[CONTRIBUTION FROM THE ORGANIC CHEMICAL AND EXPERIMENTAL THERAPEUTICS RESEARCH SECTIONS, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

16-Hydroxylated Steroids. XII.[1] The 16α,17α-Acetonides of Synthetic Non-halogenated Corticoids.

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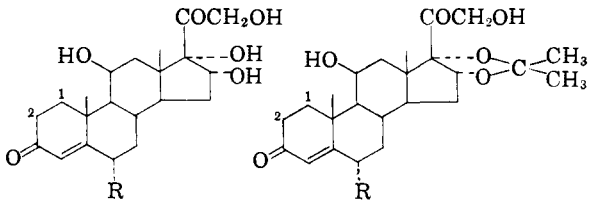
The preparation and biological activities of the 16,17-acetonides of 16α-hydroxyhydrocortisone, 16α-hydroxyprednisolone, 16α-hydroxy-6α-methylhydrocortisone and 16α-hydroxy-6α-methylprednisolone are reported.

Fried, Borman and co-workers[2] recently have reported on the marked potentiation of the glucocorticoid activity of 9α-fluoro-16α-hydroxyhydrocortisone[3] and triamcinolone[3] by 16,17-acetal and-ketal formation.[4]

We have extended and evaluated the "acetonide principle" with a variety of synthetic 16α-hydroxycorticoids.[5] In particular, a study has been made on the biological influence of acetonide formation on *non-halogenated* 16α-hydroxy-corticoids. Accordingly, the acetonides (V–VIII) of several compounds were prepared with acetone and perchloric acid[2a] or hydrochloric acid as catalysts: 16α-hydroxyhydrocortisone (I), 16α-hydroxyprednisolone (II), 16α-hydroxy-6α-methyl-hydrocortisone (III) and 16α-hydroxy-6α-methyl-prednisolone (IV).



I, R = H
II, Δ¹, R = H
III, R = CH_3
IV, Δ¹, R = CH_3

V, R = H
VI, Δ¹, R = H
VII, R = CH_3
VIII, Δ¹, R = CH_3

The biological activities of these acetonides and the parent free steroids are recorded in the table. It is concluded that acetonide formation potentiates the glucocorticoid activities of synthetic *non-halogenated*-corticoids. Moreover, all the acetonides on the adrenalectomized rat produced an appreciable natriuresis and diuresis.[6]

Finally, compounds VI, VII and VIII appear to be the most active corticoids yet synthesized.

Acknowledgment.—We wish to thank Louis M. Brancone and associates for the analytical data, William Fulmor and associate for the spectral and optical rotational data.

Experimental

Melting points.—All m.p.'s are uncorrected.

Absorption Spectra.—The ultraviolet absorption spectra were determined in methanol. The infrared absorption spectra were carried out with pressed potassium bromide.

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

16α-Hydroxyhydrocortisone 16,17-Acetonide (11β,21-Dihydroxy-16α,17α-isopropylidenedioxy-4-pregnene-3,20-dione (V).—Perchloric acid (72%, 5 drops) was added to a stirred suspension of 16α-hydroxyhydrocortisone (I, 0.5 g.)[8] in acetone (50 ml.). Solution took place rapidly and the mixture was kept at room temperature for 18 hr., when water (200 ml.) was added. The solid which separated was extracted with chloroform (3 × 50 ml.) and the combined extracts were washed with water (50 ml.) and dried. Evaporation of the solvent and then crystallization of the residue from ethyl acetate–petroleum ether gave the acetonide V as needles (300 mg.), m.p. 198–200°, raised to m.p. 205–206° upon recrystallization; $[\alpha]^{25}D$ +152° (methanol); λ_{max} 240 mμ (ε 16,100); ν_{max} 3436, 1715, 1664, 1621, 1087 and 1053 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{34}O_6$ (418.51): C, 68.87; H, 8.19. Found: C, 68.80; H, 8.43.

Improved yields were obtained by reducing the time of reaction to 2 hours.

16α-Hydroxyprednisolone 16,17-Acetonide (11β,21-Dihydroxy-16α,17α-isopropylidenedioxy-1,4-pregnadiene-3,20-dione) (VI).—A solution of 16α-hydroxyprednisolone[7] (40 mg.) in acetone (10 ml.) containing hydrochloric acid (3 drops, d. 1.19) was boiled on the steam-bath for 2 minutes and then allowed to stand for 18 hr. at room temperature. The reaction mixture was diluted with water (50 ml.) and the product was isolated essentially as above. Recrystallization of the crude product from ethyl acetate–petroleum ether gave pure VI as small plates (25 mg.), m.p. 263–266°; $[\alpha]^{25}D$ +122° (methanol); λ_{max} 242 mμ (ε 15,800); ν_{max} 3401, 1709, 1661, 1616, 1603 (inflection), 1087 and 1053 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{32}O_6$ (416.50): C, 69.21; H, 7.74. Found: C, 69.00; H, 7.82.

Improved yields were obtained by using the perchloric acid method above.

16α-Hydroxy-6α-methylhydrocortisone 16,17-Acetonide (11β,21-Dihydroxy-16α,17α-isopropylidenedioxy-6α-methyl-4-pregnene-3,20-dione) (VII).—A suspension of 16α-hy-

(1) Paper XI, S. Bernstein, M. Heller and S. M. Stolar, THIS JOURNAL, **81**, 1256 (1959).

(2) (a) J. Fried, A. Borman, W. B. Kessler, P. Grabowich and E. F. Sabo, *ibid.*, **80**, 2338 (1958); (b) J. Fried and A. Borman, *Vitamins and Hormones*, **16**, 303 (1958).

(3) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, THIS JOURNAL, **76**, 5693 (1956); **81**, 1689 (1959); S. Bernstein, *Recent Prog. in Hormone Research*, **14**, 1 (1958).

(4) The outstanding compound described was triamcinolone acetonide whose preparation was first reported by one of us (S. B.) in a talk at the Gordon Research Conference on *Chemistry of Steroids and Related Natural Products*, Aug. 8, 1957; see also ref. 3. Triamcinolone acetonide is currently being marketed as a topical anti-inflammatory agent under the trademarks of Aristocort® triamcinolone acetonide cream (Lederle Laboratories, Division of American Cyanamid Company) and KENALOG® (E. R. Squibb and Sons).

(5) Recently, H. J. Ringold, O. Mancera, C. Djerassi, A. Bowers, E. Batres, H. Martínez, E. Necoechea, J. Edwards, M. Velasco, C. C. Campillo and R. I. Dorfman, THIS JOURNAL, **80**, 6464 (1958), and J. S. Mills, A. Bowers, C. C. Campillo, C. Djerassi and H. J. Ringold, *ibid.*, **81**, 1264 (1959), have extended the "acetonide principle" to include 6α-halogeno-16α-hydroxy-corticoids.

(6) The diuretic properties of these compounds also have been demonstrated in the normal rat. We wish to thank Dr. J. R. Cummings of the Experimental Therapeutics Research Section of this laboratory for these findings.

(7) In preliminary clinical evaluation, 16α-hydroxyhydrocortisone acetonide (V) appears to be approximately 10 times more active than hydrocortisone acetate as a topical anti-inflammatory agent against a number of dermatological disorders. We are indebted to Drs. S. Blau and N. B. Kanof (New York City), and Dr. N. Orentreich (New York City) for releasing to us this observation prior to publication.

(8) The preparation of the 16α-hydroxy-derivatives of hydrocortisone and prednisolone has been described previously; see W. S. Allen and S. Bernstein, THIS JOURNAL, **78**, 1909 (1956), and ref. 3.

TABLE I

BIOLOGICAL ACTIVITIES[a]

Compound	Free steroid		16,17-Acetonide	
	Thymus involution	Liver glycogen	Thymus involution	Liver glycogen
16α-Hydroxyhydrocortisone	0.3 (0.2–0.4)	0.4 (0.2–1)	3 (2–3)[b]	3 (1–8)
16α-Hydroxyprednisolone	1 (1–2)	1 (0.5–2)	17 (10–28)	13 (7–22)
16α-Hydroxy-6α-methylhydrocortisone	1 (1–2)	1 (1–2)	14 (10–20)	10 (5–18)
16α-Hydroxy-6α-methylprednisolone	3 (2–4)	2 (1–3)	24 (12–49)	22 (11–43)

[a] Activities are on a weight basis relative to hydrocortisone = 1. Figures in parentheses represent 95% confidence limits. The assay procedure is given: Liver glycogen deposition, thymus involution, fluid diuresis and electrolyte excretion were measured in adrenalectomized immature male rats. Twenty-four hr. after adrenalectomy, the rats were injected subcutaneously with graded doses of the test compound suspended in a modified carboxymethylcellulose vehicle. The rats were injected intraperitoneally with 3 ml. of saline, and three rats placed in each metabolism cage. Urine was collected for 5 hr. and sodium and potassium were determined by flame photometry. Daily injections of the steroids then were continued for the next 4 days. The rats were fasted for 15 hr. prior to the last injection and for an additional 7 hr. afterward. At the end of this time, the animals were anesthetized with sodium pentobarbital, and the livers were quickly excised and hydrolyzed in 30% potassium hydroxide solution. Liver glycogen was measured by the anthrone method of S. Seifter, S. Dayton, B. Noric and E. Muntwyler, *Arch. Biochem.*, **25**, 191 (1950). Also, the thymi were removed and weighed. The assays were done by L. Bortle, E. Heyder, A. Monteforte, J. Perrine and E. Ross. [b] N. R. Stephenson (Department of National Health and Welfare, Ottawa, Canada) has found in his thymus involution assay (subcutaneous, corn oil) that 16α-hydroxyhydrocortisone 16,17-acetonide (V) possessed an activity of 9.26 (8.35–10.28) times that of hydrocortisone on a weight basis. On an equimolar basis its activity was 10.70 (9.64–11.87). We are indebted to Dr. Stephenson for this information.

droxy-6α-methylhydrocortisone (III, 215 mg.)[9] in acetone (10 ml.) was treated with perchloric acid (72%, 25λ) and stirred for 2.5 hr. at room temperature. Then saturated sodium bicarbonate solution (0.4 ml.) and water (15 ml.) were added to the clear solution. The product which separated was collected by filtration and washed with a copious quantity of water, wt. 210 mg., m.p. 244–246°. Recrystallization from acetone–petroleum ether lowered the m.p. to 230–232°; $[\alpha]^{24}D$ +139° (chloroform); λ_{max} 241 mμ (ε 15,400); ν_{max} 3550, 1724, 1680, 1620, 1092 and 1053 cm.$^{-1}$.

(9) The multi-stage syntheses of 16α-bydroxy-6α-methylhydrocortisone (III) and prednisolone (IV) (from the 5α,6α-epoxide of hydrocortisone bis-ethylene ketal *via* the bis-ethylene ketal of 6α-methylcortisone acetate) will be described shortly by S. Bernstein and R. Littell, manuscript in preparation.

Anal. Calcd. for $C_{25}H_{36}O_6$ (432.54): C, 69.42; H, 8.31. Found: C, 69.53; H, 8.80.

16α-Hydroxy-6α-methylprednisolone 16,17-Acetonide (11β,21-Dihydroxy-16α,17α-isopropylidenedioxy-6α-methyl-1,4-pregnadiene-3,20-dione) (VIII).—16α-Hydroxy-6α-methylprednisolone (IV, 11 g.)[9] was converted into its acetonide VIII essentially by the method above for VII, wt. 10.8 g., m.p. 265–269°. The analytical sample was obtained by recrystallization from acetone–petroleum ether; m.p. 272–275°; $[\alpha]^{25}D$ +100° (chloroform); λ_{max} 241 mμ (ε 15,500); ν_{max} 3430, 1710, 1662, 1625, 1605, 1088 and 1055 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{34}O_6$ (430.52): C, 69.74; H, 7.96. Found: C, 69.49; H, 8.22.

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[CONTRIBUTION FROM THE NATURAL PRODUCTS RESEARCH DEPARTMENT, SCHERING CORP.]

Double Bond Isomerization of Steroidal A-ring α,β-Unsaturated Ketones: 1,5-Dien-3-ones

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A method was devised for the synthesis of steroidal 1,5-dien-3-ones: introduction of bromine at C-6 of a 1,4-diene-3-one is followed by reductive debromination under neutral conditions. A 6-acetoxy-1,4-dien-3-one also was transformed in this manner. Δ⁴-Cholesten-3-one similarly was converted to the unconjugated ketone.

In a recent paper[1] we described the preparation of certain 7α-hydroxysteroids. The final step in that synthesis involved the reductive debromination of 6β-bromo-7α-hydroxy derivatives with metallic zinc. It was observed that, if the reaction were carried out for periods shorter than described, certain spectral changes occurred which indicated that the reaction proceeded by way of a structure not containing the original $\Delta^{1,4}$-dien-3-one. Thus, when 6β-bromo-7α,17α,21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione 21-acetate (I) was treated with zinc in aqueous ethanol and the reaction followed spectroscopically, the original λ_{max} at 244 mμ was seen to undergo a hypsochromic shift until it reached 224 mμ. This drift then reversed itself until a final reading at 237 mμ, attributable to 7α,17α,21-trihydroxy-$\Delta^{1,4}$-pregnadiene-3,11,20-trione 21-acetate,[1] was reached. When the reaction was repeated on a preparative scale and interrupted when the ultraviolet maximum had reached its lowest wave length, it was possible to isolate an isomer of the latter compound. This isomer showed ϵ_{224}^{EtOH} 12,000, a peak which shifted instantaneously to 237 mμ upon the addition of a drop of alkali. This indicated restoration of the original chromophore, and it appeared that the double bond originally at C-4 had not been reduced[2] but isomerized to a position which permitted facile reversion into conjugation. It was concluded that the new isomer was 7α,17α,21-trihydroxy-$\Delta^{1,5}$-pregnadiene-3,11,20-trione 21-acetate (II).[3]

(1) A. L. Nussbaum, G. Brabazon, T. L. Popper and E. P. Oliveto, THIS JOURNAL, **80**, 2722 (1958).

(2) Reduction of a conjugated double bond by zinc has been observed by J. Fajkoš, *Coll. Czech. Chem. Comm.*, **8**, 1559 (1958).

(3) Steroidal compounds possessing the same residual chromophore would be the Δ^1-3-ones. Examples listed by L. Dorfman, *Chem. Revs.*, **53**, 47 (1953), range from 224 to 231 mμ; see also, *inter alia*, E. Caspi and M. M. Pechet, *J. Biol. Chem.*, **230**, 843 (1958).