounds at 1 and 14 cm.

The oil (85 mg.) was submitted to partition chromatography on Celite (60 g.) in the same manner described above, and 25-ml. fractions were taken. Fraction 2 consisted of 15 mg., m.p. 237–244° dec. (not further investigated), whereas the desired 9α-hydroxy-compound VIIIb was in fractions 9 and 10, which were evaporated. This furnished 25 mg. (29% yield), m.p. 215.5–217°. Two crystallizations from acetone–petroleum ether gave pure VIIIb, m.p. 218–219°, $[\alpha]_D$ +171° (*c* 0.662, pyridine). Its infrared absorption spectrum was identical to that of A.

PEARL RIVER, NEW YORK

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

Steroidal Cyclic Ketals. XIX.[1] The Synthesis of 7-Keto-desoxycorticosterone

BY ROBERT H. LENHARD AND SEYMOUR BERNSTEIN

RECEIVED SEPTEMBER 2, 1955

Reaction of the bis-ethylene ketal I of desoxycorticosterone acetate with N-bromosuccinimide followed by alumina furnished the 7α,21-diol 21-acetate bis-ketal IIb. Saponification with potassium carbonate gave the 7α,21-diol bis-ketal IIa (97% yield). Oxidation of IIb with chromic anhydride–pyridine complex gave the 3,7,20-trione 21-acetate 3,20-bis-ketal IIIb (89% yield). Reduction of the latter with lithium aluminum hydride produced both the 7α,21-diol bis-ketal IIa (13% yield) and the 7β,21-diol bis-ketal IV (29% yield). Hydrolysis of either epimer resulted in $\Delta^{4,6}$-pregnadiene-21-ol-3,20-dione (Va). The latter was further characterized by conversion into its 21-acetate Vb. Saponification of the 3,7,20-trione 21-acetate 3,20-bis-ketal IIIb with potassium bicarbonate afforded Δ^5-pregnene-21-ol-3,7,20-trione 3,20-bis-ethylene ketal (IIIa) (14% yield), and also, as the major product (73% yield), $\Delta^{3,5}$-pregnadiene-3-(β-hydroxy)-ethoxy-21-ol-7,20-dione 20-ethylene ketal (VIa). The diacetate VIb was obtained on acetylation. Hydrolysis of VIa gave in 80% yield the desired 7-keto-desoxycorticosterone which exists in its enol form, $\Delta^{3,5}$-pregnadiene-3,21-diol-7,20-dione (VII).

In a recent publication[2] from this Laboratory there was described the synthesis of the bis-ethylene ketal I of desoxycorticosterone acetate which was successfully transformed into a new derivative of desoxycorticosterone, namely, pregnane-5α,21-diol-3,20-dione. Subsequently, the possible utilization of I for the synthesis of 7-oxygenated derivatives of desoxycorticosterone[3] was explored, and the results obtained form the basis of this paper.

The bis-ethylene ketal I of desoxycorticosterone acetate in carbon tetrachloride and petroleum ether in the presence of anhydrous potassium carbonate was brominated in the allylic position (C-7) with N-bromosuccinimide.[4] The bromination product was not isolated but was treated immediately (after the removal of succinimide) with ethyl acetate washed alumina, and was stirred for 2.5 hours at room temperature.[5] In this manner, Δ^5-pregnene-7α,21-diol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (IIb) was obtained. Saponification in alcohol with an aqueous solution of potassium carbonate afforded Δ^5-pregnene-7α,21-diol-3,20-dione 3,-20-bis-ethylene ketal (IIa) (97% yield) ($[\alpha]^{24}_D$ −49°).

Oxidation of the 7α,21-diol 21-acetate bis-ketal IIb with chromic anhydride–pyridine complex[6] gave in 89% yield Δ^5-pregnene-21-ol-3,7,20-trione 21-acetate 3,20-bis-ethylene ketal (IIIb) which exhibited an ultraviolet absorption maximum at 240–241 mμ, with a molecular extinction coefficient of 12,700 characteristic of a Δ^5-7-ketone.[7]

Reduction of the 3,7,20-trione 21-acetate 3,20-bis-ketal IIIb in ether with lithium aluminum hydride afforded, after chromatography on silica gel, Δ^5-pregnene-7α,21-diol-3,20-dione 3,20-bis-ethylene ketal (IIa) (13% yield) and Δ^5-pregnene-7β,21-diol-3,20-dione 3,20-bis-ethylene ketal (IV) ($[\alpha]^{24}_D$ +29°) (29% yield). It is to be noted that the epimeric alcohols conform to the generalization established with other types of steroids that 7β-derivatives are dextrorotatory whereas the corresponding 7α-epimers are levorotatory.[5b,8]

The 7β,21-diol bis-ketal IV in methanol on hydrolysis with 8% (v./v.) sulfuric acid did not afford the desired 7β-hydroxydesoxycorticosterone. Under the conditions of the hydrolysis the C_7-hydroxyl group was eliminated and $\Delta^{4,6}$-pregnadiene-21-ol-3,20-dione (Va) in a solvated state (λ_{max} 283 mμ, ε 25,500) was formed in 67% yield.[9,10] The same compound (70% yield) was obtained by hydrolysis of the 7α,21-diol bis-ketal

(1) Paper XVIII, R. Littell and S. Bernstein, THIS JOURNAL, **78**, 981 (1956).

(2) S. Bernstein and R. H. Lenhard, *ibid.*, **77**, 2233 (1955).

(3) The only such derivative known is 7α-hydroxy-desoxycorticosterone which has been synthesized *microbiologically* by Ch. Meystre, E. Vischer and A. Wettstein, *Helv. Chim. Acta*, **38**, 381 (1955).

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

(5) (a) J. A. K. Buisman, W. Stevens and J. v. d. Vliet, *Rec. trav. chim.*, **66**, 83 (1947); (b) H. J. Ringold, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **74**, 3318 (1952).

(6) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953).

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

(8) (a) A. E. Bide, H. B. Henbest, E. R. H. Jones and P. A. Wilkinson, *J. Chem. Soc.*, 1788 (1948); and (b) C. W. Greenhalgh, H. B. Henbest and E. R. H. Jones, *ibid.*, 2375 (1952).

(9) After the completion of this work, the compound Va was described by Meystre, Vischer and Wettstein.[3]

(10) Only one example of a 7-ol-Δ^4-3-one synthesized by *chemical* means has been described, namely, Δ^4-cholestene-7β-ol-3-one.[8b] This compound was synthesized in 10% yield by an Oppenauer oxidation of Δ^5-cholestene-3β,7β-diol. The principal oxidation product (40%) was $\Delta^{4,6}$-cholestadiene-3-one. A similar pathway to Δ^4-cholestene-7α-ol-3-one was unsuccessful. The $\Delta^{4,6}$-diene was again the principal product (*ca.* 75% yield).