ethanol and benzene was added to incipient crystallization; yield 20 mg., m.p. 128–133°, 109–125° dec. on admixture with starting material, m.p. 130–136° on admixture with authentic IV, R_{glue} identical with that of authentic IV.

2-Acetoxymethyl-3,4,5,6-tetra-*O*-acetyl-1-bromo-1-deoxy-D-glucitol(mannitol?) (VI). (*a*) **With Hydrogen Bromide in Acetic Acid.**—To a solution of 2 g. of the epoxide acetate II in 3 ml. of acetic acid was added 3 ml. of acetic acid nearly saturated with hydrogen bromide. After 2 min. at room temperature, the solution was poured slowly into 40 ml. of a saturated aqueous sodium bicarbonate solution. Solid sodium bicarbonate was added in small portions until the solution was slightly basic to litmus. This mixture was extracted with three 10-ml. portions of chloroform, the combined extracts dried with sodium sulfate and the solvent removed under reduced pressure to yield a thin sirup. Solution in benzene followed by removal of the solvent under reduced pressure gave a sirup which crystallized spontaneously. Recrystallization was effected from ether–petroleum ether (b.p. 30–60°); yield 1.06 g., m.p. 91–98°. Two additional recrystallizations from ether–petroleum ether (b.p. 30–60°) gave pure material, m.p. 99–101° unchanged on recrystallization from methanol, $[\alpha]^{24}D$ +30.9° (*c* 3.9, $CHCl_3$); X-ray powder diffraction data: 11.22[26] vw[27], 9.46w, 7.78w, 6.76s, 5.69vw, 4.68w, 4.47vw, 3.97m, 3.73s, 3.10m.

Anal. Calcd. for $C_{17}H_{25}O_{11}Br$: C, 42.07; H, 5.19; Br, 16.46. Found: C, 41.97; H, 5.15; Br, 17.01.

(*b*) **With Magnesium Bromide.**—To prepare active magnesium bromide,[8b] an excess of ethylene bromide was added to 10 mg. of magnesium in 50 ml. of benzene–ether (1–1 by vol.). After all the magnesium had dissolved, 200 mg. of the epoxide acetate II was added and the solution was stirred overnight at room temperature. An equal volume of water was added, the ether layer separated, and the aqueous layer was extracted with 20 ml. of chloroform. The combined ether and chloroform extracts were dried with sodium sulfate and the solvent was removed under reduced pressure to yield a sirup. Solution in benzene followed by solvent removal as before gave a sirup which crystallized spontaneously; yield 0.105 g., m.p. 96.5–98°, mixed melting point with authentic bromohydrin acetate VI undepressed, strong positive Beilstein halogen test.

2-Acetoxymethyl-3,4,5,6-tetra-*O*-acetyl-1,2-dibromo-1,2-dideoxy-D-glucitol(mannitol?) (V).—The preparation of this compound follows that of the bromohydrin acetate VI part (a), except that 6 ml. of acetic acid saturated with hydrogen bromide was used with a reaction time of 21 hr. at about 10°. After slowly pouring the reaction mixture into 40 ml. of saturated aqueous sodium bicarbonate solution, solid sodium bicarbonate was added in small portions until the solution was no longer acidic to congo red paper but was still acidic to litmus paper. The frothy white gum, initially formed, became crystalline on thorough trituration. Filtration was followed by washing with water until the filtrate was no longer acidic to litmus paper; yield 2.39 g., m.p. 79–82°. Two recrystallizations were effected from ether–petroleum ether (b.p. 30–60°) (a small amount of the bromohydrin acetate VI may be obtained from the mother liquors); m.p. 81–84°, $[\alpha]^{24}D$ +35.0° (*c* 3.3, $CHCl_3$), whose melting point range was unchanged on recrystallization from hot 1,2-dimethoxyethane.

Anal. Calcd. for $C_{17}H_{24}O_{10}Br_2$: C, 37.24; H, 4.41; Br, 29.16. Found: C, 37.94; H, 4.94; Br, 28.65.

On attempted recrystallization of this compound from hot methanol, sirups were obtained except on one occasion when a small amount of a solid was obtained, m.p. 126° dec., strongly positive Beilstein halogen test. This material was not further characterized.

2-Acetoxymethyl-3,4,5,6-tetra-*O*-acetyl-1-chloro-1-deoxy-D-iditol(gulitol?).—An amount of 1 g. of the epoxide acetate, obtained, as described above, from *keto*-L-sorbose pentaacetate, was treated with an acetic acid solution of hydrogen chloride as described above for the corresponding derivative II from *keto*-D-fructose pentaacetate except that the reaction mixture was maintained at room temperature for 4 min. The resultant crude, sirupy product was isolated in the same manner. This sirup was divided in half and each half was chromatographed on a Magnesol[32]–Celite[33] (5–1 by wt.) column (17.5 × 3.5 cm., diam.) using 350 ml. of benzene–*tert*-butyl alcohol (100–1 by vol.) as developer. Extrusion of the columns and streaking with alkaline permanganate indicator revealed a single large zone located 1–8 cm. from the column top. The zones from the two columns were dissected, combined, and eluted with acetone. On evaporation of the solvent a yellow sirup was obtained. This sirup was divided into thirds and each third was chromatographed as before except that 700 ml. of developer was used for each column. Alkaline permanganate indicator (1% potassium permanganate in 2.5 *N* sodium hydroxide) revealed a single zone on each column located 7–11 cm. from the column top. The bottom half of each of these zones was dissected, combined, and eluted with acetone. Removal of the solvent under reduced pressure gave a yellow sirup. The sirup was decolorized by two treatments with Darco G60[34] in ethanol and then dried at 78° in vacuum over phosphorus pentoxide; yield 110 mg., $[\alpha]^{24}D$ +14.2° (*c* 4.3, $CHCl_3$).

Anal. Calcd. for $C_{17}H_{25}O_{11}Cl$: C, 46.32; H, 5.73; Cl, 8.04. Found: C, 46.57; H, 5.52; Cl, 8.54.

(32) A product of the Westvaco Chemical Division of Food Machinery and Chemical Corp., South Charleston, W. Va.

(33) A siliceous filter-aid produced by the Johns-Manville Co., New York, N. Y.

(34) An activated carbon produced by the Darco Corporation, 60 East 42nd Street, New York, N. Y.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF G. D. SEARLE AND CO.]

7-Keto Steroids. I. Steroidal 3-Hydroxy-3,5-dien-7-ones

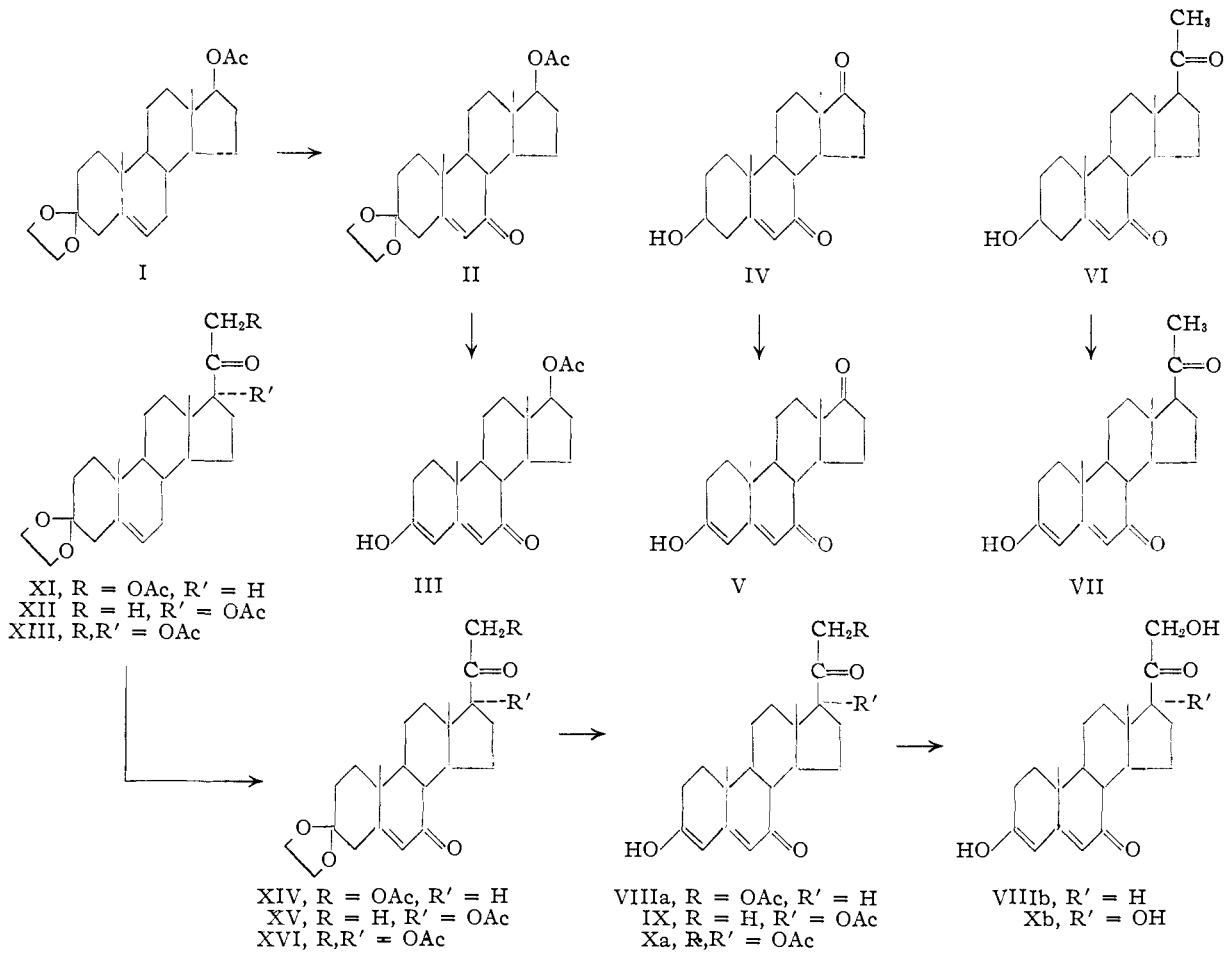
BY C. W. MARSHALL, RICHARD E. RAY, IVAR LAOS AND BYRON RIEGEL

RECEIVED JUNE 13, 1957

A series of steroidal 3-hydroxy-3,5-dien-7-ones was prepared for evaluation as anti-cortisone agents. Of the compounds studied, 7-keto-desoxycorticosterone 21-acetate appears to have the greatest biological interest. Two synthetic routes were employed. 3β-Hydroxyandrost-5-ene-7,17-dione and 3β-hydroxypregn-5-ene-7,20-dione were subjected to Oppenauer oxidation for 30 minutes; whereas the other members of the series were made by *t*-butyl chromate oxidation of the corresponding Δ^6-3-ethylene ketal with subsequent ketal cleavage.

Early in 1953 our Division of Biological Research reported that 7-ketocholesterol possessed marked anti-cortisone properties but caused toxic effects in animals. Accordingly we embarked on a concerted effort to explore this area of steroid chemistry which had long lain fallow. There were at that time few 3-hydroxy Δ^5-7-keto steroids reported in the literature, and we have since added a number of new members to this series which are concurrently reported in Paper II.[1] However, when this program was initiated in our laboratories, the chemical literature revealed only one example

(1) C. W. Marshall, Richard E. Ray, Ivar Laos and Byron Riegel, THIS JOURNAL, **79**, 6308 (1957).



of a steroidal 3-hydroxy-3,5-dien-7-one, namely, 7-ketocholestenone, (3-hydroxycholesta-3,5-dien-7-one).[2] Accordingly our first efforts were directed to this series which is described in this paper.

The synthesis and some biological properties are here reported for 7-ketotestosterone 17-acetate (17β - acetoxy - 3 - hydroxyandrosta - 3,5 - dien-7-one),[3] 7-ketoandrostendione (3-hydroxyandrosta-3,5-diene-7-17-dione),[3] 7-ketoprogesterone (3-hydroxypregna-3,5-diene-7,20-dione), 7-ketodesoxycorticosterone (3,21-dihydroxypregna-3,5-diene-7,-20-dione)[4] and its 21-acetate (21-acetoxy-3-hydroxypregna-3,5-diene-7,20-dione), 7-keto-17-acetoxyprogesterone (17α-acetoxy-3-hydroxypregna-3,5-diene-7,20-dione), 7-keto Reichstein's Substance "S" (3,17α-21-trihydroxypregna-3,5-diene-7,20-dione) and its 17,21-diacetate (17α,21-diacetoxy-3-hydroxypregna-3,5-diene-7,20-dione).

Two synthetic routes were used. The first was the modified Oppenauer oxidation[5] of 3-hydroxyandrost-5-ene-7,17-dione (IV)[6] and 3-hydroxypregn-5-ene-7,20-dione (VI)[1,7] to yield 3-hydroxyandrosta-3,5-diene-7,17-dione (V) and 7-ketoprogesterone (VII). It was found that 30 minutes slow distillation through a short Vigreux column proved optimum (65% yield). The enolic hydroxyl, present in each of the two products, allowed an elegant means of separating the products from the reaction mixture—after the aluminum salts had been removed with dilute hydrochloric acid—by extraction into cold dilute alkali.

The second approach employed the readily available Δ^4-3-keto steroids which were converted to the Δ^5-3-ethylene ketals[8] and oxidized in the allylic C-7 position with *t*-butyl chromate.[9,10] Preliminary trials with Reichstein's Substance "S" 21-acetate 3-ethylene ketal resulted in unwanted attack at C-17 by the *t*-butyl chromate. Therefore

(2) (a) J. Barnett, B. E. Ryman and F. Smith, *J. Chem. Soc.*, 526 (1946); (b) C. W. Greenhalgh, H. B. Henbest and E. R. H. Jones, *ibid.*, 2375 (1952).

(3) The 3-methyl ether of 7-ketotestosterone and 3-hydroxyandrosta-3,5-diene-7,17-dione were described recently by P. N. Rao and P. Kurath, THIS JOURNAL, **78**, 5660 (1956).

(4) R. H. Lenhard and S. Bernstein, *ibid.*, **78**, 989 (1956). These authors, employing a different route, prepared one compound in this series, 7-ketodesoxycorticosterone.

(5) Ch. Meystre and A. Wettstein, *Helv. Chim. Acta*, **30**, 1256 (1947).

(6) (a) W. Logemann and P. Giraldi, *Gazz. chim. ital.*, **81**, 548 (1951); (b) A. Butenandt and W. Logemann, U. S. Patent 2,170,124 (1939).

(7) The acetate of this compound has been described by (a) W. Klyne, *J. Chem. Soc.*, 3449 (1951), and (b) Logemann and Giraldi, ref. 6a.

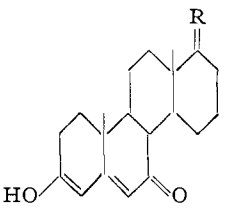
(8) (a) R. Antonucci, S. Bernstein, R. Littell, K. J. Sax and J. H. Williams, *J. Org. Chem.*, **17**, 1341 (1952); (b) Antonucci, Bernstein, R. Lenhard, Sax and Williams, *ibid.*, **17**, 1369 (1952); and (c) Antonucci, Bernstein, M. Heller, Lenhard, Littell and Williams, *ibid.*, **18**, 70 (1953).

(9) R. V. Oppenauer and H. Oberrauch, *Anales asoc. quim. Argentina*, **37**, 246 (1949).

(10) K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **35**, 284 (1952).

TABLE I

THE CHLOROFORM EFFECT ON δ-HYDROXY-α,γ-DIENONES



R =	$[\alpha]_D$ Dioxane	$[\alpha]_D$ $CHCl_3$	Ultraviolet spectra MeOH λ, mμ	MeOH ε	$CHCl_3$ λ, mμ	$CHCl_3$ ε
O	−256°	0°, USP	320	24,700		
		0, Pur.[c]			307	4500
	−207 (HCl)	−9[a]	312 (HCl)	26,000		
OAc / H	−269	?	320	24,500	306	5200
	−260					
$COCH_3$ / H	−204, Py.[d]	Insol.	321	21,200	Insol.	Insol.
$COCH_2OAc$ / H	−182	+29, USP	320	23,700	304.5	5500
$COCH_2OH$ / H	−131	+2	320	22,400		
		−267[b]				
$COCH_3$ / OAc	−208	−174, USP	318	23,000		
$COCH_2OAc$ / OAc	−258	−46, Pur.	320	21,000	300–304	2300

[a] Rao and Kurath, ref. 3. [b] Lenhard and Bernstein, ref. 4. [c] Pur. = washed with H_2O and dried. [d] Py. = pyridine.

the 17α-hydroxyl was protected by acetylation in preparing 7-keto-"S" (Xb), its diacetate Xa, and 7-keto-17α-acetoxyprogesterone (IX). Ultraviolet spectra of the total crude solids showed the formation of about 80% 7-keto derivative, based on molecular extinction coefficients in the range of 12,000 to 13,000 for the pure 7-keto-Δ^5-3-ketals. Separation of these products from the corresponding 7-desoxy starting material by chromatography on silica gel proved difficult and was only used to prepare analytical samples of the 7-keto-Δ^5-3-ketals. Thus in most cases, the desired 3-enol was obtained in 35–45% yield by subjecting the crude 80:20 mixture to ketal hydrolysis in aqueous 80% acetic acid for 30 minutes on the steam-bath. This method of hydrolysis allowed preservation of the ketol acetate side chain of 7-ketodesoxycorticosterone 21-acetate (VIIIa) and 7-keto-17α-acetoxyprogesterone (IX), as well as the diacetoxy acetone group at C-17 in the 7-keto Substance "S" diacetate (Xa).

For the preparation of 7-ketodesoxycorticosterone (VIIIb), the 7-keto-21-acetate-3 ketal was first subjected to ester exchange in methanolic potassium hydroxide for 8 minutes to form the 21-ol as a mixture of 3-ketal (*ca.* 85%) and 3-(β-hydroxy)-ethoxy-3,5-dien-7-one (*ca.* 15%) as estimated by ultraviolet spectra. The mixture, upon undergoing perchloric acid catalyzed exchange in acetone, provided pure VIIIb. It is worthy to note the extreme lability to base of the 3-ethylenedioxy-Δ^5-7-keto steroids[3,4] which represent vinylogs of β-diketone monoketals.[11]

In this series of steroidal 3-hydroxy-3,5-dien-7-ones, the justification for structure assignment was based partly on analogy with the known 3-hydroxycholesta-3,5-dien-7-one,[2] on the strong levorotatory effect of the compounds in solvents other than chloroform and the maxima at 320 mμ in the ultraviolet spectra.[12]

The instability of these 3-hydroxy-3,5-dien-7-ones has been mentioned by Rao and Kurath.[3] We have found the pure crystalline solids quite stable for periods of at least two years. A sample of 3-hydroxyandrosta-3,5-diene-7,17-dione (V) and of 7-ketotestosterone 17-acetate (III) showed no change in melting point or rotation (in dioxane) after 30 months storage in tightly stoppered bottles. However in solution these compounds show a marked instability and extreme reactivity, particularly to autoxidation, reminiscent of dihydroresorcinol. These compounds in neutral ketonic or ester solvents can be recovered unchanged after 1 to 2 hr. but after two days are largely converted to as yet unidentified adducts and/or condensation products. In chloroform solution, these compounds undergo striking changes in optical rotatory, ultraviolet and infrared absorption properties. These changes are summarized in Table I, and whether they point to adduct formation,[13] proton-catalyzed tautomeric equilibrium shifts[14] or hydrogen bonding is still uncertain and will be the subject of a future communication. It is obvious that in chloroform the characteristic enolenone structure of these compounds is largely destroyed.

The only exception in Table I is the amazingly strong levorotation (−267°) in chloroform obtained for 7-ketodesoxycorticosterone by Lenhard and Bernstein.[4]

In a number of routine biological tests conducted

(11) M. W. Cronyn and J. E. Goodrich, THIS JOURNAL, **74**, 3331 (1952).

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

(13) Ch. Weizmann, M. Sulzbacher and E. Bergmann, THIS JOURNAL, **70**, 1153 (1948).

(14) H. Rapoport and J. B. Lavigne, *ibid.*, **78**, 2455 (1956).

by our Division of Biological Research,[15] these compounds have exhibited appreciable anti-cortisone effects in that they block some of the undesirable properties of cortisone. Weak to moderate blocking of cortisone-induced fulmination of Coxsackie virus infection in mice was shown by III, VII and VIIIa. Antineoglycogenetic property was exhibited in low degree by only one compound in this series, namely, IX. When administered with cortisone, compounds III, VII, VIIIa and Xa blocked the lymph node atrophying influence of cortisone in either the rabbit or the mouse. Compound VIIIa also inhibited cortisone-induced thymus involution in mice. In anti-inflammatory properties, a few of these compounds showed mild activity, compound VIIIa being weakly active in the rabbit iritis and in the Selye type[16] pneumodermal rat pouch tests. Also VIIIa and VII were active anti-inflammatory agents in the mouse cotton pellet granuloma test, whereas compound V reduced inflammatory petechiae in the hamster cheek pouch. Anti-androgenic activity of mild order in the rat seminal vesicle test was shown by V.

Experimental[17,18]

3-Ethylenedioxy-17β-acetoxyandrost-5-en-7-one (II).[8a]—A solution of 20 g. of testosterone 17-acetate 3-ethylene ketal[8a] in 200 ml. of carbon tetrachloride was warmed to 60° with stirring, and to this over 45 minutes was added a mixture of 190 ml. of *t*-butyl chromate solution (equivalent to 28.5 g. of CrO_3),[10] 80 ml. of acetic acid and 20 ml. of acetic anhydride. The reaction mixture was stirred at 60–65° for 18 hr. and cooled to 20°. The excess chromate was reductively hydrolyzed with aqueous oxalic acid and the mixture worked up in the usual fashion,[10] using chloroform as the extraction solvent. Of the crude solids (18.5 g.), 16.5 g. was subjected to ketal hydrolysis, without further purification, as in the preparation of III. The balance of 2 g. of crude 7-keto 3-ketal was chromatographed on 168 g. of silica gel using benzene and benzene–ethyl acetate. The fraction eluted with 5% ethyl acetate in benzene provided 877 mg. of crude II, m.p. 254–256°. Two recrystallizations from acetone gave pure II,[19] m.p. 260–261°, $[\alpha]_D$ −72° (1.02% in dioxane), $\epsilon_{2403}^{CH_3OH}$ 12,000; μ^{KBr} 5.78, 6.02, 6.11 (shoulder), 7.95, 9.09 and 9.32.

Anal. Calcd. for $C_{23}H_{32}O_5$ (388.49): C, 71.10; H, 8.30. Found: C, 70.88; H, 8.25.

3-Hydroxy-17β-acetoxyandrost-3,5-dien-7-one (III).—The crude 7-keto-testosterone 17-acetate 3-ethylene ketal (16.5 g.) was ketal-hydrolyzed by heating at 100° for 25 minutes in 410 ml. of aqueous 80% acetic acid. Upon chilling, precipitation with excess cold water and filtration there was obtained 11 g. of very crude solids. Crystallization alternately from ethyl acetate and methanol provided pure III, as dimorphs, m.p. 245–246° and 268–271°, $[\alpha]_D$ −269° (0.89% in dioxane), $\epsilon_{3200}^{CH_3OH}$ 24,500, $\epsilon_{3060}^{CHCl_3}$ 5200; μ^{KBr} 3.19, 5.78, 6.18, 6.28 and 6.50.

Anal. Calcd. for $C_{21}H_{28}O_4$ (344.43): C, 73.23; H, 8.19. Found: C, 73.34; H, 8.27.

3-Hydroxyandrost-3,5-diene-7,17-dione (V).[8]—A solution of 20 g. of 3β-hydroxyandrost-5-ene-7,17-dione (IV)[6] in 1400 ml. of toluene was azeotropically dried by distilling off 300 to 400 ml. and then treated at vigorous reflux with 200 ml. of cyclohexanone and 150 ml. of a toluene solution of aluminum isopropoxide (30 g.). Slow distillation through a short Vigreux column was maintained for 25 minutes, according to Meystre and Wettstein.[20] The reaction mixture was chilled, mixed with 3 liters of cold dichloromethane, acidified with 1 liter of cold dilute sulfuric acid, agitated and the layer separated. The dichloromethane extracts were counter-extracted with two 500-ml. portions of cold aqueous 2% sodium hydroxide and the alkaline extracts acidified at once with cold dilute hydrochloric acid whereupon the desired enolic product precipitated. The solid was filtered, washed with water and air-dried to yield 15 g. of yellow powdery crude V. Crystallization from methanol and from acetone afforded pure dimorphous V,[21] m.p. 215-216° and 227–228° (the higher-melting form being invariably obtained from acetone), $[\alpha]_D$ −256° (0.82% in dioxane), $[\alpha]_D$ 0° (0.72% in $CHCl_3$, U.S.P. or freshly washed and dried), $\epsilon_{3200}^{CH_3OH}$ 24,700, $\epsilon_{3070}^{CHCl_3}$ 4500; μ^{KBr} 3.02, 5.74, 6.06, 6.28, 6.40, 8.25, 8.55 and 9.45.

Anal. Calcd. for $C_{19}H_{24}O_3$ (300.38): C, 75.97; H, 8.05. Found: C, 76.06; H, 8.15.

3-Hydroxypregna-3,5-diene-7,20-dione (VII).—To a solution of 2 g. of pure 7-ketopregnenolone (VI)[1,7] of m.p. 209–210° in 150 ml. of toluene at vigorous reflux was added 20 ml. of cyclohexanone and 15 ml. of a toluene solution of aluminum isopropoxide (3 g.). The reaction mixture was slowly distilled for 25 minutes, chilled and worked up as in the preparation of V above. The precipitated acidic (enolic) fraction, after air-drying, weighed 1.5 g. Crystallization from methanol afforded 1.2 g. of product, m.p. 258–259°, and recrystallization provided pure VII as white cottony needles, m.p. 260-261°, $[\alpha]_D$ −204° (1.09% in pyridine), $\epsilon_{3210}^{CH_3OH}$ 23,200; μ^{KBr} 3.19, 5.88, 6.18, 6.27 and 6.50. (Note this product was too insoluble in $CHCl_3$ or dioxane to obtain rotations in these solvents.)

Anal. Calcd. for $C_{21}H_{28}O_3$ (328.43): C, 76.79; H, 8.59. Found: C, 76.48; H, 8.88.

3-Ethylenedioxy-21-acetoxypregn-5-ene-7,20-dione (XIV).—A solution of 7.5 g. of desoxycorticosterone acetate 3-ethylene ketal[8b] in 400 ml. of carbon tetrachloride was subjected to oxidation with *t*-butyl chromate (120 ml. of CCl_4 solution equivalent to 11 g. of CrO_3) in the presence of 110 ml. of acetic acid and 25 ml. of acetic anhydride by a procedure similar to the preparation of II above. The reaction mixture was worked up in a like fashion, using dichloromethane as the extraction solvent, and gave a total crude steroid residue of 7.1 g. which was 70% Δ^5-7-one by ultraviolet analysis ($\epsilon_{2400}^{CH_3OH}$ 9000). The residue was divided into two portions, 5.1 g. being set aside for ketal hydrolysis (see preparation of VIIIa) and the balance chromatographed. Chromatography of the 2-g. portion of crude product on 200 g. of silica gel with benzene–ethyl acetate mixtures provided, from the 10% ethyl acetate elutions, 800 mg. of crude crystalline product, $\epsilon_{2380}^{CH_3OH}$ 11,500. Two recrystallizations from acetone furnished pure XIV, m.p. 252–254°, $[\alpha]_D$ +11° (1.06% in dioxane), $\epsilon_{2400}^{CH_3OH}$ 12,400; μ^{KBr} 5.75, 5.80, 6.01, 6.15, 8.07, 9.05, 9.31 and 9.48.

Anal. Calcd. for $C_{25}H_{34}O_6$ (430.53): C, 69.74; H, 7.96. Found: C, 69.51; H, 8.17.

3-Hydroxy-21-acetoxypregna-3,5-diene-7,20-dione (VIIIa).—The 5.1-g. portion, set aside above, of crude 7-keto-3-ketal (XIV) was hydrolyzed by heating on the steam-bath for 25 minutes in 200 ml. of aqueous 80% acetic acid. Upon chilling, precipitation with cold water and filtration, there was obtained 3.2 g. of tan powder. After decolorization in methanol solution with Darco G-60 charcoal and alternate recrystallizations from acetone and methanol, fine needles of pure VIIIa were obtained, m.p. 229–

(15) Details of the biological testing methods are not given here as this work will be reported elsewhere. We are indebted to Dr. Gregory Pincus of the Worcester Foundation for Experimental Biology (Worcester, Mass.) for some of the biological testing here reported. For most of the testing we gratefully acknowledge the contributions from our Division of Biological Research, under the direction of Dr. Victor A. Drill, and especially to our biological colleagues, Drs. J. Clampit, R. Craig, L. Hershberger and F. Saunders.

(16) H. Selye, *Proc. Soc. Exper. Biol. Med.*, **82**, 328 (1953).

(17) All analytical data, optical rotations and spectra were determined by the Analytical Department under the direction of Dr. Robert T. Dillon.

(18) All melting points are uncorrected. Ultraviolet spectra were determined in methanol unless otherwise specified. Infrared spectra were determined, as noted, in either chloroform or as 0.5% compound in pressed potassium bromide. Silica gel used for chromatography was Davison Chemical Corporation No. 923 (80-200 mesh). Petroleum ether refers to Skellysolve-B (boiling range 60–70°) unless specified. Optical rotations were taken at 24–26°.

(19) Rao and Kurath, ref. 3, reported m.p. 260–262°, $[\alpha]_D$ −95° (chloroform), log ϵ_{2410}^{EtOH} 4.1.

(20) Ch. Meystre and A. Wettstein, *Helv. Chim. Acta*, **30**, 1256 (1947).

(21) Rao and Kurath, ref. 3, reported one form, m.p. 225–227°, $[\alpha]_D$ −9.6° (chloroform), log ϵ_{3200}^{EtOH} 4.3.

229.5°, $[\alpha]_D$ −182.5° (0.46% in dioxane), $[\alpha]_D$ +29° (0.5% in $CHCl_3$), $\epsilon_{3200}^{CH_3OH}$ 23,700, $\epsilon_{3580}^{CH_3OH}$ 960, $\epsilon_{3060}^{CHCl_3}$ 5500; μ^{KBr} 3.17, 5.72, 5.81, 6.19, 6.25, 6.48 and 8.08.

Anal. Calcd. for $C_{23}H_{30}O_5$ (386.47): C, 71.48; H, 7.82. Found: C, 71.58; H, 7.91.

3,21-Dihydroxypregna-3,5-diene-7,20-dione (VIIIb).—The pure 7-keto-3-ketal 21-acetate (XIV) (2.96 g.) was subjected to base-catalyzed ester exchange[22] using 0.05 *N* potassium hydroxide (1.05 equiv.) in dioxane–methanol 2:1 for 7 minutes under nitrogen. After acidification with dilute acetic acid, precipitation with cold water and extraction with dichloromethane, a solvent-free residue was obtained weighing 2.66 g. and exhibiting ultraviolet peaks $\epsilon_{2400}^{CH_3OH}$ 9,000 and $\epsilon_{3100}^{CH_3OH}$ 4500. This result indicated a mixture of approximately 75% 7-keto-Δ^5-3-ketal and about 15–20% of 3-(β-hydroxy)-ethoxy-3,5-dien-7-one.[23] The crude mixture was dissolved in 400 ml. of acetone, 20 ml. of aqueous 3 *N* perchloric acid added and the solution allowed to stand 2 hr. at room temperature. After the usual work-up, the crude solids were crystallized alternately from methanol and acetone to afford 0.61 g. of pure VIIIb,[24] m.p. 226–228°, $[\alpha]_D$ −131° (1.11% in dioxane), $\epsilon_{3200}^{CH_3OH}$ 22,400; μ^{KBr} 2.88, 3.15, 5.85, 6.05, 6.28 and 6.45–6.50 (broad).

Anal. Calcd. for $C_{21}H_{28}O_4$ (344.43): C, 73.23; H, 8.19. Found: C, 72.74; H, 8.06.

3-Ethylenedioxy-17α-acetoxypregn-5-en-20-one (XII).—17α-Acetoxyprogesterone[25] (24 g.) was subjected to ethylene ketal formation by the Antonucci–Bernstein modification[8] of the standard procedure (using benzene–ethylene glycol–*p*-toluenesulfonic acid) in the belief that, as in the case of 21-acetoxy-20-keto steroids, the 17α-acetoxy group would sterically prevent ketalization of the 20-carbonyl. This proved to be the case. After 7 hr. of azeotropic reflux-distillation, the reaction mixture was diluted with water and extracted with ethyl acetate. From the washed and dried extracts, there were obtained crude solids which were recrystallized from methanol to give 18.1 g. of crude XII, m.p. 233–235°. Two recrystallizations from methanol afforded pure XII as the methanol solvate, m.p. 239–241°, ϵ^{CH_3OH} transparent; μ^{KBr} 2.93, 5.78, 5.87, 6.08, 6.22 and 8.0. The solvate, on drying at 110° under 0.1 mm. pressure for 3 hr., showed loss of the hydroxyl band at 2.93 μ in the infrared.

Anal. Calcd. for $C_{25}H_{36}O_5$ (416.52): C, 72.08; H, 8.71. Found: C, 71.98; H, 9.04.

3-Ethylenedioxy-17α-acetoxypregn-5-ene-7,20-dione (XV).—A solution of 9.8 g. of 3-ethylenedioxy-17α-acetoxypregn-5-en-20-one (XII) in 375 ml. of carbon tetrachloride was oxidized with *t*-butyl chromate (124 ml. of CCl_4 solution equivalent to 12.4 g. of CrO_3) in the presence of 100 ml. of acetic acid and 25 ml. of acetic anhydride by a procedure identical with the preparation of II above. The reaction mixture was worked up in a like fashion, using carbon tetrachloride as additional extraction solvent, and, after solvent removal, a crude solid residue was obtained weighing 8.6 g., $\epsilon_{2400}^{CH_3OH}$ 8000 (61% Δ^5-7-one). This material was reoxidized in 200 ml. of carbon tetrachloride with 60 ml. of a carbon tetrachloride solution of *t*-butyl chromate (equiv. to 6 g. of CrO_3) with acetic acid and acetic anhydride added as before. Upon similar work-up, a solid residue was found weighing 7.0 g., $\epsilon_{2400}^{CH_3OH}$ 9400 (72% Δ^5-7-one). Crystallization from ethyl acetate provided pure XV, m.p. 242–243°, $[\alpha]_D$ −89° (0.92% in dioxane), $\epsilon_{2400}^{CH_3OH}$ 13,000; μ^{KBr} 5.77, 5.86, 6.04, 6.17, 8.05, 9.00, 9.34 and 9.56.

Anal. Calcd. for $C_{25}H_{34}O_6$ (430.51): C, 69.74; H, 7.96. Found: C, 69.60; H, 7.70.

3-Hydroxy-17α-acetoxypregna-3,5-diene-7,20-dione (IX).—A solution of 1.4 g. of near pure (ε 12,600) 7-keto-17α-acetoxy 3-ketal (XV) in 65 ml. of aqueous 80% acetic acid was hydrolyzed by heating on the steam-bath for 25 minutes. Upon chilling, precipitation with cold 5% brine, extraction with dichloromethane and then the usual work-up, there was provided a crude solid residue weighing 1.2 g. Two recrystallizations from methanol furnished 0.78 g. of pure IX, m.p. 225–227°, $[\alpha]_D$ −174° (1.15% in $CHCl_3$), $\epsilon_{3180}^{CH_3OH}$ 22,900; μ^{KBr} 2.93, 5.77, 5.89, 6.07, 6.20, 6.30 and 8.08.

Anal. Calcd. for $C_{23}H_{30}O_5$ (386.47): C, 71.48; H, 7.82. Found: C, 71.52; H, 8.22.

The 3-Ethylenedioxy-17α,21-diacetoxypregn-5-en-20-one (XIII).—Reichstein's Substance "S" Diacetate[25] (31 g.) was subjected to ethylene ketal formation by the Antonucci–Bernstein modification[8] of the standard procedure (using benzene–ethylene glycol–*p*-toluenesulfonic acid). After 6 hr. of azeotropic reflux-distillation, the reaction mixture was diluted with water and extracted with ethyl acetate. From the washed and dried extracts, there were obtained crude solids which were crystallized from acetone–petroleum ether and recrystallized from acetone–methanol to yield 15.8 g. of crude XIII, m.p. 105–115°. Upon recrystallization from methanol or acetone–methanol, the pure XIII was obtained as dimorphic platelets, m.p. 115–128° and 164–166°.[26] The following analysis was on the solvated high melting form (m.p. 164–166°).

Anal. Calcd. for $C_{27}H_{38}O_7 \cdot CH_3OH$ (506.62): C, 66.38; H, 8.36. Found: C, 66.44; H, 8.27.

After desolvation at 100° *in vacuo*, the two forms exhibited identical optical rotations, ultraviolet and infrared spectra, $[\alpha]_D$ −34° (1.2% in $CHCl_3$), $\epsilon_{2300-3000}^{CH_3OH}$ transparent μ^{KBr} 5.71, 5.78, 8.07 and 9.25. The following analysis was on desolvated low melting form (m.p. 112–120°).

Anal. Calcd. for $C_{27}H_{38}O_7$ (474.57): C, 68.33; H, 8.07. Found: C, 68.06; H, 7.97.

3-Ethylenedioxy-17α,21-diacetoxypregn-5-ene-7,20-dione (XVI).—A solution of 9.6 g. of XIII in 200 ml. of carbon tetrachloride was oxidized with *t*-butyl chromate (100 ml. of CCl_4 solution equivalent to 12 g. of CrO_3) in the presence of 60 ml. of acetic acid and 20 ml. of acetic anhydride by a procedure identical with that used for the preparation of II. The reaction mixture was worked up in a like fashion, using dichloromethane as additional extraction solvent, and, after solvent removal, a crude solid residue was obtained weighing 9.2 g., $\epsilon_{2400}^{CH_3OH}$ 9200 (76% Δ^5-7-one). Half (4.6 g.) of these solids were chromatographed on 370 g. of silica gel using benzene–ethyl acetate mixtures. From the late 15% ethyl acetate eluates there was obtained 1.40 g. of crude crystalline XVI, m.p. 140–144° resolidifying and remelting 153–157°. Upon three recrystallizations from methanol–acetone 2:1, there was obtained pure dimorphic XVI, m.p. 142–144° and 157–159°, $[\alpha]_D$ −76° (0.79% in dioxane), $\epsilon_{2400}^{CH_3OH}$ 12,100; μ^{KBr} 5.72, 5.75–5.79 (broad), 6.02, 6.15, 8.10, 9.02 and 9.28.

Anal. Calcd. for $C_{27}H_{36}O_8$ (488.56): C, 66.37; H, 7.43. Found: C, 66.06; H, 7.49.

3-Hydroxy-17α,21-diacetoxypregna-3,5-diene-7,20-dione (Xa).—The 4.6 g. portion, set aside above, of crude 7-keto 17,21-diacetate 3-ketal (XVI) was hydrolyzed by heating on the steam-bath for 25 minutes in 200 ml. of aqueous 80% acetic acid. Upon chilling, precipitation with cold water and filtration, there was obtained 2.8 g. of tan powder. Several recrystallizations alternately from acetone and from methanol provided pure Xa, m.p. 251–252°, $[\alpha]_D$ −258° (0.5% in dioxane), $[\alpha]_D$ −46° (1.01% in $CHCl_3$), $\epsilon_{3200}^{CH_3OH}$ 21,000, $\epsilon_{3000-3040}^{CHCl_3}$ 2300; μ^{KBr} 3.03, 5.77, 5.85, 6.05, 6.23, 6.34, 7.95 and 8.10; μ^{CHCl_3} 2.67 (very weak), 5.77, 5.98, 6.18 and 8.04–8.08 (broad).

Anal. Calcd. for $C_{25}H_{32}O_7$ (444.51): C, 67.55; H, 7.26. Found: C, 67.32; H, 7.21.

3,17α,21-Trihydroxypregna-3,5-diene-7,20-dione (Xb).—A solution of 0.5 g. of pure 7-keto-Substance "S" diacetate (Xa) in 50 ml. of dioxane was titrated over a 9-minute period at 25° with 25 ml. of aqueous 0.75 *N* potassium hydroxide (2.1 mole equiv.), chilled, acidified with acetic acid and diluted with 1 liter of cold 10% brine. The precipitated steroid was extracted with dichloromethane, and the extracts were washed, dried and distilled *in vacuo* to yield 0.24 g. of Xb as a light-buff colored amorphous powder. All efforts to crystallize this material failed. The amorphous

(22) H. Minlon and M. Tishler, U. S. Patent 2,634,277 (1951).

(23) Predictable by vinylogy with monoketals of β-diketones, ref. 11.

(24) R. Lenhard and S. Bernstein, ref. 4, report a m.p. of 232–235°, $[\alpha]_D$ −267° ($CHCl_3$), $\epsilon_{3200}^{CH_3OH}$ 23,600.

(25) R. B. Turner, THIS JOURNAL, 75, 3489 (1953).

(26) Both forms are methanol monosolvated. The lower melting form resolidifies above 130° and melts again at 162–165°. Dried *in vacuo* at 100° either form loses its solvent but undergoes no change in melting point.

product does not show melting up to 310° and was characterized as follows: $\epsilon_{3190}^{CH_3OH}$ 21,000; μ^{KBr} 2.90, 3.12, 5.80, 6.10, 6.25 and 6.47.

Anal. Calcd. for $C_{21}H_{28}O_5$ (360.43): C, 69.97; H, 7.83. Found: C, 69.91; H, 7.54.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF G. D. SEARLE AND CO.]

7-Keto Steroids. II.[1] Steroidal 3β-Hydroxy-Δ⁵-7-ones and Δ³,⁵-7-Ones

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A series of steroidal 3β-hydroxy-Δ⁵-7-ones and Δ³,⁵-7-ones were prepared as potential anti-cortisone agents. Biological evaluation showed 7-ketopregnenolone, 7-keto-21-acetoxypregnenolone 3-acetate and pregna-3,5-diene-7,20-dione to have a mild order of activity. The 3β-hydroxy (or acetoxy)-Δ⁵-7-ones were prepared by oxidation in the allylic (C-7) position with either *t*-butyl chromate or sodium chromate. The Δ³,⁵-diene-7-ones were prepared by boiling the 3β-acyloxy-Δ⁵-7-ones one hour in glacial acetic acid with 0.5% (w./v.) of *p*-toluenesulfonic acid.

In a concurrent publication[1] from this Laboratory, there was described the synthesis of a series of steroidal 3-hydroxy-3,5-diene-7-ones which were biologically evaluated as anti-cortisone agents. That study and the currently reported investigation were instituted upon the finding by our Division of Biological Research early in 1953 that 7-ketocholesterol possesses striking anti-cortisone properties in experimental animals. Accordingly we studied two additional series of 7-keto steroids, namely, the 3β-hydroxy-Δ⁵-7-ones and the Δ³,⁵-7-ones, which are described in this paper.

At the time this study was initiated the only steroidal 3-hydroxy-7-ones or 3β-hydroxy-Δ⁵-7-ones in the chemical literature, other than cholic and chenodesoxycholic acid derivatives, were those with C-17 side chains of the following types: cholesterol[2a,b] stigmasterol,[2c] sapogenin; as well as with these C-17 substituents: 17-keto,[3] 17β-acyloxy[3a,b,4] and 17β-hydroxy.[3a,b,4] There also had been reported 7-ketopregnenolone as the 3β-acetate,[3a,d,5] but not as the 3-ol and more recently 3β,20β-diacetoxypregn-5-ene-7-one.[6]

In planning the synthesis of a series of 3β-hydroxy-Δ⁵-7-one steroids, embracing C-17 side chains of the 20,21-ketol and 17α-21-dihydroxy-20-ketone types, it was essential, prior to the oxidative introduction of the 7-carbonyl, to protect the 3-hydroxy function with a blocker that could be removed under conditions comparable to the mild conditions required for safe cleavage of the protective esters in these alkali- and acid-sensitive side chains at C-17. Pregnenolone acetate was oxidized to the 7-keto derivative (III)[3a,d,5] by the *t*-butyl chromate procedure[4b,7] and also by the sodium chromate method.[8] In a like manner were made the following: 3β,17α-diacetoxypregn-5-ene-7,20-dione (IV), 3β,21-diacetoxypregn-5-ene-7,20-dione (IXa), 3β-ethoxycarbonyloxy-21-acetoxypregn-5-ene-7,20-dione (IXb), 3β-trifluoroacetoxy-21-acetoxypregn-5-ene-7,20-dione (IXc), 3β,17α,21-triacetoxypregn-5-ene-7,20-dione (Xa), 3β-ethoxycarbonyloxy-17α,21-diacetoxypregn-5-ene-7,20-dione (Xb), 3β-acetoxy-17β-trifluoroacetoxy-17-methylandrost-5-en-7-one (XV) and 3β-acetoxy-17β-trifluoroacetoxy-17-ethylandrost-5-en-7-one (XVI).

The esterc leavage of these 3β,17α-, 3β,21- and 3β,17β(17α-alkyl)-diesters and the 3β,17α,21-triesters required carefully controlled conditions. 7-Ketopregnenolone acetate, on standing 24 hr. at 25°, or boiling 30 minutes, in 0.5 *N* alkali or 0.5 *N* mineral acid, undergoes dehydration at C-3 to produce pregna-3,5-diene-7,20-dione.[3a] Accordingly III, IV, XV and XVI were saponified by the action of 0.2 *N* alkali for 2.5 to 3 hr. at room temperatures. Titrations of aliquots from the saponification of III indicated 95% completion in 2.5 hr. Despite these precautions, after crystallization had provided a 75% yield of the 3β-ol (V), the mother liquors furnished a 10% yield of pregna-3,5-diene-7,20-dione. By these means were prepared 7-ketopregnenolone (3β-hydroxypregn-5-ene-7,20-dione) (V), 3β,17α-dihydroxypregn-5-ene-7,20-dione (VI), 7-keto-17α-methylandrostenediol (3β,17-dihydroxy-17α-methylandrost-5-en-7-one) (XVIIb) and 7-keto-17α-ethylandrostenediol (3β,17-dihydroxy-17α-ethylandrost-5-en-7-one) (XVIIIb).

XVIIb and XVIIIb were selectively monoacetylated to form, respectively, 3β-acetoxy-17-hydroxy-17α-methylandrost-5-en-7-one (XVIIa) and 3β-acetoxy-17-hydroxy-17α-ethylandrost-5-en-7-one (XVIIIa).

Employing the fast base-catalyzed exchange conditions of Huang-Minlon,[9] using 1.05 times theory of potassium hydroxide at 0.05 *N* concentration for 5 to 10 minutes at 25°, the following were completely de-esterified: IXb and IXc to give 3β,21-dihydroxypregn-5-ene-7,20-dione (XIb) and Xb to provide 3β,17α-21-trihydroxypregn-5-ene-7,20-dione (XII). XIb was monoacetylated to 21-acetoxy-3β-hydroxypregn-5-ene-7,20-dione (XIa).

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