[CONTRIBUTION FROM MERCK SHARP & DOHME RESEARCH LABORATORIES]

Alkylated Adrenal Hormones. The Synthesis of 6α-Methyl Cortical Steroids

BY JOHN H. FRIED, GLEN E. ARTH AND LEWIS H. SARETT

RECEIVED SEPTEMBER 11, 1958

Cortisone has been converted to 6α-methylhydrocortisone, 6α-methyl*allo*dihydrohydrocortisone and Δ¹-6α-methylhydrocortisone. The synthesis of these compounds, proceeding *via* Grignard addition to a suitably protected 6-ketone, V, is described.

The synthesis of modified cortical steroids has been of particular interest since the report of greatly enhanced activity in a series of 9α-halogen derivatives of hydrocortisone.[1] Further modifications have included the introduction of the C-1 double bond[2] and the 2α-methyl[3] group. The increased activity due to these latter changes has been attributed[4] to protection of the A-ring against inactivation *via* enzymatic reduction. It seemed reasonable to suppose that a 6α-methyl group would be similarly effective.

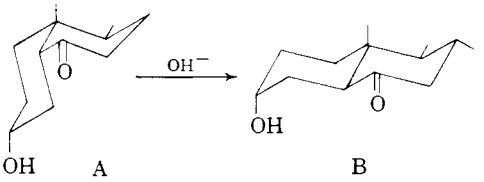
This paper describes the synthesis of 6α-methylhydrocortisone, Δ¹-6α-methylhydrocortisone and 6α-methyl*allo*dihydrohydrocortisone. Near the completion of this work an alternate route to the first two of these compounds was reported by Spero[5] and co-workers.

Beyler, *et al.*, have recently described the preparation of 17α,20,20,21-bismethylenedioxy-4-pregnene-3,11-dione, cortisone BMD[6] I, as well as 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5-pregnene-3,11-dione, cortisone BMD-3-dioxolane[6,7] (II). The latter, possessing 5,6-unsaturation susceptible to attack by peracid[8] and a well protected dihydroxy acetone side-chain, appeared to be an ideal starting material for the projected synthesis.

Peracid oxidation of II afforded a mixture of 5,6-oxides which could be separated by fractional crystallization into the pure epimers, the α-oxide IIIa (four parts) and the β-oxide IIIb (three parts). Configurational assignments of IIIa and IIIb were made on the basis of molecular rotation differences, analogous to those found for the cholesterol 5,6-oxides.[9]

For further synthetic purposes the epimeric oxides were not separated but treated sequentially with formic acid and alkali[10] to yield the trione IV. The same reagents converted the pure α-oxide IIIa to IV in equivalent yield. Formic acid cleaved the dioxolane and opened the oxides to mixed hydroxy formates, which subsequently were hydrolyzed and dehydrated by alkali. Formulation of IV as an A/B *trans* structure follows by analogy with other 6-keto steroids, including 3,6-diones, which have been shown to be stable in the *allo*-series.[11]

Reaction of butanone dioxolane[12,13] and IV afforded a key intermediate, the 3-monodioxolane V. The position of the ethylenedioxy function in V is based on the analogous 3-ethylenedioxyandrostane-6,17-dione reported by Rosenkranz,[13] *et al.* Our compound formed under equilibrating conditions was also assigned the A/B *trans* structure, an intuitive conclusion in agreement with that of Rosenkranz, *et al.*, in the 6-ketodihydrotestosterone series. The same conclusion was reached by the more reliable inferential argument cited below. Wieland and Dane[14] effected the transformation A to B, a change which involves



conversion of the 3α-hydroxyl initially equatorial to a 3α-axial substituent. From this experiment it may be concluded that the A/B *trans* 6-keto structure is more stable than the corresponding *cis* structure by an energy factor which is greater than the difference between an axial and an equatorial hydroxyl (*ca.* 1 kcal.[15]). In the present case since one bond of the 3-dioxolane is axial and the other equatorial in either the A/B *cis* or *trans* form, any change in going from A/B *cis* ⇄ A/B *trans* involves a net energy change of zero insofar as calculated conformational interactions are concerned. Since these interactions are the only ones which need be considered, the *trans* form is the more stable. This conclusion was strengthened by an experiment carried out in the present case. The base stable ketodioxolane V on modified Wolff-Kishner[16] reduction at C-6 followed by lithium aluminum hydride reduction of the 11-

(1) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953).

(2) H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet, *Science*, **121**, 176 (1955).

(3) J. A. Hogg, F. H. Lincoln, R. W. Jackson and W. P. Schneider, THIS JOURNAL, **77**, 6401 (1955).

(4) R. S. Ely, A. K. Done and V. C. Kelley, *Proc. Soc. Exp. Biol. and Med.*, **91**, 503 (1956); G. W. Liddle and J. E. Richard, *Science*, **123**, 324 (1956).

(5) G. B. Spero, J. L. Thompson, B. J. Magerlein, A. R. Hanze, H. C. Murray, O. K. Sebek and J. A. Hogg, THIS JOURNAL, **78**, 6213 (1956).

(6) R. E. Beyler, R. M. Morlarty, Frances Hoffman and L. H. Sarett, *ibid.*, **80**, 1517 (1958).

(7) A detailed description of the preparation and properties of II is given in the Experimental section of this paper.

(8) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(9) P. A. Plattner, T. Petrzilka and W. Lang, *Helv. Chim. Acta*, **27**, 513 (1944); Z. Hattori, *J. Pharm. Soc. Japan*, **60**, 125 (1940).

(10) C. Amendolla, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 1226 (1954); J. Romo, G. Rosenkranz, C. Djerassi and F. Sondheimer, *J. Org. Chem.*, **19**, 1501 (1954).

(11) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publishing Co., New York, N. Y., 1949, p. 621.

(12) H. J. Dauben, B. Loken and H. J. Ringold, THIS JOURNAL, **76**, 1359 (1954).

(13) G. Rosenkranz, M. Velasco and F. Sondheimer, *ibid.*, **76**, 5024 (1954).

(14) H. Wieland and E. Dane, *Z. physiol. Chem.*, **212**, 41 (1932).

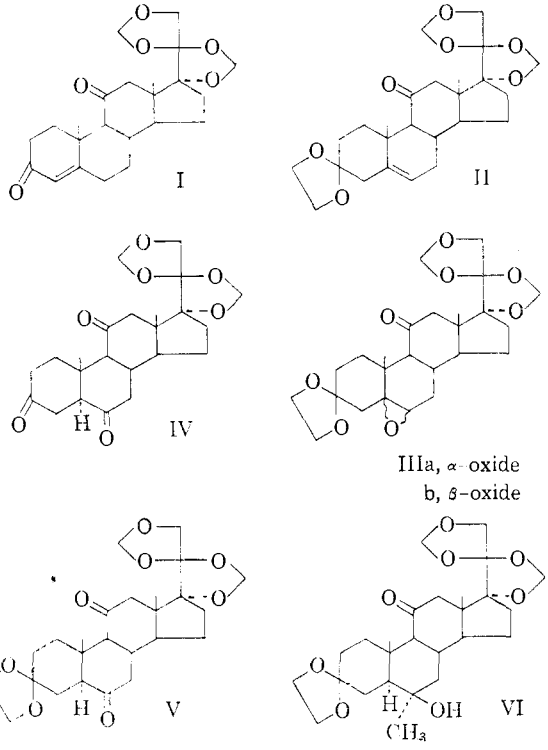
(15) E. L. Eliel and R. S. Ro, THIS JOURNAL, **79**, 5992 (1957).

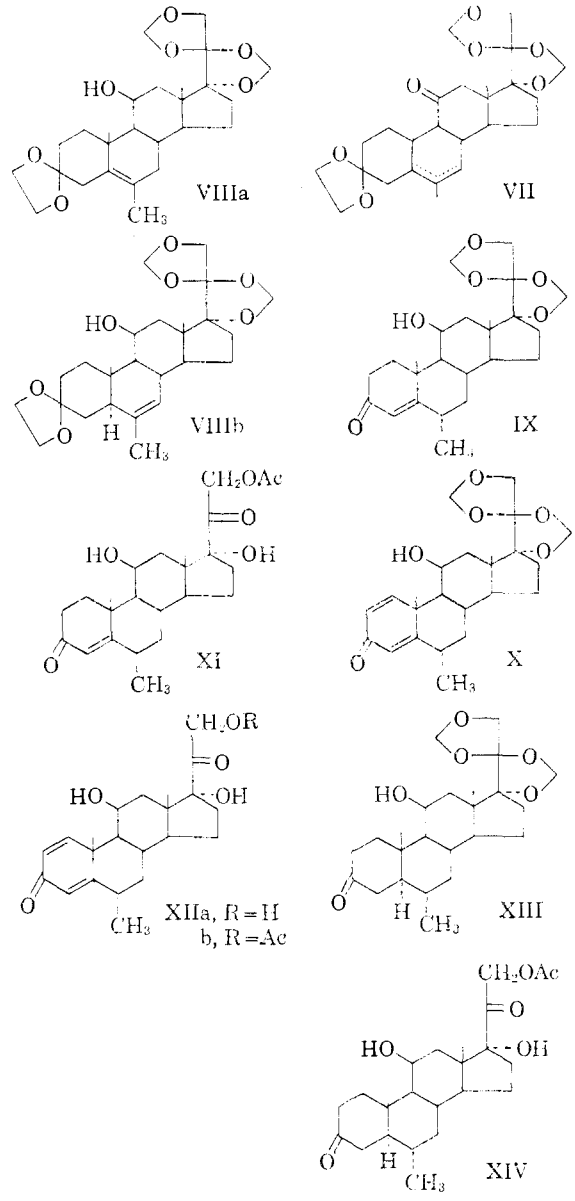
(16) Huang-Minlon, *ibid.*, **71**, 3301 (1949).

carbonyl and reversal of the 3-dioxolane yielded 17α,20,20,21-bismethylenedioxy*allo*pregnane-11β-ol-3-one identical with an authentic sample.[17] Assuming the stable configuration of the A/B-ring juncture to be the same for the intermediate hydrazone formed during Wolff-Kishner reduction as for the ketonic precursor, then V must also be in the *allo* series.

Treatment of the keto dioxolane V with methylmagnesium iodide led to the carbinol VI. Conversion of only one of the carbonyls was indicated by the analytical data and the infrared spectrum which showed both hydroxyl and carbonyl functions at 3420 and 1685 cm.$^{-1}$. Since the unreactive character of the 11-ketone is well substantiated,[18] the formation of the mono-Grignard adduct can only be construed as due to reaction at C-6. The newly introduced methyl group is undoubtedly alpha, since approach to the carbonyl at six is relatively unhindered from the alpha in contrast to strong steric hindrance encountered on the beta face. The same conclusion can also be drawn by analogy with metal hydride reductions of keto steroids.[19]

Dehydration of the carbinol with thionyl chloride–pyridine led to a mixture of Δ^5- and Δ^6-olefins, VII. Separation at this stage was not practical. However, after lithium aluminum hydride reduction to the 11β-ols, a separation was achieved by repeated triturations with ether. The more soluble Δ^5-olefin VIIIa predominated by a ratio of *ca.* 8:2 over the Δ^6-olefin VIIIb. Removal of the





3-dioxolane in VIIIa led to 6α-methylhydrocortisone BMD. The methyl group was assigned the stable equatorial configuration, since under the conditions of the reaction equilibration could be achieved. Examination of models indicates the 6β-methyl group to be particularly crowded due to a 1,3-diaxial interaction with the angular methyl on C-10.

Hydrolysis of the bismethylenedioxy protecting group in IX followed by acetylation afforded 6α-methylhydrocortisone acetate, XI. Introduction of the 1-double bond was achieved by treatment of IX with selenium dioxide.[20,30] Subsequent cleavage of the bismethylenedioxy function with 60% aqueous formic acid led to Δ^1-6α-methylhydrocortisone (XIIa), which afforded the corresponding acetate XIIb on acetylation.

Lithium–ammonia reduction of 6α-methylhydrocortisone BMD (IX) led to the saturated ketone

(17) We are indebted to Dr. D. Hoff for supplying the samples used in this comparison.

(18) Reference 11, p. 409; see, however, H. J. Ringold, E. Batres and J. A. Zderic, *Tetrahedron*, **2**, 164 (1958).

(19) C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 1790, 3374 (1952); see, however, R. A. Sneen, THIS JOURNAL, **80**, 3982 (1958).

(20) Ch. Meystre, H. Frey, W. Voser and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956).

XIII. The thermodynamically stable A/B *trans* structure was assigned to XIII by analogy with other metal–ammonia reductions.[21] Finally, 6α-methyl*allo*dihydrocortisone acetate was obtained after hydrolysis and acetylation of XIII.

The biological activities of 6α-methylhydrocortisone acetate and Δ¹-6α-methylhydrocortisone acetate were evaluated in the Merck Institute for Therapeutic Research.[22] The liver glycogen[23] activities (oral administration) of XI and XIIb were 1–2 and 1.4–3.3 times hydrocortisone, respectively. The anti-inflammatory activity (oral administration) of XIIb as determined by systemic granuloma inhibition[24] was 3-4 times prednisolone acetate. Both XI and XIIb cause sodium and water diuresis in adrenalectomized rats. The reported[5] liver glycogen activities of the 21-alcohols corresponding to XI and XIIa were four and sixteen. The apparent discrepancy presumably is due to differences in the method[25] used for carrying out this test.

Experimental[26]

17α,20,20,21-Bismethylenedioxy-4-pregnene-3,11-dione (Cortisone BMD)[6] (I).—To a solution of 132 g. of cortisone in 5 l. of chloroform was added 1300 ml. of concd. hydrochloric acid and 1300 ml. of 37% formalin. The resulting system was stirred for 76 hr. at room temperature. The aqueous layer was separated and discarded. The chloroform solution was washed with aqueous sodium bicarbonate until neutral, dried over sodium sulfate and concentrated *in vacuo*. A slurry of the residue in hot methanol afforded crude crystalline I which was separated by filtration, washed once with methanol and recrystallized from the minimum amount of methylene chloride by addition of methanol. The yield of cortisone BMD (I), m.p. 249–253°, was 94 g. (64%).

17α,20,20,21-Bismethylenedioxy-3-ethylenedioxy-5-pregnene-11-one (Cortisone BMD dioxolane) (II).—A solution containing 16.2 g. of cortisone BMD (I) dissolved in 800 cc. of benzene was refluxed overnight with 40 cc. of ethylene glycol and 1.6 g. of *p*-toluenesulfonic acid in a 3-l. flask fitted with a water separator. The cooled solution was washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The crude product was adsorbed on 400 g. of acid-washed alumina (Merck) from benzene. Elution with 1:1 ether–petroleum ether yielded 8.5 g. (47%) of product, m.p. 200–210°. The analytical sample[27] of 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5-pregnene-11-one was crystallized from methanol, m.p. 210–212°, $[\alpha]_D$ −87° (*c* 0.5). Calcd. for $C_{28}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 67.05; H, 7.55.

17α,20,20,21-Bismethylenedioxy-3-ethylenedioxy-5,6α-oxido-*allo*pregnene-11-one (IIIa) and 17α,20,20,21-Bismethylenedioxy-3-ethylenedioxy-5,6β-oxido-pregnane-11-one (IIIb).—A solution containing 16.5 g. of cortisone BMD (II, 37 mmoles) and 45 meq. of perbenzoic acid in a total volume of 350 ml. of benzene was stored in the dark at room temperature for 45 hours. The solution was decanted from 6.3 g. of crystalline product, washed with saturated aqueous sodium bicarbonate solution and dried over sodium sulfate. Concentration *in vacuo* yielded 12.5 g. of additional material, a total of 18.8 g. of mixed oxides. The product from a similar reaction utilizing 3.8 g. of II was crystallized from benzene to yield 1.8 g. (46%) of α-oxide IIIa, m.p. 285–300°. The analytical sample of 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5,6α-oxido-*allo*pregnane-11-one was crystallized from benzene, m.p. 293–296°, $[\alpha]_D$ −104°. Calcd. for $C_{28}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 64.97; H, 7.10.

The solution remaining after removal of the α-oxide was concentrated to yield 1.3 g. (33%) of β-oxide, m.p. 206–219°. The analytical sample of 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5,6β-oxido-pregnane-11-one was crystallized from benzene, m.p. 220–224°, $[\alpha]_D$ −73°. Calcd. for $C_{28}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 64.97; H, 7.47.

17α,20,20,21-Bismethylenedioxy-*allo*pregnane-3,6,11-trione (IV).—The mixed oxides III above were dissolved in 300 cc. of 98–100% formic acid at room temperature and allowed to stand 2.5 hours. The solution was poured into water and extracted with chloroform. The chloroform solution was washed with saturated aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*.

The crude mixed formates dissolved in 1400 cc. of methanol were refluxed 0.5 hour under nitrogen with a solution consisting of 27 g. of potassium hydroxide and 135 cc. of water. The cooled solution was neutralized with 37 cc. of acetic acid and concentrated *in vacuo* at 30°. The resulting solution was poured into water and extracted with chloroform. The chloroform layer was washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. Crystallization from methanol yielded 8.8 g. (57%) of the triketone IV. The analytical sample of 17α,20,20,21-bismethylenedioxy-*allo*pregnane-3,6,11-trione was crystallized from methanol, m.p. 265–268°, $[\alpha]_D$ −87°. Calcd. for $C_{23}H_{30}O_7$: C, 66.01; H, 7.23. Found: C, 66.20; H, 7.55.

Similarly, starting with pure α-oxide IIIa there was obtained 73% of 17α,20,20,21-bismethylenedioxy-*allo*pregnane-5,6β-diol-3,11-dione 6-formate. The analytical sample was crystallized from methanol, m.p. 254–256°. Calcd. for $C_{24}H_{32}O_9$: C, 62.05; H, 6.94. Found: C, 62.52; H, 6.99. Base treatment of this material yielded 67% of the trione IV.

17α,20,20,21-Bismethylenedioxy-3-ethylenedioxy-*allo*pregnane-6,11-dione[12,13] (V).—A solution consisting of 125 mg. of *p*-toluenesulfonic acid in 2 cc. of butanone dioxolane was added to a boiling suspension containing 4.94 g. of the trione IV in 115 cc. of butanone dioxolane. After a reflux period of 9 minutes, during which time solution occurred, the reaction mixture was cooled quickly in an ice-bath, washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The material on trituration with ether afforded 4.6 g. of crude product. Crystallization from ethyl acetate yielded 2.92 g. (54%) of V, m.p. 248–260°. The analytical sample of 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-*allo*pregnane-6,11-dione was recrystallized from ethyl acetate, m.p. 263–266°, $[\alpha]_D$ −66°. Calcd. for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 65.30; H, 7.37.

6α-Methyl-17α,20,20,21-Bismethylenedioxy-3-ethylenedioxy-*allo*pregnane-6-ol-11-one (VI).—The methyl Grignard reagent was prepared by adding with stirring a solution containing 3 cc. of methyl iodide in 10 cc. of ether to 300 mg. of magnesium covered with 10 cc. of ether. After the magnesium had been consumed an additional 20 cc. of ether was added.

To this solution was added with stirring over a period of 10 minutes a solution consisting of 2.91 g. of the keto dioxolane V in 40 cc. of benzene. A copious precipitate was observed. The suspension was allowed to stir an additional 0.5 hour, then decomposed with 40 cc. of water. After the addition of 100 cc. of benzene the layers were separated, and the aqueous layer was extracted twice with 100 cc. of chloroform. The organic layers were combined, dried over sodium sulfate and concentrated *in vacuo*. The crude product was crystallized from ether to yield 2.40 g. of material, m.p. 178–180° (79%). The analytical sample of 6α-methyl-17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-*allo*pregnane-6-ol-11-one was crystallized from ether, m.p. 182–185°, $[\alpha]_D$ −55°. Calcd. for $C_{26}H_{36}O_8$: C, 65.25; H, 8.00. Found: C, 65.23; H, 7.87.

(21) D. H. R. Barton and C. H. Robinson, *J. Chem. Soc.*, 3045 (1954); *cf.* A. Bowers, H. J. Ringold and R. I. Dorfman, THIS JOURNAL, **79**, 4556 (1957).

(22) We are indebted to Drs. R. H. Silber, H. C. Stoerk and their associates for carrying out these determinations.

(23) R. M. Reinecke and E. C. Kendall, *Endocrinol.*, **31**, 673 (1942).

(24) Modification of the method of R. Meier, W. Schuler and P. Desaulles, *Experientia*, **6**, 469 (1950). Intact male Holtzman rats (*ca.* 125 g.) are dosed orally each day for a week.

(25) C. C. Porter and R. H. Silber, *Endocrinol.*, **53**, 73 (1953).

(26) Melting points were determined on a Kofler micro hot-stage and are corrected. Rotations were determined at 24° in chloroform at concn. = 10 mg./ml., unless otherwise noted. We wish to thank Mr. R. Boos and his associates for microanalyses, Mr. H. Murphy and his associates for ultraviolet absorption spectra and Mr. R. Walker for the infrared spectra (Baird model B) herein reported.

(27) Physical properties were determined by Dr. R. Beyler.

6-Methyl-17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5-pregnene-11-one and 6-Methyl-17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-6-pregnene-11-one (VII).—A solution of thionyl chloride in pyridine was prepared by adding 7.5 cc. of freshly distilled thionyl chloride to 38 cc. of ice-cold anhydrous pyridine. This was added dropwise to a stirred solution of VII, 7.0 g., in 45 cc. of anhydrous pyridine. The rate of addition was controlled such that the reaction mixture was maintained at approximately 40°. The solution was stirred an additional 30 minutes after the addition of the reagent was completed, subsequently cooled in ice and poured into 250 cc. of ice-water. The mixture was extracted with chloroform and the chloroform layer washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The crude product dissolved in benzene was chromatographed on 200 g. of acid-washed alumina (Merck). Elution with ether–petroleum ether, 2:8 yielded 6.2 g. (92%) of material, m.p. >200°, which was used in the next step. The analytical sample, mainly 6-methyl-17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5-pregnene-11-one,[28] was crystallized from benzene, m.p. 223–228°, $[\alpha]_D$ −61°. Calcd. for $C_{26}H_{36}O_7$: C, 67.80; H, 7.88. Found: C, 67.93; H, 7.65.

6-Methyl-17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5-pregnene-11β-ol (VIIIa).—To a stirred suspension consisting of 6.0 g. of lithium aluminum hydride in 1.2 liters of anhydrous ether was added 6.2 g. of VII dissolved in 100 cc. of benzene. The suspension was refluxed 4 hours, cooled, and treated with 40 cc. of ethyl acetate to destroy excess lithium aluminum hydride. After hydrolysis with 120 cc. of water the ether layer was decanted and the aqueous layer diluted with an additional 200 cc. of water and extracted with chloroform. The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. Six grams of crude crystalline material was obtained. This material dissolved in 300 cc. of benzene was adsorbed on 180 g. of acid-washed alumina (Merck). Elution with ether–petroleum ether, 4:6, yielded 5.3 g. of crystalline material melting at 170–210°. The mixture was purified by partial solution in ether to yield 3.8 g., 61%, of material, m.p. 180–190°. The analytical sample of 6-methyl-17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5-pregnene-11β-ol was crystallized from ether, m.p. 188–193°, $[\alpha]_D$ −105°. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28. Found: C, 67.67; H, 8.24.

6-Methyl-17α,20,20,21-bismethylenedioxy-6-pregnene-11β-ol (VIIIb).—The ether-insoluble material from above was crystallized from benzene to yield 0.80 g. (13%) of a compound, m.p. 235–250°. The analytical sample of 6-methyl-17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-6-pregnene-11β-ol was recrystallized from benzene, m.p. 250–255°, $[\alpha]_D$ −80°. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28. Found: C, 67.67; H, 8.26.

6α-Methyl-17α,20,20,21-bismethylenedioxy-4-pregnene-11β-ol-3-one (IX).—A solution consisting of 3.8 g. of VIIIa, 400 cc. of anhydrous acetone and 400 mg. of *p*-toluenesulfonic acid monohydrate was allowed to stand overnight. The solution which had turned green was poured into water and extracted with chloroform. The chloroform solution was washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The crude product was crystallized twice from benzene to yield 0.90 g. of material, m.p. 255–270°. The benzene mother liquors were chromatographed on 90 g. of acid-washed alumina (Merck). Elution with ether–petroleum ether, 8:2, yielded an additional 1.25 g. of material, m.p. 240–260°, for a total of 2.15 g. (63%). The analytical sample of 6α-methyl-17α,20,20,21-bismethylenedioxy-4-pregnene-11β-ol-3-one was crystallized from benzene, m.p. 275–279°, $[\alpha]_D$ +1°; ultraviolet λ_{max} 242 mμ, *E* 13,700. Calcd. for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19. Found: C, 68.30; H, 8.23.

6α-Methylhydrocortisone Acetate[5,6] (XI).—A suspension of 97 mg. of 6α-methylhydrocortisone BMD (IX) and 18 cc. of 50% aqueous acetic acid was carefully purged with nitrogen and heated 8 hours on the steam-bath. After storing at room temperature overnight the solution was taken to dryness *in vacuo*. A solution consisting of 1 cc. of acetic anhydride and 1 cc. of pyridine was added and the mixture heated 15 minutes on the steam-bath. The solution was cooled and poured into ice-water. The aqueous suspension was extracted with chloroform, washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The crude product dissolved in benzene was adsorbed on 5 g. of acid-washed alumina (Merck), and eluted with ether–chloroform, 1:1. Crystallization from methanol afforded 50 mg. (52%) of 6α-methylhydrocortisone acetate. The analytical sample was recrystallized from methanol, m.p. 208–211°, $[\alpha]_D$ +136°; ultraviolet λ_{max} 242 mμ, *E* 14,800. Calcd. for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19. Found: C, 68.99; H, 8.21.

6α-Methyl-17α,20,20,21-bismethylenedioxy-1,4-pregnadiene-11β-ol-3-one[29] (X).—A solution of 0.38 g. of 6α-methylhydrocortisone BMD in 75 cc. of *t*-butyl alcohol containing 0.2 cc. of acetic acid and 0.12 g. of selenium dioxide was refluxed for a period of 87 hours under an atmosphere of nitrogen. Additions of 0.12 g. of selenium dioxide were made after 26 hours and 68 hours. The cooled reaction mixture was filtered and concentrated under vacuum. A chloroform solution of the residue was washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The crude product dissolved in 25 cc. of benzene was stirred with *ca.* 1 ml. of mercury overnight[29], filtered through Super-cel and chromatographed on 20 g. of acid-washed alumina (Merck). Elution with benzene–chloroform mixtures afforded 0.23 g. of material which was crystallized from chloroform–benzene to yield 0.10 g. (27%) of 6α-methyl-17α,20,20,21-bismethylenedioxy-1,4-pregnadiene-11β-ol-3-one, m.p. 308–313°, $[\alpha]_D$ −33°; ultraviolet λ_{max} 244 mμ, *E* 13,550; infrared λ_{max}^{Nujol} 6.01, 6.15, 6.20 μ. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 69.49; H, 7.82.

6α-Methylprednisolone[5,6] (XII).—A suspension consisting of 0.74 g. of 6α-methylprednisolone BMD (X) in 21 cc. of 60% aqueous formic acid was purged with nitrogen and heated inside a steam-cone for 10 minutes. The resulting solution was cooled, diluted with ice-water and extracted with chloroform. The organic layer was washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. Crystallization from ethyl acetate yielded 0.35 g. (55%) of 6α-methylprednisolone, m.p. 229–234°. The analytical sample was recrystallized from ethyl acetate, m.p. 226–237°, $[\alpha]_D$ + 94° (*c* 0.5 dioxane); ultraviolet λ_{max} 244 mμ, *E* 14,500 infrared λ_{max}^{Nujol} 5.83, 6.01, 6.19, sh.6.21 μ. Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.99; H, 8.05.

6α-Methylprednisolone Acetate (XIIb).—Acetylation of XIIa with acetic anhydride–pyridine under standard conditions yielded the acetate XIIb. The analytical sample of 6α-methylprednisolone acetate was crystallized from ethyl acetate, m.p. 205–208°, $[\alpha]_D$ +95° ultraviolet λ_{max} 243 mμ, *E* 14,685; infrared λ_{max}^{Nujol} sh. 5.72, 5.79, 6.03, 6.18 μ. Calcd. for $C_{24}H_{32}O_5$: C, 69.21; H, 7.74. Found: C, 68.83; H, 7.71.

6α-Methyl-17α,20,20,21-bismethylenedioxy-*allo*pregnane-11β-ol-3-one (XIII) and 6α-Methyl-17α,20,20,21-bismethylenedioxy-*allo*pregnane-3β,11β-diol.—A solution consisting of 420 mg. of 6α-methylhydrocortisone BMD (IX) in 20 cc. of tetrahydrofuran (partially dried by storage over potassium hydroxide pellets) was added to 35 cc. of liquid ammonia containing 75 mg. of lithium. After stirring for 10 minutes *ca.* 10 cc. of ethanol was added and the ammonia allowed to evaporate. The residue was poured into aqueous sodium dihydrogen phosphate solution and extracted with chloroform. The chloroform solution was washed with water, dried over sodium sulfate and concentrated *in vacuo*. A benzene solution of the concentrate was adsorbed on 25 g. of acid-washed alumina (Merck) and eluted with ether to yield 183 mg. (43%) of 6α-methyl-17α,20,20,21-bismethylenedioxy-*allo*pregnane-11β-ol-3-one, m.p. 210–220°. Two crystallizations from methanol afforded the analytical sample, m.p. 219–224°, $[\alpha]_D$ −53°. Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.67; H, 8.60.

On further elution with chloroform 65 mg. of 6α-methyl-17α-20,20,21-bismethylenedioxy-*allo*pregnane-3β,11β-diol, m.p. 208–212° (ether–methanol), was obtained. The analytical sample was recrystallized from ether–methanol, m.p. 130°, 213–215°, $[\alpha]_D$ −54° (*c* 0.3). Calcd. for $C_{24}H_{38}O_6$: C, 68.22; H, 9.07. Found: C, 68.00; H, 9.33.

6α-Methyl*allo*pregnane-11β,17α,21-triol-3,20-dione 21-Acetate[6] (XIV). A suspension containing 80 mg. of 6α-

(28) Pure 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5-pregnene-11-one has been obtained by Dr. D. Hoff. The properties of this compound differed from the analytical sample prepared above.

(29) The use of mercury to scavenge some of the selenium containing contaminants afforded a more tractable crude product.

methyl*allo*dihydrohydrocortisone BMD (XIII) and 30 cc. of 60% aqueous formic acid was heated inside a steam-cone for 30 minutes. The resulting solution was cooled and extracted with chloroform. The chloroform layer was washed with aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The crude concentrate was dried by azeotropic distillation with benzene, and then subsequently treated for 15 minutes with 0.07 meq. of sodium methoxide in 1.0 ml. of methanol to cleave any formate esters. After neutralization with acetic acid the solution was diluted with water and extracted with chloroform. The chloroform layer was dried over sodium sulfate and concentrated *in vacuo*. The material was acetylated with acetic anhydride in pyridine under standard conditions and chromatographed on 7 g. of acid-washed alumina (Merck). The column was eluted with ether–chloroform, 7:3, to remove an unsaturated fraction, m.p. 225–235°, presumably 6α-methyl-9,11-*allo*pregnene-17α,21-diol-3,20-dione 21-acetate. Elution with ether–chloroform, 4:6, afforded 46 mg. of crude 6α-methyl*allo*pregnane-11β,17α,21-triol-3,20-dione 21-acetate (XIV). The analytical sample was crystallized from benzene, m.p. 183–186°. Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.87; H, 8.92.

17α,20,20,21-Bismethylenedioxy-3-ethylenedioxy-*allo*pregnane-11-one.[16]—A suspension containing 100 mg. of the diketodioxolane V, 700 mg. of potassium hydroxide pellets, 1.0 ml. of 85% hydrazine hydrate and 10 ml. of redistilled diethylene glycol was refluxed at 170° for 30 minutes. The temperature was raised to 210° by removing low boiling components, and reflux continued for two hours. The cooled reaction mixture was diluted with water and extracted with ether. After drying the ether layer over sodium sulfate and concentrating, the crude material in benzene solution was adsorbed on 7.0 g. of acid-washed alumina (Merck). Elution with ether and crystallization from methanol yielded 40 mg. of 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-*allo*pregnane-11-one, m.p. 190–200°. The analytical sample was recrystallized from methanol, m.p. 197–201°, $[\alpha]_D$ −37°. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 66.20; H, 7.97.

17α,20,20,21-Bismethylenedioxy-*allo*pregnane-11β-ol-3-one.—A solution containing 30 mg. of 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-*allo*pregnane-11-one in 5 cc. of benzene dried by azeotropic distillation was added to a suspension consisting of 100 mg. of lithium aluminum hydride in 20 ml. of dry tetrahydrofuran, and refluxed for 4 hours. Excess lithium aluminum hydride was decomposed with ethyl acetate. After hydrolysis of aluminum alkoxides with 1.3 cc. of water, the organic layer was separated by filtration, and concentrated. The crude product which showed no absorption due to carbonyl in the infrared was treated with 5 cc. of acetone, containing 10 mg. of *p*-toluenesulfonic acid, at room temperature overnight. The reaction mixture was diluted with water, neutralized with aqueous sodium bicarbonate solution, and extracted with chloroform. The chloroform layer was dried over sodium sulfate and concentrated *in vacuo*. The crude material in benzene solution was adsorbed on 5.0 g. of acid-washed alumina (Merck). Elution with ether yielded 16 mg. of 17α,20,20,-21-bismethylenedioxy-*allo*pregnane-11β-ol-3-one, m.p. 205–215° after crystallization from ether. This material did not depress the melting point of an authentic[17] sample. A melting point depression was observed, however, with the A/B *cis* compound, 17α,20,20,21-bismethylenedioxy-pregnane-11β-ol-3-one.[17] Infrared analysis of these compounds confirmed the conclusions drawn from the melting point data.

Acknowledgment.—We are indebted to Dr. J. Van de Kamp and Mr. S. M. Miller for preparation of supplies of some of the intermediates used in this synthesis.

RAHWAY, N. J.

[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, HEBREW UNIVERSITY AND THE MEDICAL RESEARCH LABORATORIES, MEDICAL CORPS, ISRAEL DEFENSE FORCES]

The Synthesis and Biological Availability of Some Lower Homologs of Cholesterol

BY ERNST D. BERGMANN, MORDECAI RABINOVITZ AND ZWI H. LEVINSON

RECEIVED JULY 10, 1958

Several analogs of cholesterol containing shorter side-chains have been synthesized from the chloride of 3-acetoxy-etiocholenic acid (II) and di-*n*-butyl-, di-*n*-pentyl- and diphenylcadmium and from pregnenolone acetate (III) and *n*-butyl-, *n*-pentyl- and phenylmagnesium bromide. In the former case, the ketones obtained were reduced, in the latter the tertiary carbinols dehydrated and subsequently hydrogenated. The structure of these dehydration products and the configuration of the hydrogenation products has been established, the latter by the observation that the analogous series of reactions with 4-methylpentylmagnesium bromide leads to cholesteryl acetate. These "unnatural" sterols show an effectiveness of at most 43% of that of cholesterol, as growth promoters of housefly larvae. No pupation occurred when these "unnatural" sterols were added to the sterile medium on which the larvae were reared.

It has been known for some time that insect larvae cannot themselves synthesize the sterols they require but have to rely on their food for the supply of these essential factors.[1,2] According to Bloch[3] and co-workers, larvae of the beetle *Dermestes vulpinus* transform acetate normally to squalene but cannot cyclize the latter to lanosterol (and cholesterol).

In the case of the housefly *Musca vicina Macq.*[4] cholesterol and sitosterol, and to a lesser extent ergosterol[5] and stigmasterol, were found effective in promoting larval growth and pupation. Also a number of minor changes of the molecular structure of cholesterol was found compatible with the biological functions of the compound, while more drastic changes destroyed its ability to regulate growth and pupation and produced either biologically inactive substances or even antagonists of the natural sterols. In particular, the removal of the sterol side chain destroyed the activity completely. In view of these effects,[6] it was thought interesting to synthesize analogs of cholesterol in which the side-chains are similar to the natural one, but shorter, and to study the biological availability of these compounds.

(1) H. Lipke and G. Fraenkel, *Ann. Rev. Entomol.*, **1**, 17 (1956).

(2) Another case recently studied is that of *Tribolium confusum* Duval; St. D. Beck and G. G. Kapadia, *Science*, **126**, 258 (1957).

(3) R. G. Langdon and K. Bloch, *J. Biol. Chem.*, **200**, 135 (1953); R. B. Clayton and K. Bloch, *ibid.*, **218**, 319 (1956); T. T. Tchen and K. Bloch, THIS JOURNAL, **78**, 1516 (1956); K. Bloch, R. G. Langdon, A. I. Clark and G. Fraenkel, *Biochim. Biophys. Acta*, **21**, 176 (1956); J. D. Johnston and K. Bloch, THIS JOURNAL, **79**, 1145 (1957).

(4) Z. H. Levinson and E. D. Bergmann, *Biochem. J.*, **65**, 254 (1957).

(5) Ergosterol is also less active than cholesterol in promoting larval growth of the flies *Musca domestica* L. (C. S. Hammen, *Ann. Entom. Soc. Amer.*, **50**, 125 (1957)), *Lucilia sericata* Meig. (R. P. Hobson, *Biochem. J.*, **29**, 2023 (1935)) and *Phormia regina* Meig (M. Brust and G. Fraenkel, *Physiol. Zool.*, **28**, 186 (1955)).

(6) J. Glover and C. Green (*Biochem. J.*, **67**, 308 (1957)) have ascribed the specificity of the sterols for many insects, which require either phyto- or zoösterols, to the fact that the intestinal membranes through which the sterols have to pass are adapted specifically to either of these sterol types.