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Cortisone Isomers. The Epimeric 16-Hydroxy-16-acetoxyacetyl-Δ^4-androstene-3,11-diones[1]

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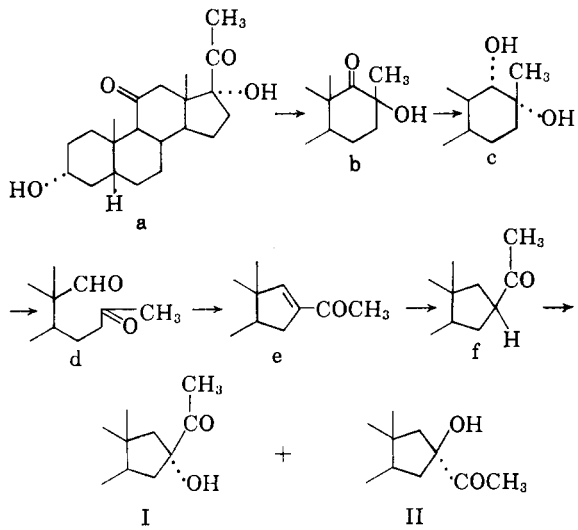
The synthesis of the two isomers, VIII and IX, of cortisone acetate having the dihydroxyacetone side chain attached to C-16 is described.

We have previously reported the preparation and characterization of the C-16 epimeric ketols I and II[2,3] by a sequence of reactions starting with the well known 3α,17α-dihydroxypregnane-11-20-dione (a)[4] and proceeding through the intermediates b–f.



Both C-16 epimers of f were obtained, and the common enol acetate was converted by the peracid procedure[5] to a mixture of the ketols I and II separable by alumina chromatography.[2]

The transformation of I and II into the respective C-16 substituted cortisone analogs VIII and IX[6] is described below and follows standard procedures for introduction of the 21-acetoxy[5,7] and Δ^4-3-keto part structures.[8]

Bromination of 3α,16α-dihydroxy-16β-acetyl-5β-androstane-11-one (I) in chloroform gave the corresponding 21-bromo compound IIIa. Treatment of the latter with sodium iodide–potassium acetate in acetone–acetic acid afforded the 21-acetate IIIb. Oxidation of the 3α-hydroxy group of IIIb with chromic oxide in aqueous acetic acid gave 16α - hydroxy - 16β - acetoxyacetyl - 5β-androstane-3,11-dione (IV) in moderate yield as well as considerable 5β-androstane-3,11,16-trione (V).

The same sequence in the C-16 epimeric series proceeded similarly to give 16β-hydroxy-16α-acetoxyacetyl-5β-androstane-3,11-dione (VII) and 5β-androstane-3,11,16-trione (V). The fact that (V) was obtained from both series is confirmatory evidence for the validity of the present formulations.[9]

The ease of chromium trioxide cleavage of the 21-acetoxydihydroxyacetone side chain of either C-16 epimer is in contrast with the relative inertness of this part structure when attached to C-17 and demonstrates the greater steric accessibility of the side chain in the C-16 series. Under the same conditions, 21-acetoxy-3α,17α-dihydroxypregnane-11,20-dione gave the corresponding 3-ketone in excellent yield with 5β-androstane-3,11,17-trione formed only in trace amounts. It proved preferable to utilize *N*-bromsuccinimide in *t*-butyl alcohol[5] for oxidation of the 3α-hydroxyl group in both C-16 substituted series.

Monobromination of each 3-ketone (IV, VII) followed by semicarbazone formation and reversal[8] gave respectively 16α-hydroxy-16β-acetoxyacetyl-

(1) Presented in part at the Symposium on Steroids and Related Natural Products, The Gordon Research Conferences, New Hampton, N. H., July 30–August 3, 1956.

(2) N. L. Wendler and D. Taub, *J. Org. Chem.*, **23**, 953 (1958).

(3) D. Taub, R. D. Hoffsommer, H. L. Slates, and N. L. Wendler, *J. Org. Chem.*, **26**, 2852 (1961).

(4) L. H. Sarett, *J. Am. Chem. Soc.*, **70**, 1454 (1948).

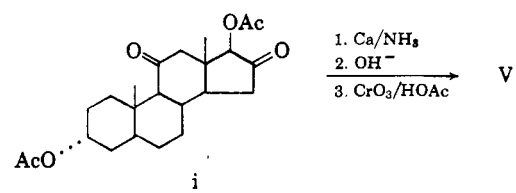
(5) Method of T. H. Kritchevsky and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 184 (1951).

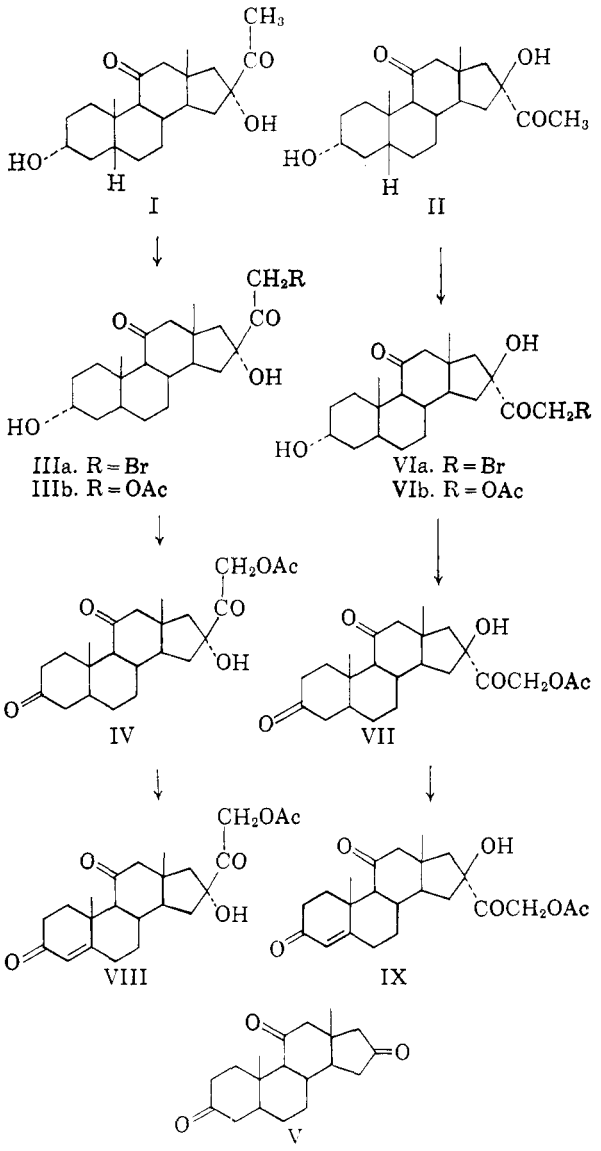
(6) C-16 substituted analogs of progesterone and desoxycorticosterone have been synthesized by another route by J. Fajkŏs and F. Sŏrm, *Chem. Listy*, **51**, 579 (1957).

(7) *Cf.* G. Rosenkranz, J. Pataki, St. Kaufmann, J. Berlin, and C. Djerassi, *J. Am. Chem. Soc.*, **72**, 4081 (1950).

(8) *Cf.* W. F. McGucken and E. C. Kendall, *J. Am. Chem. Soc.*, **74**, 5811 (1952).

(9) 5β-Androstane-3,11,16-trione (V) was also prepared by the following independent sequence (C. H. Kuo, D. Taub, and N. L. Wendler, North Jersey Section, American Chemical Society, Meeting-in-Miniature, Feb. 1, 1960; paper in preparation) in which the 17β-acetoxy function in i was removed by the calcium-liquid ammonia method of J. H. Chapman, J. Elks, G. H. Phillipps, and L. J. Wyman, *J. Chem. Soc.*, 4344 (1956).





Δ^4-androstene-3,11-dione (VIII) and 16β-hydroxy-16α-acetoxyacetyl-Δ^4-androstene-3,11-dione (IX). It is noteworthy that in each series the standard procedure for mono-semicarbazone preparation resulted in the formation of considerable 3,20-disemicarbazone as indicated by an ultraviolet maximum at 232–235 mμ as well as at 269 mμ.

The various C-16 epimeric pairs had essentially identical paper chromatographic mobilities. The cortisone analogs VIII and IX had significantly different optical rotations, ΔM_D (IX–VIII) = +120°. This value is in general agreement with the positive values (+60° to +157°) observed by Fajkŏs and Sŏrm in their series.[6]

Neither VIII nor IX showed significant cortisone-like activity in the local anti-inflammatory (rat) and liver glycogen deposition (mouse) assays.[10]

(10) We are grateful to Dr. R. H. Silber and his associates of the Merck Institute for Therapeutic Research for the biological data.

EXPERIMENTAL[11]

3α,16α-Dihydroxy-16β-acetoxyacetyl-5β-androstane-11-one (IIIb). To a stirred solution of 350 mg. of 3α-16α-dihydroxy-16β-acetyl-5β-androstane-11-one (I) in 10 ml. of chloroform maintained at 45° was added 175 mg. of bromine in 9 ml. of chloroform over a period of 20 min. The nearly colorless solution was cooled to 10°, dilute aqueous potassium bicarbonate was added, and the mixture was extracted with chloroform. The organic extract was washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated to dryness to give 400 mg. of *3α,16α-dihydroxy-16β-bromacetyl-5β-androstane-11-one* (IIIa); positive blue tetrazolium test; single spot on paper chromatography (benzene-formamide system). A portion crystallized from acetone-ether had m.p. 200–220° dec., $\lambda_{max}^{Nu.ol}$ 2.88, 3.00 μ (3α,16α —OH), 5.80 μ (20 C = O adjacent to 21-Br), 5.91 μ (11 C = O).

To 340 mg. of the bromacetylandrostane IIIa in 15 ml. of acetone and 0.1 ml. acetic acid was added 350 mg. of potassium acetate and 175 mg. of sodium iodide. The stirred mixture was refluxed 4 hr., 10 ml. of water was added, and the acetone removed under vacuum. Additional water was added and the mixture was extracted with chloroform. The latter extract was washed with dilute aqueous sodium bisulfite, water, and saturated sodium chloride solution and dried over magnesium sulfate.

Removal of the solvent and crystallization of the residue from acetone-ether gave 3α,16α-dihydroxy-16β-acetoxyacetyl-5β-androstane-11-one (IIIb); prismatic needles, m.p. 176–178°; $\lambda_{max}^{CHCl_3}$ 2.9–3.0 μ (3α,16α —OH), 5.79 μ broad (20—C=O and 21—OAc), 5.87 μ (11—C=O), 8.1 μ (—OAc); R_f 0.24 benzene formamide system—slightly more mobile than the corresponding C-17 substituted analog [3α,17α-dihydroxy-21-acetoxypregnane-11,20-dione].

Anal. Calcd. for $C_{23}H_{34}O_6$: C, 67.96; H, 8.43. Found: C, 68.14; H, 8.50.

16α-Hydroxy-16β-acetoxyacetyl-5β-androstane-3,11-dione (IV) *and 5β-androstane-3,11,16-trione* (V) *by chromium trioxidation of* IIIb. To a solution of 205 mg. of IIIb in 5 ml. of acetic acid was added 42 mg. of chromium trioxide (20% equivalent excess) in 0.1 ml. of water and 2 ml. of acetic acid. After 17 hr. at 25° dilute sodium sulfate solution was added and the mixture was extracted with chloroform. The latter extract was washed with dilute aqueous potassium bicarbonate, water, and saturated sodium chloride solution and dried over magnesium sulfate. The residue (195 mg.) following removal of the solvent showed the presence of considerable starting material IIIb by paper chromatography and the oxidation was repeated utilizing 30 mg. of chromium trioxide. The product (150 mg.) following the repeated oxidation no longer contained starting material. However, it was separated into two substances by chromatography on 9 g. of neutral alumina. From the 50% petroleum ether–(b.p. 30–60°) benzene to 100% benzene eluates was obtained 51 mg. of 5β-androstane-3,11,16-trione (V); prismatic needles from acetone-ether, m.p. 189–191°; $\lambda_{max}^{CHCl_3}$ 5.75 μ (16-C=O), 5.85 μ (3,11—C=O). This material was identical by mixed melting point and infrared criteria with a sample obtained by chromium trioxide oxidation of VIb and with a sample obtained from 3α,17α-diacetoxy-5β-androstane-11,16-dione.[9]

Anal. Calcd. for $C_{19}H_{26}O_5$: C, 75.44; H, 8.67. Found: C, 75.17; H, 8.55.

From the 50% benzene-chloroform to 100% chloroform eluates was obtained 62 mg. of 16α-hydroxy-16β-acetoxyacetyl-5β-androstane-3,11-dione (IV); hexagonal prisms from acetone-ether, m.p. 186–187°; λ_{max}^{Nujol} 2.95 μ (OH),

(11) Melting points were taken on a micro hot stage apparatus and are corrected. Paper chromatograms were run on strips of Whatman No. 1 or No. 4 filter paper using the benzene-formamide system of A. Zaffaroni, R. B. Burton, and E. H. Keutmann, *Science*, 111, 6 (1950).

5.72 μ (21—OAc); 5.80 μ (20—C=O), 5.91 μ (11—C=O), 8.1 μ (OAc). R_f 0.58 benzene-formamide system.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.30; H, 7.98. Found: C, 68.47; H, 7.68.

16α-Hydroxy-16β-acetoxyacetyl-5β-androstane-3,11-dione (IV) by *N-bromsuccinimide oxidation of* IIIb. To a stirred solution of 200 mg. of IIIb in 10 ml. of *t*-butyl alcohol and 2 ml. of water maintained at 10° was added 200 mg. of *N*-bromsuccinimide. After 17 hr. at 10° water was added followed by a few milligrams of sodium sulfite and the mixture was extracted with chloroform. The latter extract was washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated to dryness. Crystallization of the residue from acetone-ether gave 115 mg. of the 3-ketone IV, m.p. 182–185° identical with a sample obtained as described in the previous experiment. An additional 15 mg. of IV was obtained on chromatography of the mother liquors on 2 g. of neutral alumina.

16α-Hydroxy-16β-acetoxyacetyl-Δ⁴-androstene-3,11-dione (VIII). To a stirred solution of 85 mg. of the 3-ketone IV in 3 ml. of acetic acid was added 0.3 ml. of 0.5*N* *p*-toluenesulfonic acid followed by 1.05 ml. (added dropwise) of a stock solution consisting of 320 mg. of bromine and 181 mg. of sodium acetate in 10 ml. of acetic acid. Sodium acetate (30 mg.) in 2 ml. of water was added to the nearly colorless solution followed by 20 ml. of water. The mixture was extracted with chloroform, the latter extract washed with aqueous potassium bicarbonate, saturated sodium chloride solution and dried over magnesium sulfate. The solvent was removed under vacuum to give 105 mg. of crude partly crystalline *4-bromo-16α-hydroxy-16β-acetoxyacetyl-5β-androstane-3,11-dione*. The latter was dissolved in 5 ml. of acetonitrile under nitrogen and 100 mg. of semicarbazide base in 2 ml. of acetonitrile and 0.3 ml. of acetic acid added. The deep yellow solution slowly became nearly colorless. After 17 hr. the mixture was concentrated to a small volume, 5 ml. of water was added and the granular precipitate filtered, washed with water and dried in air. The crude semicarbazone had m.p. >275°; $\lambda_{max}^{CH_3OH}$ 267 mμ, $E_{cm}^{1\%}$ 430; 232 mμ, $E_{cm}^{1\%}$ 446 indicative of largely a 3,20-disemicarbazone. The semicarbazone was kept in a mixture of 4 ml. of acetic acid, 0.4 ml. of pyruvic acid, and 1.35 ml. of water for 18 hr. and 1 hr. at 50°. Water was added and the mixture was extracted with chloroform. The latter extract was washed with aqueous potassium bicarbonate, saturated sodium chloride solution, dried over magnesium sulfate and the solvent removed under vacuum. The residue (60 mg.) was chromatographed on 5 g. of neutral alumina. From the 50% benzene-chloroform to 100% chloroform eluates was obtained 37 mg. of 16α-hydroxy-16β-acetoxyacetyl-Δ⁴-androstene-3,11-dione (VIII); plates (19 mg.) from acetone-ether; m.p. 204–207°; $[\alpha]_D^{CHCl_3}$ +115°; M_D +460°; $\lambda_{max}^{CH_3OH}$ 238 mμ (14,800); $\lambda_{max}^{CHCl_3}$ 2.9–3.0 μ (OH), —5.73 μ (—OAc), 5.78 μ (20—C=O) 5.85 μ (11—C=O), 6.00, 6.18 μ (3—C=O—Δ⁴). R_f 0.35 benzene-formamide—essentially the same as cortisone acetate.

Anal. Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.79; H, 7.29.

The mother liquors and earlier chromatographic fractions contained a slightly more mobile ultraviolet absorbing species gave a positive blue tetrazolium test and may therefore be the corresponding Δ¹-3-ketone.

21-Bromination and acetylation of 3α,16β-dihydroxy-16α-acetyl-5β-androstane-11-one (II). The 16β-hydroxy ketol II (210 mg.) was brominated by the same procedure utilized to brominate the 16α-hydroxy ketol I. The resulting 16α-bromacetyl compound VIa (230 mg.) was obtained as a colorless amorphous solid; $\lambda_{max}^{CHCl_3}$ 3.00 μ broad (3α,16β—OH), 5.80 μ (20—C=O adjacent to 21 Br), 5.90 μ (11—C=O)—different from IIIa in the fingerprint region, + blue tetrazolium test. It was converted by the procedure described above for the preparation of IIIb to *3α,16β-dihydroxy-16α-acetoxyacetyl-5β-androstane-11-one* (VIb) (190 mg.) which was also obtained in noncrystalline form; $\lambda_{max}^{CHCl_3}$ 2.76, 2.90 μ (3α,16β—OH), 5.72 μ (21-OAc), 5.78 μ (20—C=O), 5.84 μ (11—C=O)—different from IIIb in the fingerprint region; R_f 0.24 essentially the same mobility on paper as IIIb.

16β-Hydroxy-16α-acetoxyacetyl-5β-androstane-3,11-dione (VII) *and 5β-androstane-3,11-16-trione* (V) *by chromium trioxide oxidation of* VIb. The amorphous 16α-acetoxyacetyl compound VIb (180 mg.) was treated with chromium trioxide (40 mg.) in acetic acid (5 ml.) and water (0.1 ml.) as described above for the oxidation of IIIb. Chromatography of the product on neutral alumina (8 g.) gave 5β-androstane-3,11,16-trione (V) (30 mg.), m.p. 188–190° identical with a sample obtained from IIIb and 16β-hydroxy-16α-acetoxyacetyl-5β-androstane-3,11-dione (VII) (90 mg.) obtained as an amorphous solid $\lambda_{max}^{CHCl_3}$ 2.9 μ broad (16—βOH), 5.74 μ (21—OAc), 5.80 μ (20—C=O), 5.84 μ (3,11—C=O), 8.1 μ (—OAc); R_f, 0.60 benzene-formamide—essentially the same as IV.

Compound VII was also obtained from VIb by *N*-bromsuccinimide oxidation as described for IIIb → IV.

16β-Hydroxy-16α-acetoxyacetyl-Δ⁴-androstene-3,11-dione (IX). The 3,11,20-trione VII (120 mg.) was successively monobrominated, converted to the semicarbazone ($\lambda_{max}^{CH_3OH}$ 269 mμ $E_{cm}^{1\%}$ 400; 235 mμ $E_{cm}^{1\%}$ 310—probably mainly 3,20-disemicarbazone) and the latter reversed in aqueous acetic acid–pyruvic acid as described under the preparation of VIII. The product of this sequence, crude IX (63 mg.) was chromatographed on 5 g. of neutral alumina. Combination of the 50% benzene-chloroform eluates (40 mg.) and crystallization from ether-acetone gave 25 mg. of 16β-hydroxy-16α-acetoxyacetyl-Δ⁴-androstene-3,11-dione (IX) as prisms, m.p. 182–186°; $[\alpha]_D^{CHCl_3}$ +145; M_D +580°; $\lambda_{max}^{CH_3OH}$ 238 mμ (14,500) $\lambda_{max}^{CHCl_3}$ 2.9–3.0 μ (—OH), 5.72 μ (21—OAc), 5.79 μ (20—C=O), 5.88 μ (11—C=O) 6.00, 6.19 μ (3—C=O—Δ⁴); R_f 0.36 benzene-formamide—essentially the same as cortisone acetate and VIII.

Anal. Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.43; H, 7.47.

Alternatively, the 3,11,20-trione VII (190 mg.) was monobrominated and dehydrobrominated by lithium chloride (100 mg.) in dimethylformamide (5 ml.) at 100° for 2 hr.[12]

The product was purified by chromatography on Whatman No. 3 filter paper to give IX, m.p. 181–185° as the major component.

Chromium trioxide oxidation of 3α,17α-dihydroxy-21-acetoxypregnane-11,20-dione. A solution of 205 mg. of 3α,17α-dihydroxy-21-acetoxypregnane-11,20-dione in 5 ml. of acetic acid was treated with 42 mg. of chromium trioxide in 2 ml. of acetic acid and 0.2 ml. of water as described above for IIIb → IV. The corresponding 3-ketone (17α-hydroxy-21-acetoxypregnane-3,11,20-trione[4] was obtained in over 80% yield. Paper chromatography of the mother liquors (benzene-formamide system) revealed the presence of traces of 5β-androstane-3,11,17-trione.

RAHWAY, N. J.

(12) Procedure of R. Holyz, *J. Am. Chem. Soc.*, **75**, 4432 (1953).