[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE CHEMICAL DIVISION, MERCK & CO., INC.]

Synthesis of 16β-Chlorocortisone Acetate and Related 16-Substituted Steroids

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The synthesis of 16β-chlorocortisone acetate has been described. The cleavage of 16,17(α)-oxidopregnanes with formic and acetic acids has also been investigated.

Because of the enhanced glucocorticoid and mineralocorticoid activities of 9α-halo adrenal cortical hormones,[1] it was of interest to prepare a compound with the halogen atom (preferably fluorine or chlorine) substituted at other positions in the steroid nucleus. This communication describes the synthesis of 16β-chlorocortisone acetate and related compounds. Julian[2] has recently reported the synthesis of 16β-bromocortisone acetate by an analogous route.

16-Pregnene-3α-ol-11,20-dione acetate (I)[2,3] was converted to the 16,17(α)-oxide (IIa) and then to 16,17(α)-oxidopregnane-3α,21-diol-11,20-dione 21-acetate (III) by reactions similar to those used by Julian and co-workers.[2] Oxidation with the chromium trioxide-pyridine reagent[4] or chromic acid in acetic acid yielded 16,17(α)-oxidopregnan-21-ol-3,-11,20-trione acetate (IV).[5] The oxide function was cleaved with hydrogen chloride in acetic acid, giving 16β-chloropregnane-17α,21-diol-3,11,20-trione 21-acetate (V). Bromination yielded a non-crystalline 4-bromo compound which was dehydrobrominated with lithium chloride-dimethylformamide[6] to 16β-chlorocortisone acetate (VI).

Proof of the structure of VI was provided by reclosure of the oxide, using potassium acetate, to the known 16,17(α)-oxido-4-pregnene-21-ol-3,11 20-trione acetate (VII).[7] Attempts to dechlorinate at C_{16} with Raney nickel returned starting material. As might be predicted the chloro compound is more difficult to dehalogenate than are the 16-bromo compounds which are reported in the literature.[2,8]

16β-Chlorocortisone acetate showed no appreciable activity in glycogen deposition, granuloma inhibition, adrenal atrophy or thymus involution. Furthermore it was found to be inactive when tested for ability to retain sodium in adrenalectomized rats.[9]

Cleavage of the 16,17(α)-oxide function by a number of other acids was also investigated. It proved to be a rather difficult oxide to cleave. A useful diagnostic tool for these ring openings was provided when it was discovered that 16,17(α)-oxido ketols *do not* give a positive test with blue tetrazolium reagent (five examples) whereas the ring-cleaved products give the expected positive test (four examples). It is generally considered that the presence of a 20,21-ketol grouping is the only requisite for a positive test and we have no satisfactory explanation for the 16,17-oxide anomaly.

We were unable to obtain any 16-fluoro compounds by using hydrogen fluoride under a variety of conditions which have been used in these laboratories to open 9,11(β)-oxides. In this connection, a recent patent claims the preparation of 9α,16β-difluoro-4-pregnene-11β,17α-diol-3,20-dione as a gum on treatment of the corresponding 9,11(β),16,-17(α)-*bis*-epoxide with hydrogen fluoride.[10]

We also investigated cleavage of the 16,17(α)-oxides with formic and acetic acids. Treatment of IIa with formic acid-sulfuric acid at room temperature (or formic acid at 100°) followed by potassium bicarbonate hydrolysis of the ester groups gave a *triol*. The analysis corresponds to that of pregnane-3α,16β,17α-triol-11,20-dione. Heusler and Wett-

(1) Fried and Sabo, *J. Am. Chem. Soc.*, **75**, 2273 (1953); Fried and Sabo, *J. Am. Chem. Soc.*, **76**, 1455 (1954); Fried, Herz, Sabo, Borman, Sinder, and Numeroff, *J. Am. Chem. Soc.*, **77**, 1069 (1955).

(2) Julian, Cole, Meyer, and Regan, *J. Am. Chem. Soc.*, **77**, 4601 (1955).

(3) Nes and Mason, *J. Am. Chem. Soc.*, **73**, 4765 (1951). Julian and Karpel, U. S. Patent 2,671,794 (March 9, 1954); Schock and Karpel, U. S. Patent 2,684,963 (July 27, 1954).

(4) Poos, Arth, Beyler, and Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).

(5) Colton, Nes, Van Dorp, Mason, and Kendall, *J. Biol. Chem.*, **194**, 235 (1952).

(6) Holysz, *J. Am. Chem. Soc.*, **75**, 4432 (1953).

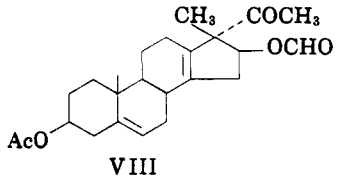
(7) McGuckin and Mason, *J. Am. Chem. Soc.*, **77**, 1822 (1955).

(8) Adams, Patel, Petrow, and Stuart-Weber, *J. Chem. Soc.*, 1825 (1954); Barkley, Farrar, Knowles, and Raffelson, *J. Am. Chem. Soc.*, **76**, 5017 (1954).

(9) We are indebted to Drs. Winter, Porter and Stoerk of the Merck Institute for Therapeutic Research for these results.

(10) Bergstrom, U. S. Patent 2,703,799 (March 8, 1955). The 16,17-oxide function presumably reclosed during treatment of the 9,16-difluoro compound with chromium trioxide-pyridine, whereupon 9α-fluoro-16,17(α)-oxido-4-pregnene-3,11,20-trione was obtained. No evidence that the 16,17-oxide had been cleaved by HF was given although a fluorine analysis of the product would provide this information.

stein[11] found that treatment of 16,17(α)-oxidopregnenolone acetate with formic acid-sulfuric acid caused Wagner-Meerwein rearrangement to an 18-norsteroid (VIII). This compound corresponds to



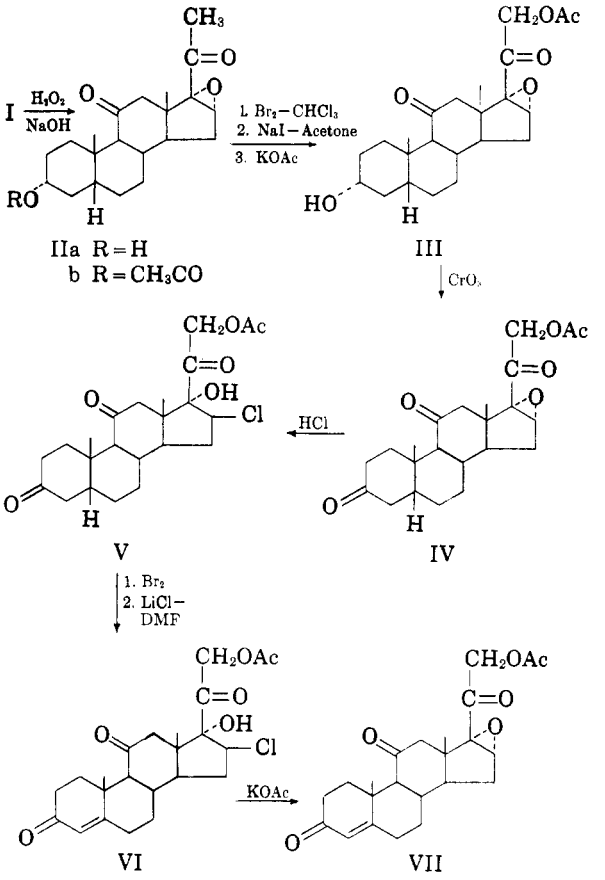
VIII

loss of a molecule of water from the expected 16-formoxy-17-hydroxypregnene. If IIa had undergone this rearrangement we should have isolated a *diol.*

Treatment of IV with formic acid at 100° (or formic acid-sulfuric acid at room temperature) gave a formate ester. The structure of this product is presumed to be pregnane-16β,17α,21-triol-3,11,20-trione 16-formate 21-acetate.

Cleavage of IIa with acetic acid-sulfuric acid followed by acetylation at C_3 yielded pregnane-3α,-16β,17α-triol-11,20-dione 3,16-diacetate and IIb which were separated chromatographically. Inhoffen and associates[12] cleaved 16,17(α)-oxido-20-ketopregnanes with acetic acid-sulfuric acid. Heusler and Wettstein subsequently showed that only with a short reaction time could D-homo rearrangement be avoided and the 16β-acetoxy-17α-hydroxypregnanes be obtained. The 21-acetoxy-20-ketones seem to be less prone to rearrangement than the 20-ketones, however.[11] Our reactions were run for even shorter periods than used by these authors and so it is assumed that they are not D-homo steroids.

Similar treatment of IV yielded pregnane-16β,-17α,21-triol-3,11,20-trione 16,21-diacetate. The reactions with acetic or formic acid on IV have not produced yields comparable with those of HBr or HCl. Consequently introduction of the C_{4-5} double bond to give other C_{16} substituted adrenal cortical steroids was not carried out.



EXPERIMENTAL[13]

16,17(α)-Oxidopregnan-21-ol-3,11,20-trione acetate (IV). To 142 mg. of 16,17(α)-oxidopregnane-3α,21-diol-11,20-dione 21-acetate in 1.4 ml. of pyridine was added 140 mg. of chromium trioxide in 1.4 ml. of pyridine and the mixture was allowed to stand at room temperature for 16 hours. Then it was added to water-ether, separated, and extracted twice more with ether. The combined ether extract was washed with dilute hydrochloric acid and aqueous sodium bicarbonate. After drying and concentration 145 mg. of oil was obtained. This was dissolved in ether (with a trace of benzene) and after cooling 108 mg. of crystals were collected in two crops (79 mg., m.p. 128–131°; 29 mg., m.p. 120–127°). Recrystallization from acetone-ether and benzene-ether gave prisms, m.p. 133–135°, $[\alpha]_D^{25}$ +117° ± 4° (*c*, 0.5 in $CHCl_3$),[5] BT-negative.

Anal. Calc'd for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.78; H, 7.77. λ_{max}^{nujol} 5.72, 5.79, 5.85–5.90, 8.15 μ.

16β-Chloropregnane-3,11,20-trione-17α,21-diol 21-acetate (V). Compound IV (1.5 g.) was dissolved in 6.1 ml. of acetic acid; 1.2 ml. of dry HCl in acetic acid (11% HCl by weight) was added and the mixture was let stand for three hours at room temperature. Water was added and the mixture was extracted with methylene chloride. The organic extract was washed with aqueous sodium bicarbonate, and water, then was dried and concentrated. The 1.7 g. of oil obtained was dissolved in methylene chloride-ether (1:10) and kept at 0° overnight. The resultant crystals amounted to 613 mg., m.p. 183–186°, $[\alpha]_D^{23}$ +61 ± 4° (*c*, 0.5 in $CHCl_3$), BT—slow pink. $AgNO_3$ test—slight precipitate after 30 minutes at 80°.

Anal. Calc'd for $C_{23}H_{31}ClO_6$: C, 62.93; H, 7.12; Cl, 8.07. Found: C, 63.12; H, 7.20; Cl, 8.32. λ_{max}^{nujol} 3.1, 5.70, 5.75, 5.90, 8.1 μ.

16β-Chlorocortisone acetate (VI). The 16-chloro compound V (56 mg.) was dissolved in 1.0 ml. of dimethylformamide containing 10 mg. of *p*-toluenesulfonic acid. To the stirred solution was added 1.28 ml. of 0.1 *M* bromine in dimethylformamide—half of it during the first 5 minutes and the other half 30 minutes later. Decolorization did not occur after 30 minutes longer nor after maintaining at 45 to 60° for 15 minutes. Aqueous sodium thiosulfate and ether were added; further extraction with ether and concentration yielded 72 mg. of oil which could not be crystalllized. It gave an immediate precipitate with alcoholic silver nitrate.

(11) Heusler and Wettstein, *Ber.*, **87**, 1301 (1954).

(12) Inhoffen, Blomeyer, and Brückner, *Ber.*, **87**, 593 (1954).

(13) Melting points were determined on the Kofler micro hotstage. The elemental analyses were done by R. N. Boos and associates and the infrared spectra by R. W. Walker to whom we are indebted.

The oil was dissolved in 0.7 ml. of dimethylformamide, 34 mg. of lithium chloride was added, and the mixture was heated under nitrogen on the steam-bath for 1.5 hours. Water and ether were added and after separation the ether was washed with dilute hydrochloric acid and water. Drying and concentration gave 40 mg. of residue which crystallized when triturated with methylene chloride-ether. Recrystallization from acetone gave the analytical sample, m.p. 249–255° dec., $[\alpha]_D^{22}$ +155° ± 2° (*c*, 0.98 in $CHCl_3$). BT-positive.

Anal. Calc'd for $C_{23}H_{29}ClO_6$: C, 63.22; H, 6.69; Cl, 8.12. Found: C, 63.37; H, 6.70; Cl, 8.10. λ_{max}^{MeOH} 238 mμ, E 15,700 λ_{max}^{nujol} 3.1, 5.71, 5.75, 5.87, 6.05, 6.20, 8.1 μ.

16,17(α)-Oxido-4-pregnene-21-ol-3,11,20-trione acetate (VII). 16β-Chlorocortisone acetate (20 mg.) and 40 mg. of potassium acetate in 5 ml. of acetone were refluxed with stirring for four hours. The mixture was filtered, concentrated, and water-methylene chloride was added to the residue. After separation and evaporation crystalline VII was obtained, m.p. 186–190°. Recrystallization from acetone-ether gave a sample, m.p. 193–196°.[7] Beilstein test—negative. BT—negative. λ_{max}^{nujol} 5.70, 5.75, 5.86, 6.0, 6.1, 8.1 μ.

Oxide cleavages with formic acid. A. 16,17(α)-Oxidopregnane-3α-ol-11,20-dione (IIa). IIa (100 mg.) was dissolved in 1 ml. of formic acid containing 5% (by volume) of concentrated sulfuric acid. After keeping for four hours at room temperature the mixture was poured into water-chloroform, separated, and the chloroform was washed with aqueous bicarbonate. Distillation of the chloroform gave 132 mg. of oil. This crude formate was dissolved in 1 ml. of methanol and 1 ml. of 5% potassium bicarbonate in methanol-water (1:1) was added. After one hour at room temperature the methanol was distilled *in vacuo* and the residue was extracted into chloroform. Solvent removal yielded 112 mg.; acetone trituration gave 56 mg. of pregnane-3α,16β,17α-triol-11,20-dione, m.p. 245–253°. Recrystallization from methanol afforded an analytical sample, m.p. 249–253°.

Anal. Calc'd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.18; H, 8.61. λ_{max}^{nujol} 2.97, 3.02, 3.11, 5.86, 5.92 μ.

The same compound was prepared by heating IIa with 98% formic acid for two hours at 100° followed by bicarbonate hydrolysis.

B. 16,17(α)-Oxidopregnan-21-ol-3,11,20-trione acetate (IV). Compound IV (100 mg.), was heated for two hours on the steam-bath with 1 ml. of 98% formic acid. After cooling the mixture was poured into water-chloroform, separated, and extracted twice more with chloroform; an aqueous bicarbonate wash and concentration gave 113 mg. of oil. Chromatography on Florisil gave crystalline material (37 mg.) in the chloroform-acetone (9:1 and 8:2) effluents in addition to IV (39 mg.) in the chloroform effluent. The former was recrystallized from ether to give pregnane-3α,16β,17α-triol-11,20-dione 16-formate 21-acetate, m.p. 208–210°. BT—positive.

Anal. Calc'd for $C_{24}H_{32}O_8$: C, 64.27; H, 7.19. Found: C, 64.47; H, 7.68. λ_{max}^{nujol} 2.95–3.0, 5.73, 5.80 (strong), 5.90, 8.0, 8.5 (formate) μ.

Oxide cleavages with acetic acid. A. 16,17(α)-Oxidopregnan-3α-ol-11,20-dione (IIa). To 346 mg. of IIa in 3.5 ml. of acetic acid was added 3.5 ml. of 5% (by volume) sulfuric acid in acetic acid. After 2.5 hours at room temperature the mixture was treated with excess saturated sodium bicarbonate and concentrated to dryness *in vacuo*. Thorough extraction of the oil and salts with ether gave 465 mg. of oil. This was acetylated overnight at room temperature with acetic anhydride-pyridine. Standard workup gave 367 mg. of oil. Chromatography on acid-washed alumina gave 50 mg. of crude 16,17(α)-oxidopregnan-3α-ol-11,20-dione acetate (IIb) in the ether-petroleum ether (2:3) effluent. Recrystallization from ether gave an analytical sample, m.p. 130–135° and 151–153°, $[\alpha]_D^{24}$ +122.2 ± 4° (*c*, 0.5 in $CHCl_3$).

Anal. Calc'd for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.39; H, 8.20. λ_{max}^{nujol} 5.78, 5.90, 8.05 μ.

The ether-petroleum ether (1:1 to 4:1) fractions yielded 153 mg. of crystalline material. Recrystallization from ether afforded pure pregnane-3α,16β,17α-triol-11,20-dione 3,16-diacetate, m.p. 101–110° and 145–147°, $[\alpha]^{23}D$ +79.3° ± 4° (*c*, 0.5 in $CHCl_3$).

Anal. Calc'd for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.84; H, 8.10. λ_{max}^{nujol} 2.91, 5.78, 5.86, 8.05 μ.

B. 16,17(α)-Oxidopregnane-21-ol-3,11,20-trione acetate (IV). Compound IV (100 mg.) was dissolved in 1 ml. of acetic acid and 1 ml. of 5% (by volume) sulfuric acid in acetic acid was added. After 3.5 hours at room temperature the reaction was worked up as in A. Chromatography on acid-washed alumina gave 24 mg. of starting material followed by 36 mg. of crude product. Recrystallization from acetone-ether gave a small amount of pregnane-16β,17α,21-triol-3,11,20-trione 16,21-diacetate, m.p. 120° and 186–188°. On some samples a melting point of 94–96° was observed. BT—positive.

Anal. Calc'd for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 65.17; H, 8.55. λ_{max}^{nujol} 2.92, 5.72, 5.78, 5.86, 5.92, 8.0–8.1 μ.

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