1670, 1600 and 1250 cm.$^{-1}$; rotatory dispersion curve (c 0.0675 in dioxane): $[\alpha]_{700}$ −44°, $[\alpha]_{589}$ −50°, $[\alpha]_{500}$ −62°, $[\alpha]_{332.5}$ −5,510°.

Anal. Calcd. for $C_{21}H_{29}O_3I$: C, 55.26; H, 6.40; I, 27.81. Found: C, 55.54; H, 6.39; I, 27.24.

(b) **By the Treatment of Testosterone Acetate Enol Ether (XVI) with N-Iodosuccinimide.**—N-Iodosuccinimide (845 mg.) and acetic acid (0.4 cc.) were added to a solution of testosterone acetate enol ether[33] (XVI) (1.0 g., m.p. 129–132°, $[\alpha]_D$ −150°, λ_{max}^{EtOH} 240–242, ϵ 20,900) in acetone (20 cc.) and water (4.0 cc.) containing sodium acetate (400 mg.) at 0–5°. After stirring at 0–5° for 1 hour the excess of reagent was destroyed by the addition of sodium sulfite solution. Addition of ice-water and crystallization of the precipitate from methylene dichloride–hexane afforded 6β-iodotestosterone (XV) (770 mg.), m.p. 98–102° dec. raised by one further crystallization from methylene dichloride–hexane to 105–108° dec., $[\alpha]_D$ −52°, λ_{max}^{EtOH} 252–254 mμ, ϵ 15,300. The m.p. was undepressed upon admixture with a sample prepared as in method a and the infrared spectra were identical.

(33) E. Batres and H. J. Ringold, forthcoming publication from these laboratories.

APARTADO POSTAL 2679, MEXICO, D. F. MEX.

[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES]

16β-Methyl Cortical Steroids[1]

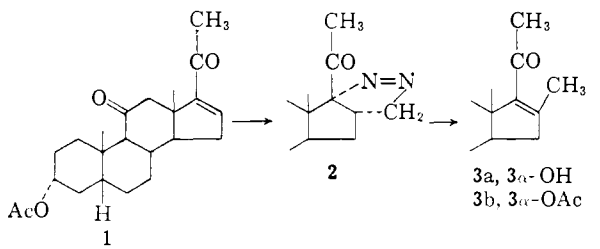
BY D. TAUB, R. D. HOFFSOMMER, H. L. SLATES, C. H. KUO AND N. L. WENDLER

RECEIVED DECEMBER 30, 1959

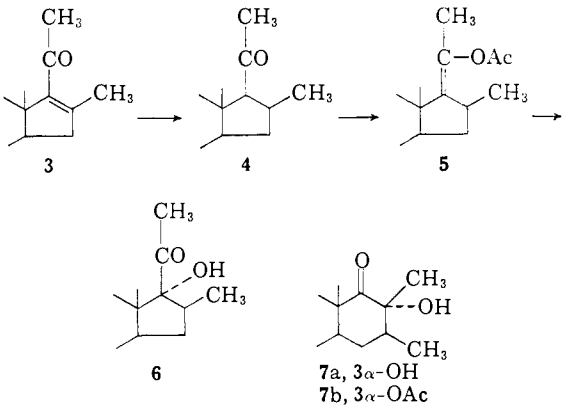
The partial synthesis of a number of 16β-methyl cortical steroids is described.

In this paper we wish to report in detail the preparation of 16β-methyl homologs of cortisone and its congeners. These substances are the first β-substituted cortical steroid derivatives to be described which are more potent anti-inflammatory agents than the corresponding parent steroids. Previously reported potentiating substituents have been attached to the α-face of the steroid nucleus.[2]

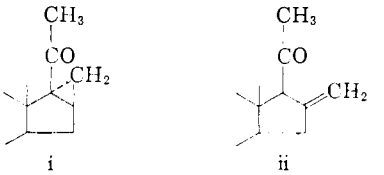
We utilized the readily available 3α-acetoxy-16-pregnene-11,20-dione (1)[3] as starting material, and introduced the 16-methyl substituent by reaction of 1 with diazomethane[4] and pyrolysis of the intermediate pyrazoline (2) followed by treatment with methanolic potassium hydroxide to give 16-methyl-3α-hydroxy-16-pregnene-11,20-dione (3a) in good over-all yield. Omission of the methanolic potassium hydroxide treatment yielded the 3-acetate 3b in somewhat smaller yield.[5] As is shown in the sequel, the 16-methyl-16-pregnene 3 is a versatile intermediate suitable for the preparation of both 16α-methyl- and 16β-methyl-substituted steroids.



Several approaches for the introduction of the 17α-hydroxyl group in the 16β-methyl series were next investigated. Catalytic hydrogenation of the 3α-acetoxy-16-methyl-16-pregnene 3b over palladium-on-calcium carbonate[4] led in excellent yield to 3α-acetoxy-16β-methylpregnane-11,20-dione (4). The β-configuration is assigned to the



C_{16}-methyl group and C-17 side chain in 4 as a consequence of the well established addition of hydrogen and other reactants to the α-side of the methylenepregnane-11,20-dione (ii). Since, as these authors have shown, ii is isomerized to the 16-methyl-16-pregnene (3) under alkaline conditions, the yield of the latter in the pyrolysis step was increased by alkaline treatment of the pyrolysis product before isolation.



(1) (a) A preliminary account of a portion of this work was communicated earlier: D. Taub, R. D. Hoffsommer, H. L. Slates and N. L. Wendler, THIS JOURNAL, **80**, 4435 (1958). See also (b) E. P. Oliveto, R. Rausser, A. L. Nussbaum, W. Gebert, E. B. Hershberg, S. Tolksdorf, M. Eisler, P. L. Perlman and M. M. Pechet, *ibid.*, **80**, 4428 (1958); (c) E. P. Oliveto, R. Rausser, H. L. Herzog, E. B. Hershberg, S. Tolksdorf, M. Eisler, P. L. Perlman and M. M. Pechet, *ibid.*, **80**, 6627 (1958).

(2) For recent discussion and review of structure activity relationships in this field see J. Fried, *Vitamins and Hormones*, **16**, 304 (1958); L. H. Sarett, *Ann. N. Y. Acad. Sci.*, **82**, 802 (1959).

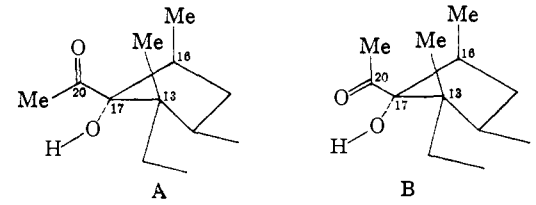
(3) P. L. Julian and W. J. Karpel, U. S. Patent 2,671,794 (1954), H. L. Slates and N. L. Wendler, *J. Org. Chem.*, **22**, 498 (1957).

(4) *Cf.* A. Wettstein, *Helv. Chim. Acta*, **27**, 1803 (1944).

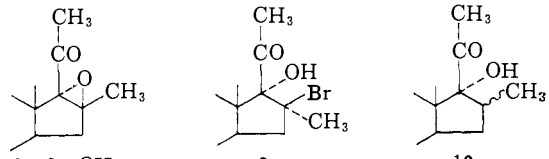
(5) This sequence is described in detail by H. L. Slates and N. L. Wendler, THIS JOURNAL, **81**, 5472 (1959), who also isolated and characterized the isomeric cyclopropane, 3α-acetoxy-16α,17α-methylenepregnane-11,20-dione (i), and exocyclic olefin, 3α-acetoxy-16-

C_{16}–C_{17}-double bond.[6,7,8] The 16β-methyl-11,20-dione 4 was converted into the corresponding $\Delta^{17(20)}$-enol acetate 5 by treatment with acetic anhydride–perchloric acid in chloroform–carbon tetrachloride.[9] The yield of $\Delta^{17(20)}$-enol acetate 5 was about 50% as judged by acetyl group analyses, whereas in the unmethylated pregnane or allopregnane series excellent yields are obtained under the same conditions.[9] The relatively low yield in the present case obviously may be attributed to steric hindrance of the 20-carbonyl function by the 16β-methyl group. It is noteworthy that the attempted formation of a C_{20}-cyanohydrin failed although this is a facile reaction in the parent 16-desmethyl series.[10]

The crude enol acetate 5 on reaction with perbenzoic acid, then alkaline hydrolysis,[11] gave 3α,17α-dihydroxy-16β-methylpregnane-11,20-dione (6), m.p. 192–197°, in small yield by crystallization of the crude product. The 16β-methyl-17α-hydroxy-20-ketone 6 was quite sensitive to Lewis acid-catalyzed D-homo-rearrangement. Thus chromatography of crudes rich in 6 on neutral alumina led in large part to a new compound, m.p. 213–215°, formulated as 3α,17α-dihydroxy-16β,17β-dimethyl-D-homo-5β-androstane-11,17a-dione (7a) by analogy with the Lewis acid-catalyzed rearrangement in the unmethylated parent series.[12] Neither the 16α-methyl analog of 6 nor the parent unmethylated compound was rearranged under these mild conditions. Similarly 6 underwent thermal D-homo-rearrangement to 7a at its melting point. Under these conditions the 16α-methyl analog was stable. The greater tendency toward rearrangement in the 16β-methyl series than in the 16α-methyl or unmethylated series probably is a consequence of the relatively greater oppositional forces in the 16β-methyl series, involving the three adjacent ring D β-groupings, the 13β- and 16β-methyl groups and the 17β-side chain. Models of the compounds under discussion indicate that in the 16β-methyl series the rotational conformation illustrated in A is strongly favored for the 17β-side chain with free rotation about the 17–20-bond restricted because of van der Waals interaction among the 13β, 16β and side chain methyl groups in rotational conformation B. Such restriction appears to be a relatively minor consideration in the 16α-methyl and unmethylated parent series.



Simultaneously with the above investigations we studied the properties and possible utility of the 16β-methyl-16α,17α-oxide. The 16-methyl-16-pregnene (either 3a or 3b) reacted readily with hydrogen peroxide in aqueous methanolic sodium hydroxide[13] to form 16α,17α-oxido-16β-methylpregnane-3α-ol-11,20-dione (8a), which on treatment with acetic anhydride in pyridine gave the 3α-acetate 8b.

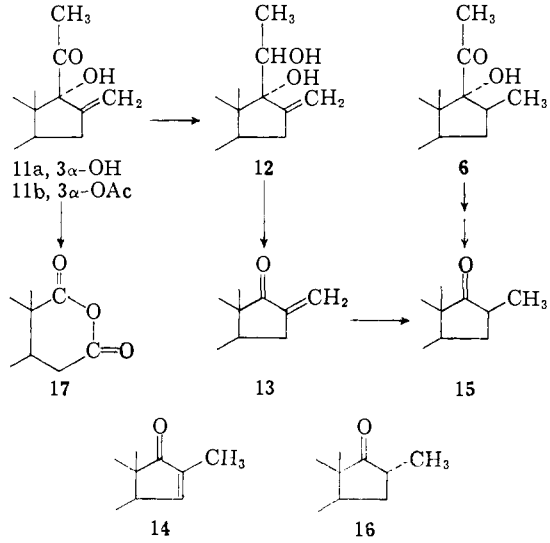


8a, 3α-OH
8b, 3α-OAc

9

10

By analogy with the work of Julian and his colleagues in the unmethylated series,[13] reaction of the oxide-acetate 8b with hydrogen bromide might be expected to yield the *trans*-bromohydrin 9 [$C_{24}H_{35}O_5Br$] which on reductive removal of the bromine would give a 16-methyl-17α-hydroxy ketol 10 in which the configuration of the methyl group could not be predicted with certainty. However, on treatment of 8b with hydrogen bromide in acetic acid two substances, $C_{24}H_{34}O_5$, m.p. 198–200°, and $C_{24}H_{33}O_4Br$, m.p. 138–148° dec.,[14] were obtained. The compound $C_{24}H_{34}O_5$ possessed an olefinic double bond as indicated by reaction with osmium tetroxide. It proved to be



(6) L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948).

(7) T. F. Gallagher and T. H. Kritchevsky, This Journal, **72**, 882 (1950).

(8) The assigned 16β-methyl configuration is consistent with all other lines of evidence which bear on this point: *i.e.*, the degradation of the 17α-hydroxy-20-ketone 6 to the corresponding 16β-methyl-17-ketone 15 which was synthesized by an independent route (see below) and the non-identity of 4 and all subsequent 16β-methyl compounds with the corresponding 16α-epimers synthesized by Arth and his colleagues.[18]

(9) Procedure of D. H. R. Barton, R. M. Evans, J. C. Hamlett, P. G. Jones and T. Walker, *J. Chem. Soc.*, 747 (1954).

(10) L. H. Sarett, This Journal, **70**, 1454 (1948).

(11) Method of T. Kritchevsky and T. F. Gallagher, *ibid.*, **73**, 184 (1951).

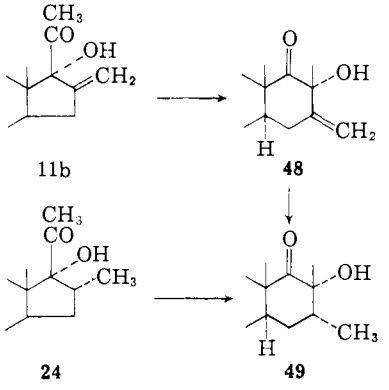
(12) (a) N. L. Wendler, D. Taub, S. Dobriner and D. K. Fukushima, *ibid.*, **78**, 5027 (1956); (b) D. K. Fukushima, S. Dobriner, M. S. Heffler, T. H. Kritchevsky, F. Herling and G. Roberts, *ibid.*, **77**, 6585 (1955).

(13) Method of P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Walker, *ibid.*, **72**, 5145 (1950).

(14) Trace amounts of a dienone λ_{max} 304 were also isolated in larger runs (see below).

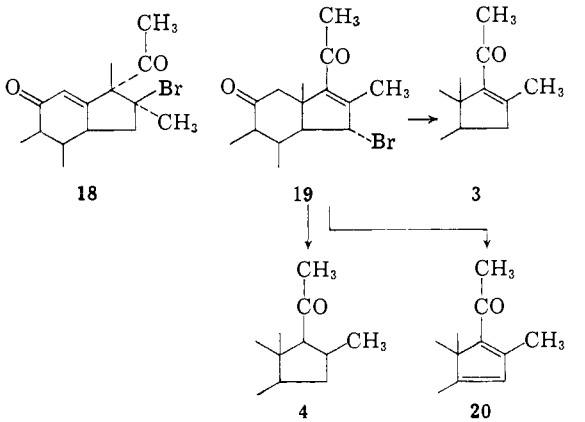
the exocyclic olefin 3α-acetoxy-16-methylenepregnane-17α-ol-11,20-dione (11b) since, on successive treatment with lithium aluminum hydride and sodium periodate, it was converted *via* 12 into 16-methylene-5β-androstane-3α,11β-diol-17-one (13). The 16-methylene-17-ketone part structure in 13 is clearly demonstrated by its spectral properties. It absorbed in the ultraviolet at 227 mμ, ε 8,700, and in the infrared at 5.79 and 6.07 μ.[15] It is noteworthy that the carbonyl band of exocyclic cyclopentenones occurs near 5.79 μ. The isomeric endocyclic cyclopentenone 14 would be expected to absorb near 5.85 μ in the infrared and near 245 mμ in the ultraviolet.[16] The n.m.r. spectrum of 13 is also in accord with its formulation and clearly shows the presence of the exocyclic methylene group as a doublet at 3.73 and 4.51 τ. Finally, an independent synthesis of 13 was achieved by application of the Mannich reaction[15] to 3α,11β-dihydroxy-5β-androstane-17-one.[17]

Catalytic reduction of the 16-methylene-17-ketone 13 led to 16β-methyl-3α,11β-dihydroxy-5β-androstane-17-one (15) identical with a specimen obtained from the 16β-methyl-17α-hydroxy-20-ketone (6) by the lithium aluminum hydride–sodium periodate side-chain degradation sequence.[17] The 16α-methyl-17-ketone 16 was prepared analogously from 3α,17α-dihydroxy-16α-methylpregnane-11,20-dione[18] and clearly differed from 15. In keeping with the exocyclic olefin formulation, potassium permanganate oxidation of 11b and then acetylation led to 3α-acetoxy-11-ketoetiobilianic acid anhydride (17).[12a]



On contact with neutral alumina 11b was converted in part to an isomer formulated as the 16-methylene D-homo compound 48. The structural assignment was confirmed by catalytic hydrogenation of 48 which gave 3α,17α-dihydroxy-16α,17β-dimethyl-D-homo-5β-androstane-11,17a-dione 3α-acetate (49), identical with a sample prepared by Lewis acid-catalyzed D-homoannulation of the 16α-methyl-17α-hydroxy-20-ketopregnane 24. It is of interest that catalytic hydrogenation of the double bond of 48 occurs from the front side of the molecule exclusively. Possibly, interaction with the catalyst from the rear is impeded by the C-14 axial hydrogen or, more probably, ring D in 48 exists primarily in the boat form[19] in which the exocyclic double bond is more accessible from the front side rather than the rear.

The bromine-containing substance was more mobile both on paper and alumina than the unsaturated ketol 11b. This indicated the absence of an hydroxyl group which was confirmed by the infrared spectrum. Its ultraviolet spectrum [λ_{max}^{MeOH} 250 mμ (9,000)] was characteristic of a fully substituted conjugated carbonyl group. Structure 18 formed by opening of the oxide 8b to the intermediate bromohydrin 9 followed by Kägi–Miescher rearrangement[20a,b] of the C-18 methyl group to C-17 may be rejected since the Δ^{12}-11-keto chromophore absorbs near 237 mμ.[20c] The substance was shown to be 15α-bromo-16-methyl-3α-acetoxy-



16-pregnene-11,20-dione (19) by the following evidence. Zinc–acetic acid debromination gave the 16-methyl-16-pregnene 3b and catalytic reduction over palladium produced the 16β-methylpregnane 4. These results fix the structure except for the position of the bromine atom. Since 19 is smoothly dehydrobrominated to the dienone 20, λ_{max}^{MeOH} 304 mμ (10,000),[21] under very mild conditions (pyridine at 25°) and the n.m.r. spectrum of 19 shows the presence of the unsubstituted C-16 and side chain methyl groups, the bromine is placed at C-15, and assigned the 15α-configuration for reasons discussed below.

The 16β-methyl-16α,17α-oxide (8a or 8b) was cleaved readily by perchloric acid in aqueous diox-

(15) (a) *Cf.* P. L. Julian, E. W. Meyer and H. C. Printy, THIS JOURNAL, **70**, 3872 (1948); (b) F. Neumann, O. Mancera, G. Rosenkranz and F. Sondheimer, *ibid.*, **77**, 5076 (1955).

(16) J. Fajkos, *Coll. Czech. Chem. Comm.*, **23**, 1559 (1958), reports 3β-hydroxy-15-androstene-17-one to absorb at 5.86 μ in the infrared and 233 mμ (6900) in the ultraviolet. A 16-methyl group would be expected to shift the ultraviolet maximum to 243–245 mμ.

(17) N. L. Wendler and D. Taub, *Chemistry & Industry*, 415 (1958); N. L. Wendler, D. Taub and R. P. Graber, *Tetrahedron*, **6**, 173 (1960);

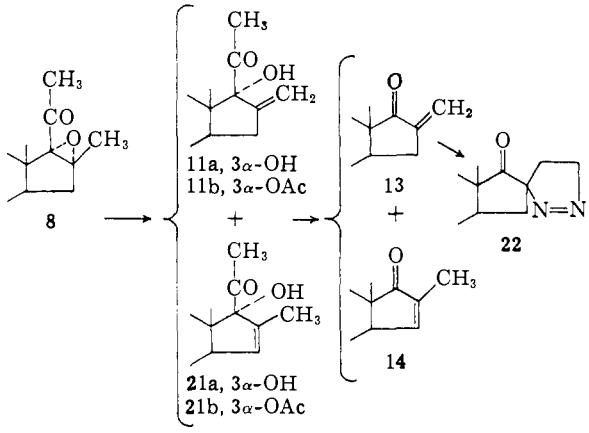
(18) G. E. Arth, D. B. R. Johnston, J. Fried, W. W. Spooncer, D. R. Hoff and L. H. Sarett, *ibid.*, **80**, 3160 (1958).

(19) In this connection see N. L. Wendler, *Chemistry & Industry*, 1662 (1958).

(20) (a) K. Miescher and H. Kägi, *Helv. Chim. Acta*, **32**, 761 (1949); (b) K. Heusler and A. Wettstein, *Chem. Ber.*, **87**, 1301 (1954), observed this type of rearrangement on acid treatment of 16α,17α-oxides unmethylated at C-16. (c) N. L. Wendler, D. Taub and H. L. Slates, THIS JOURNAL, **77**, 3559 (1955).

(21) (a) Pl. A. Plattner, L. Ruzicka, H. Heusser and R. Anliker [*Helv. Chim. Acta*, **30**, 385 (1947)] report for 3α-acetoxy-14,16-allopregnadiene-20-one λ_{max}^{MeOH} 309 mμ, log ε 4.1; (b) F. B. Colton and E. C. Kendall [*J. Biol. Chem.*, **194**, 247 (1952)] report for 3α,21-diacetoxy-12α-bromo-14,16-pregnadiene-11,20-dione λ_{max}^{MeOH} 305 mμ (9000). The 14,16-pregnadiene-20-ones have unusually high specific rotations. Compound 20 has $[\alpha]_D^{Chf}$ +341°; by comparison the 16-pregnene 20-one 3b has $[\alpha]_D^{Chf}$ +75°.

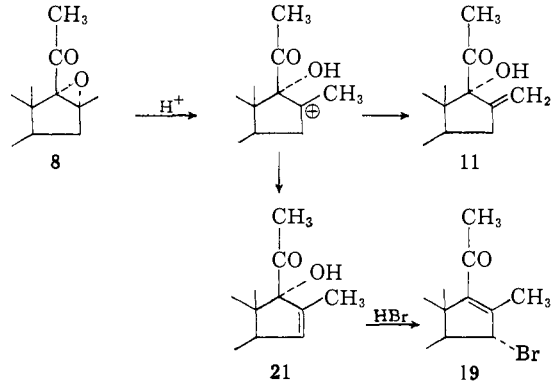
ane to give in excellent yield an unsaturated ketol product, m.p. 158–167° (3α-acetate, m.p. 206–217°) which apparently was different from the exocyclic ketol 11 (3α-ol, m.p. 160–162°; 3α-acetate, m.p. 198–200°). Although there was no melting point depression and the mobilities on paper were identical, significant differences were present in the respective infrared spectra. The perchloric acid ketol was shown to be a mixture of exocyclic (11a) and endocyclic (21a) forms[21c] by



cleavage ($LiAlH_4$ followed by $NaIO_4$) to the corresponding C-17 ketone, the infrared spectrum of which clearly showed the presence of exocyclic (13) and endocyclic (14) cyclopentenones in roughly equivalent amounts. In the double bond stretching region pure 13 absorbed at 5.79 and 6.07 μ (see above) while the present mixture absorbed at 5.79, 5.87 (equal intensity), 6.07 and 6.21 μ. The 5.87 μ band clearly pertains to an endocyclic α,β-unsaturated cyclopentenone carbonyl group.[16] The ultraviolet maximum at 232.5 mμ also is indicative of a mixture; pure exocyclic cyclopentenone 13 absorbed at 227 mμ, whereas the endocyclic structure 14 would be expected to absorb near 245 mμ.[16] It proved possible to resolve the cyclopentenone mixture by reaction with diazomethane. Only the exocyclic form reacted to give a pyrazoline derivative[22] which was separated readily from the unreacted endocyclic cyclopentenone 14 by alumina chromatography. Pure 16-methyl-Δ^{15}-5β-androstene-3α,11β-diol-17-one (14) absorbed at 243 mμ in the ultraviolet and at 5.88 and 6.20 μ in the infrared as expected.

Subsequently the perchloric acid ketol mixture itself was resolved into the two ketols 11a and 21a by partition chromatography and the endocyclic ketol 21a was independently cleaved to the endocyclic cyclopentenone 14.

In connection with the problem of the formation of the 15-bromo-16-methyl-Δ^{16}-20-ketone 19 from the 16α,17α-oxide 8 it is of interest that the exocyclic ketol 3α-acetate 11b is inert to hydrogen bromide–acetic acid under the conditions of formation of 19 and therefore cannot be an intermediate in this reaction. However, the perchloric acid–ketol mixture 3α-acetate (11b + 21b) with HBr–acetic acid was transformed into a mixture of 11b and the unsaturated bromoketone 19. This clearly indicates that the endocyclic ketol 21b is an intermediate in the formation of 19; and finally pure 21b was smoothly converted into 19. The following formulation of the HBr opening of the oxide 8 may now be written

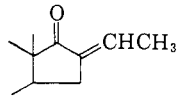


The formation of the exocyclic olefin 11 is uneventful whereas that of 19 is regarded as an allylic displacement of the protonated 17α-hydroxyl group of 21 by bromide ion. Reaction of allylic alcohols with hydrogen halides to form the rearranged allylic halides is of course well known[23a]; the driving force for the present reaction probably resides in the formation from 21 of the more stable conjugated Δ^{16}-20-ketone system of 19.[23b] To the extent that the reaction is concerted [SN2′ or SNi′] the configuration of the 15-bromine should be α,[23a,24] and, moreover, this is the thermodynamically more favorable quasi-equatorial configuration.

Reaction of the oxide 8b with hydrogen chloride in acetic acid led to the exocyclic unsaturated ketol 11b as well as a compound $C_{24}H_{33}O_4Cl$, λ_{max}^{MeOH} 244, ε 9,100, formulated as the 15α-chloro compound 23 (25% ultraviolet yield) by analogy with the HBr reaction. A third product was the dienone 20, λ_{max}^{MeOH} 304 mμ (19% ultraviolet yield). Dehydrochlorination of 23 also led to 20. Reaction of 8b with hydrogen fluoride in tetrahydrofuran gave 11b and a mixture of two substances λ_{max}^{MeOH} 240 and 304 mμ, evidently the 15α-fluoro-Δ^{16}-20-ketone (~15% ultraviolet yield) and the dienone 20 (~25% ultraviolet yield). The endocyclic unsaturated ketol 21 can react therefore

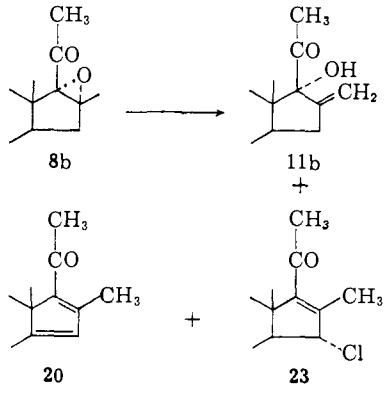
(21c) Note Added in Proof.—Since the submission of this article, G. Nominé, D. Bertin and A. Pierdet [*Tetrahedron*, **8**, 217 (1960)] have described the preparation of the oxide 8b and its behavior with acidic reagents. Treatment of 8b with hydrochloric acid in acetone at 20° was stated to produce the $\Delta^{15,16}$-methylpregnene 21b in 90% yield. However, in our hands the product of this procedure was a mixture of 11b and 21b.

(22) Pyrolysis of this pyrazoline led to an amorphous product, which on the basis of its spectral properties [λ_{max}^{MeOH} 237 mμ, $E_{cm}^{1\%}$, 102; λ_{max}^{Chf} 5.80 μ (strong), 6.05 μ (medium)] consists largely of the 16-ethylidene-17-ketone iii.



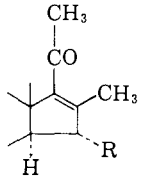
(23) (a) For a recent review see R. H. DeWolfe and W. G. Young, *Chem. Revs.*, **56**, 753 (1956). (b) Compare reaction of ArCHOHCH=CH2 with HX which leads usually to ArCH=CHCH2X [(a), p. 806].

(24) *Cf.* G. Stork and W. N. White, This Journal, **78**, 4609 (1956).



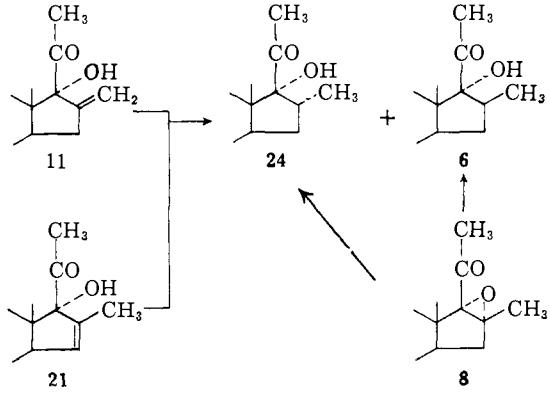
by loss of the C-14α proton (and the 17α-hydroxyl group) to give the dienone 20 as well as by addition of halogen to C-15. It is of interest that as the nucleophilicity of the halogen decreases [$Br^- > Cl^- > F^-$] the yield of 15-halide decreases and the yield of dienone increases.

A further point of interest is the hypsochromic shift in the absorption maximum of the Δ^{16}-20-keto chromophore produced by the 15α (quasi-equatorial) halogen substituent, the effect increasing with the electronegativity of the halogen atom. An analogous effect exists for the similarly



R	λmax, mμ
H	249
Br	249
Cl	244
F	240

γ-substituted 6α-(equatorial) halo-Δ^4-3-ketones,[25] while 6β-halo substitution results in a considerably lessened hypsochromic effect in the case of chlorine and a slight bathochromic effect in the case of bromine. The ultraviolet absorption data are therefore also in conformity with the 15α-halogen formulation. The effects of γ-substitution are in striking contrast with the large bathochromic shifts produced by bromine substitution on the α- and β-carbon of α,β-unsaturated ketones (about +23 and +30 mμ, respectively).[26]

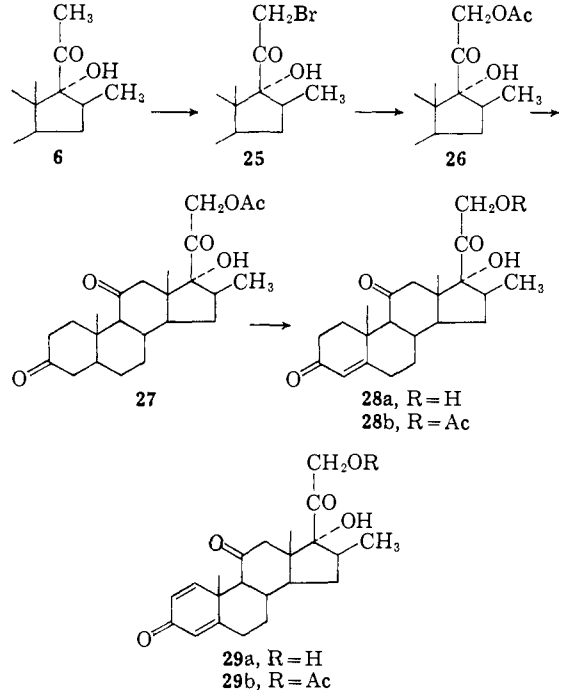


(25) For example see data on 6-halo-Δ^4-cholestenones in L. Dorfman, *Chem. Revs.*, **53**, 47 (1953); see also C. W. Bird, R. C. Cookson and S. H. Dandegaonker, *J. Chem. Soc.*, 3675 (1956).

(26) A. L. Nussbaum, O. Mancera, R. Daniels, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 3263 (1951).

We return now to the main line of the synthesis. Catalytic reduction of the perchloric acid unsaturated ketol product (11 and 21) or of pure 11 over palladium-on-calcium carbonate led to a 7:3 mixture of 16α-methyl-(24) and 16β-methylpregnane-3α,17α-diol-11,20-dione (6). The mixture was resolved by paper chromatography and by column chromatography on Florisil and the composition determined by infrared spectroscopy and by phase solubility analysis. The 16α,17α-oxide 8 could be converted directly into the same 7:3 mixture of 24 and 6 by hydrogenolysis in aqueous perchloric acid–dioxane over palladium-on-charcoal.[27] In contrast with the hydrogenolytic cleavage of the oxide function in 8 this grouping was inert to lithium aluminum hydride[28] since the 3,11,20-triol product of reduction was not cleaved by periodate.

The further transformation of the key intermediate 6 into 16β-methylcortisone (28a) and 16β-methylprednisone (29a) was achieved without undue difficulty. Bromination of 6 in chloroform[29] gave the 21-bromide 25 in good yield, although the rate of bromination was considerably slower than in the parent unmethylated series. Treatment of 25 with potassium iodide and potassium acetate in acetone–acetic acid[30] led to 16β-methylpregnane-3α,17α,21-triol-11,20-dione 21-ace-



(27) The isomer ratio of the 16α-methyl ketol 24 to the 16β-methyl ketol 6 was dependent on the conditions of the hydrogenation and could be varied to produce essentially pure 24 or 6 as desired. This aspect of the problem will be reported elsewhere by our colleagues Drs. R. Chamberlin, E. Tristram and their associates.

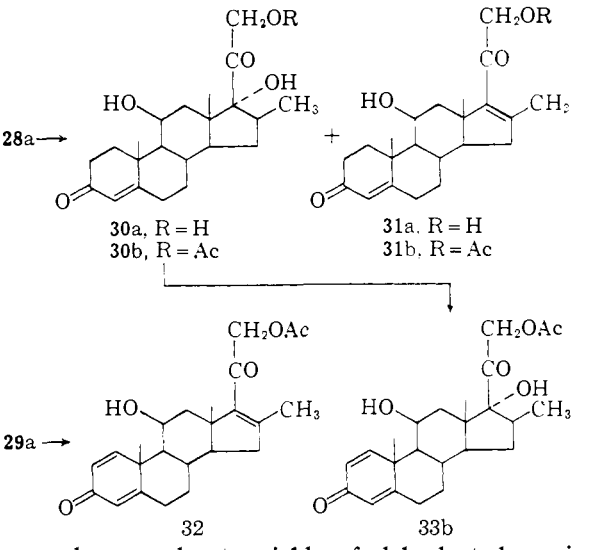
(28) In the 16-unsubstituted series the 16α,17α-oxide function is reduced readily by lithium aluminum hydride to the 17α-hydroxyl group [Pl. A. Plattner, H. Heusser and M. Feurer, *Helv. Chim. Acta*, **31**, 2210 (1948)].

(29) *Cf.* T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **73**, 184 (1951).

(30) *Cf.* G. Rosenkranz, J. Pataki, St. Kaufmann, J. Berlin and C. Djerassi, *ibid.*, **72**, 4081 (1950).

tate (26). It is noteworthy that 26 and all subsequent 16β-methyl compounds possessing the dihydroxyacetone side chain gave a much slower and weaker test with blue tetrazolium reagent than either the 16α-methyl- or 16-unsubstituted analogs. Oxidation of the 3α-hydroxyl group of 26 occurred readily with a variety of oxidants such as N-bromosuccinimide, sodium dichromate-in-acetic acid or chromium trioxide-in-acetic acid leading to the 16β-methyl-3,11,20-trione 27. Monobromination of this compound followed by dehydrobromination by the lithium chloride[31] or semicarbazone[32] procedures led to 16β-methylcortisone acetate (28b). Dibromination of 27 followed by dehydrobromination in dimethylformamide–dimethylaniline[32a] gave 16β-methylprednisone acetate (29b). The corresponding 21-alcohols 28a and 29a were obtained from the 21-acetates 28b and 29b by treatment with either potassium bicarbonate–aqueous methanol or sodium methoxide–methanol.

Application of the disemicarbazone sequence[33] for the reduction of 16β-methylcortisone (28a) led to 16β-methylhydrocortisone (30a) only in small yield. The principal product was 16-methyl-4,16-pregnadiene-11β,21-diol-3,20-dione (31a) isolated as the 21-acetate 31b, λ_{max}^{MeOH} 245 mμ (22,200), which was formed during the standard room temperature pyruvic acid–aqueous acetic acid reversal of the reduced 3,20-disemicarbazone. In the 16β-methylprednisone series the analogous 1,4,16-pregnatriene 32 was the sole isolable product. Since the 16β-methyl-17α-hydroxy-20-keto steroids are stable to the reversal conditions, the species which loses the 17α-hydroxyl group must be the reduced semicarbazone.[34] The lability of the 17α-hydroxyl group in the 16β-methyl series is striking; the *trans* relationship of the 17α-hydroxyl and 16β-methyl groups precludes conventional *trans* elimination of water and supports the mechanism indicated in footnote 34. In the parent 16-unmethylated series refluxing acetic acid is required



to produce moderate yields of dehydrated semicarbazone.[34] The driving force for the dehydration in the 16β-methyl series probably involves the decrease in steric oppositional forces among the three ring D β-substituents on planarization of the 16-methyl and 17-side-chain groups.

16β-Methylprednisolone (33a), therefore, was not obtained initially from the readily available 16β-methylprednisone (29a). The initial samples of 33 were prepared by selenium dioxide dehydrogenation[35] of 16β-methylhydrocortisone acetate (30b).

Practical methods for reducing the 11-carbonyl group in the 16β-methyl series were developed utilizing the 17,20:20,21-bismethylenedioxy (BMD) derivatives[36] to protect the side chain. 16β-Methylcortisone (28a) was converted readily into the corresponding BMD derivative 34 which was reduced at 3 and 11 by lithium aluminum hydride in tetrahydrofuran to yield the 3,11β-diol 35 together with a minor amount of a more polar by-product—presumably the 11α-hydroxy epimer. Sodium borohydride reduction of 34 in refluxing aqueous tetrahydrofuran was incomplete at C-11 after 3 hours in contrast with the 16α-methyl or the parent unmethylated series in which reduction of the C-11 carbonyl group is complete within one hour. The additional hindrance at C-11 in the 16β-methyl series may reasonably be interpreted to be a result of C-13-methyl–C-16β-methyl opposition with concomitant deformation of the C-13 methyl group toward the β-face of ring C. This would act to increase the magnitude of the 1:3 interaction between the C-13 methyl and the developing C-11β substituent, thereby increasing the activation energy for the hydride reduction reaction.[37]

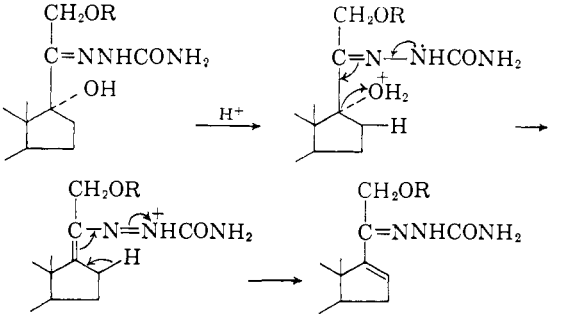
(31) *Cf.* R. P. Holyz, This Journal, **75**, 4432 (1953).

(32) *Cf.* W. F. McGuckin and E. C. Kendall, *ibid.*, **74**, 5811 (1952).

(32a) *Cf.* J. Day, R. Erickson and R. Pettebone, U. S. Patent 2,873,284 (1959).

(33) *Cf.* (a) N. L. Wendler, Huang-Minlon and M. Tishler, *ibid.*, **73**, 3818 (1951); (b) R. E. Jones and S. A. Robinson, *J. Org. Chem.*, **21**, 586 (1956). Unlike the unmethylated parent series it was necessary to utilize the 21-alcohol 28a rather than the 21-acetate 28b in order to get good yields of 3,20-disemicarbazone.

(34) *Cf.* H. L. Slates and N. L. Wendler, *J. Org. Chem.*, **22**, 498 (1957). The elimination reaction is depicted as

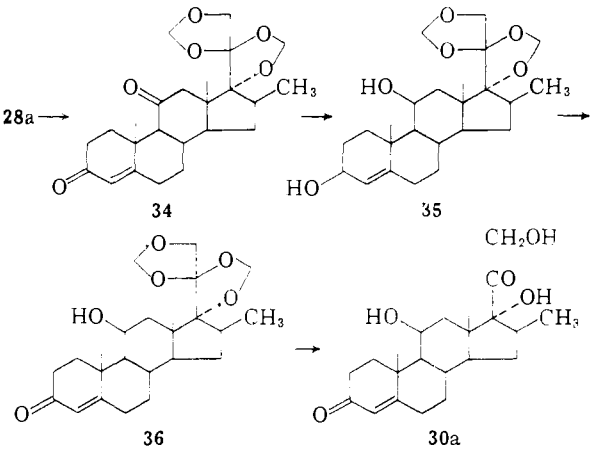


Compare V. R. Mattox and E. C. Kendall, This Journal, **72**, 2290 (1950).

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

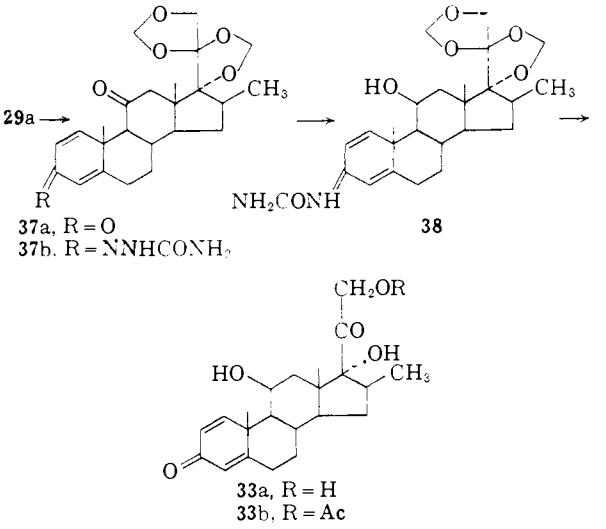
(36) The use of this derivative to protect the steroid dihydroxyacetone side chain was first described by R. E. Beyler, R. M. Moriarty, F. Hoffman and L. H. Sarett, This Journal, **80**, 1517 (1958).

(37) The effect of the 16β-methyl group on the rate of reduction of the 11-carbonyl group appears to be a striking example of axial buttressing; see D. H. R. Barton, *Experientia*, Suppl. II, 121 (1955); D. H. R. Barton, A. J. Head and P. J. May, *J. Chem. Soc.*, 935 (1957); D. H. R. Barton, F. McCapra, P. J. May and F. Thudium, *ibid.*, 1297 (1960).



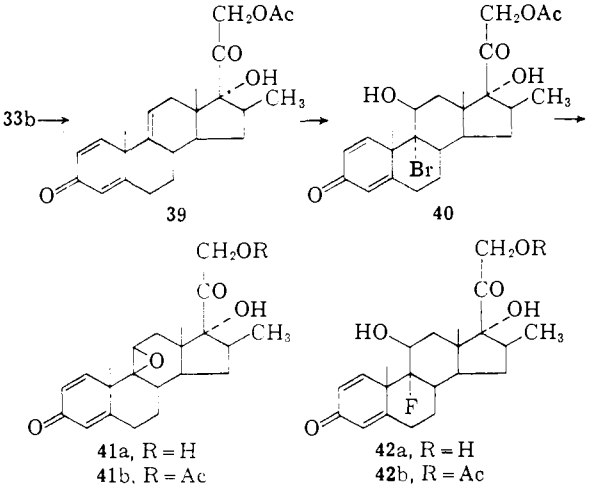
The lithium aluminum hydride product 35 was oxidized smoothly to the Δ^4-3-ketone 36 by manganese dioxide in acetone–benzene.[38] However, the back-oxidized sodium borohydride reduction product had a considerably lower extinction at 242 mμ than the back-oxidized lithium aluminum hydride product indicative of partial saturation of the Δ^4-double bond in the case of the former reducing agent.[39] Removal of the BMD protecting group of 36 by hot 50% aqueous acetic acid led to 16β-methylhydrocortisone (30a) in reasonably good over-all yield from 16β-methylcortisone (28a).

In the 1,4-dienone series manganese dioxide oxidation of the intermediate 1,4-diene-3-ol is a poor process and recourse was made to the BMD-3-semicarbazone (37b) prepared in good yield by conversion of 16β-methylprednisone (29a) into its BMD derivative 37a and thence into the BMD-3-semicarbazone 37b.[40] The latter substance was reduced at C-11 by prolonged treatment with sodium borohydride[41] in aqueous tetrahydrofuran to yield the 11β-hydroxy-BMD-3-semicarbazone 38. Both protecting groups were removed by hot 50% aqueous acetic acid to yield 16β-methylprednisolone (33a) isolated as the 21-acetate 33b (35–40% yield from 16β-methylprednisone). It is noteworthy that the 1,4-dienone



3-semicarbazone group in this and other cases is hydrolyzed under these mild conditions in good yield. Previously described hydrolytic procedures for this group are unsatisfactory[42] except for the method of Day.[43]

There remained the task of preparing the 9α-fluoro analogs, 16β-methyl-9α-fluoroprednisolone (42a) and 16β-methyl-9α-fluorohydrocortisone (46a), which was accomplished by essentially standard procedures. 16β-Methylprednisolone 21-acetate (33b) on treatment with methylchloro-



sulfite in dimethylformamide–pyridine[44] was converted into 17α,21-dihydroxy-16β-methyl-1,4,9(11)-pregnatriene-3,20-dione 21-acetate (39).[45] Reaction of 39 with N-bromosuccinimide in aqueous perchloric acid–acetone[46] led to 16β-methyl-9α-

(38) Procedure of F. Sondheimer, C. Amendola and G. Rosenkranz, THIS JOURNAL, **75**, 5930 (1953).

(39) Reduction of Δ^1- and Δ^4-bonds during the sodium borohydride reduction of the corresponding unsaturated 3-ketones was first reported by F. Sondheimer, M. Velasco, E. Batres and G. Rosenkranz, *Chemistry & Industry*, 1482 (1954). A possible alternative to direct reduction of the olefinic double bond by sodium borohydride involves migration of the double bond in the reduced Δ^4-3-ol toward C-3 in the alkaline reaction medium to give a saturated 3-ketone which is then reduced further.

(40) Compound 37b and other 1,4-diene-3-one monosemicarbazones were observed to have *two* maxima in the ultraviolet in methanol. Compound 37b had $\lambda\lambda_{max}^{MeOH}$ 294 mμ (23,400), 244 mμ (11,600). The presence of the 244 mμ band does not seem to have received general notice. It is recorded without comment, for example, in the spectra of the semicarbazones of 17α-acetoxy-1,4-androstadiene-3-one [H. H. Inhoffen G. G. Zuhlsdorf and H. Minlon, *Chem. Ber.*, **73B**, 454 (1940)], 1,4-cholestadiene-3-one, methyl 3-keto-1,4-etiocholadienate [H. H. Inhoffen and G. Stoek, *Ann.*, **563**, 128 (1949)], and 16α,17α-isopropylidenedioxy-1,4,9(11)-pregnatriene-3,20-dione [G. R. Allen, Jr., and M. J. Weiss, THIS JOURNAL, **81**, 4968 (1959)]. The band is not mentioned in Dorfman's survey of steroid ultraviolet spectra [L. Dorfman, *Chem. Revs.*, **53**, 85 (1953)].

(41) Lithium aluminum hydride could not be used because of concomitant reduction of the C=N bond.

(42) *Cf.* H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto and E. B. Hershberg, THIS JOURNAL, **77**, 4781 (1955).

(43) J. Day, Belgian Patent 568,694 (1958).

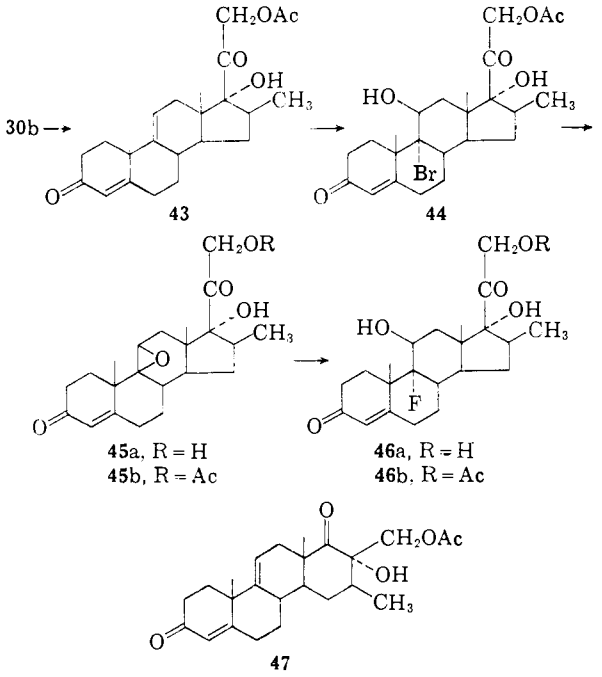
(44) Procedure of E. M. Chamberlin, E. W. Tristram, T. Utne and J. M. Chemerda, *J. Org. Chem.*, **25**, 295 (1960).

(45) There were also obtained two by-products: the tetraene, 21-hydroxy-16β-methyl-1,4,9(11),16-pregnatetraene-3,20-dione 21-acetate, and the 11β-methyl sulfite ester of 33b (see Rxperimental).

(46) *Cf.* (a) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953); **79**, 1130 (1957); (b) L. B. Barkley, M. W. Farrar, W. S. Knowles and H. Raffelson, *ibid.*, **76**, 5017 (1954).

bromoprednisolone 21-acetate (40) which, on successive treatment with methanolic sodium methoxide and acetic anhydride–pyridine, was converted into the 9β,11β-oxide 41b. Hydrofluorination of the latter in tetrahydrofuran–chloroform[46a,47] led to 16β-methyl-9α-fluoroprednisolone 21-acetate (42b) which with sodium methoxide in methanol gave 16β-methyl-9α-fluoroprednisolone (42a).

A similar series of reactions starting with 16β-methylhydrocortisone 21-acetate (30b) proceeded through the intermediates 43, 44 and 45 to 16β-methyl-9α-fluorohydrocortisone 21-acetate (46b) and the free alcohol 46a. A point of interest is the partial conversion of the 4,9(11)-diene 43 to the D-homo compound 47 on alumina chromatography, which did not occur in the 1,4-dienone series (*i.e.*, 39).



The activity of the various 16β-methyl cortical steroids (hydrocortisone = 1) in rats is summarized in the table.[48] None of the compounds exhibited sodium retention in adrenalectomized rats.

Compound, 16β-methyl-	Liver glycogen	Systematic granuloma
Cortisone (28a)	0.4	2
Hydrocortisone (30a)	0.6	4
Prednisone (29a)	1	26
Prednisolone (33a)	1.5	23
9α-Fluorohydrocortisone (46a)	8.5	23
9α-Fluoroprednisolone (42a)	11	70

Medical data in man indicate 16β-methylprednisone, 16β-methylprednisolone and 16β-methyl-9α-fluoroprednisolone to have similar metabolic properties to their 16α-methyl counterparts and to be equipotent anti-inflammatory agents.[49]

Experimental[50]

3α-Acetoxy-16α,17α-methyleneazopregnane-11,20-dione (2).—Diazomethane was generated by adding an ether solution of N-methyl-N-nitroso-*p*-toluenesulfonamide to a warm solution of potassium hydroxide in aqueous methanol–ether[51] and swept by means of nitrogen into a solution of 20 g. of 3α-hydroxy-16-pregnene-11,20-dione 3-acetate (1) in 100 ml. of tetrahydrofuran and 120 ml. of ether. Intermittent addition of diazomethane was continued until the steroid solution remained yellow for 16 hours. The pyrazoline 2 largely precipitated from solution and was collected by filtration, washed with ether and dried in air; 14.24 g., m.p. 186–190° dec. On concentration of the filtrate 5.77 g. (total yield 90%) of additional pyrazoline, m.p. 180–190° dec., was obtained.

Anal. Calcd. for $C_{24}H_{34}O_4N_2$: C, 69.55; H, 8.27. Found: C, 69.37; H, 8.01.

3α-Acetoxy-16-methyl-16-pregnene-11,20-dione (3b).—Pyrazoline 2 (37.4 g.) was placed in a 500-ml. round-bottom flask and heated by an oil-bath *in vacuo* (pressure 0.6 mm.). A manometer and 12-l. surge flask were in the line between the reaction flask and pump trap. When the bath temperature reached 180° the pyrazoline began to melt with evolution of nitrogen. The maximum pressure reached was 83 mm. After 10 minutes at 180–182° the melt was cooled. It had $\lambda_{max}^{CH_3OH}$ 249, $E_{cm}^{1\%}$, 191, and was taken up in ~150 ml. of acetone, filtered through Celite, concentrated to ~100 ml., and ether slowly added to the boiling solution until crystallization occurred; yield in two crops 24.3 g. (70%), λ_{max}^{MeOH} 249 mμ, $E_{cm}^{1\%}$ >210. The analytical sample had m.p. 165–167°, $[\alpha]^{chf}D$ +75°; λ_{max}^{MeOH} 249 mμ (9,300); λ_{max}^{chf} 5.78, 5.85, 6.03, 6.20 μ.

Anal. Calcd. for $C_{24}H_{34}O_4$: C, 74.57; H, 8.87. Found: C, 74.30; H, 8.60.

The mother liquors contained additional 3b as well as the corresponding isomeric cyclopropane and exocyclic olefin.[5] The latter was transformed by treatment with methanolic potassium hydroxide to **3α-hydroxy-16-methyl-16-pregnene-11,20-dione (3a)**, m.p. 225–228°; λ_{max}^{MeOH} 248 mμ (9,300), also obtained similarly from 3b.[5]

Anal. Calcd. for $C_{22}H_{32}O_3$: C, 76.68; H, 9.34. Found: C, 76.80; H, 9.21.

3α-Acetoxy-16β-methylpregnane-11,20-dione (4).—A solution of 2.00 g. of 3α-acetoxy-16-methyl-16-pregnene-11,-20-dione (3b) in 100 ml. of methanol was reduced at atmospheric pressure and 25° over 1.00 g. of 25% Pd-on-$CaCO_3$ catalyst. Hydrogen uptake ceased in 2 hours; the catalyst was filtered and the filtrate taken to dryness. The residue was crystallized from ether–petroleum ether to yield 1.73 g. (87%) of the 16β-methylpregnane (4) in 2 crops. The analytical sample had m.p. 160–163°, $[\alpha]^{chf}D$ +96°; λ_{max}^{chf} 5.80, 5.86 μ.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.33. Found: C, 74.15; H, 9.15.

Compound 4 was recovered unchanged on attempted cyanohydrin formation with hydrogen cyanide and triethylamine.

3α,17α-Dihydroxy-16β-methylpregnane-11,20-dione (6) from 4 by the Peracid Procedure.—To a stirred solution of 1.63 g. of 3α-acetoxy-16β-methylpregnane-11,20-dione (4) in 5 ml. of chloroform and 25 ml. of carbon tetrachloride at 0° was added dropwise a cold mixture of 0.13 ml. of 60% perchloric acid and 2.60 ml. of acetic anhydride. The cooling bath was withdrawn and the mixture stirred for 2 hours at room temperature and kept overnight at 0°. The reaction

(47) *Cf.* R. F. Hirschmann, R. Miller, J. Wood and R. E. Jones, This Journal, **78**, 4956 (1956).

(48) Private communications from Drs. R. H. Silber, S. L. Steelman and H. C. Stoerk, Merck Institute for Therapeutic Research. References to the assay procedures and comparative data for the corresponding 16α-methyl compounds are given by G. E. Arth, J. Fried, D. B. Johnston, D. R. Hoff, L. H. Sarett, R. H. Silber, H. C. Stoerk and C. A. Winter, *ibid.*, **80**, 3161 (1958).

(49) Private communication from Dr. E. Alpert, Medical Division, Merck & Co., Inc. Similar findings for 9α-fluoroprednisolone acetate (42b) are reported by Oliveto, *et al.*[10]

(50) 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 formamide systems of A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950). We are indebted to Dr. N. L. Trenner and his associates, R. W. Walker and B. Arison, for the infrared and nuclear magnetic resonance spectra, respectively; to A. Kalowsky for the ultraviolet spectra; and to Dr. R. N. Boos and associates for the elemental analyses.

(51) T. J. DeBoer and H. J. Backer, *Rec. trav. chim.*, **73**, 229 (1954).

mixture was washed with cold 5% sodium carbonate solution, water, and dried over sodium sulfate. The amorphous residue (1.86 g.) contained about 50% of the 17(20)-enol acetate 5 as indicated by acetyl group analysis. It was dissolved in 3 ml. of benzene and 25 ml. of 0.2 *M* perbenzoic acid in benzene was added. After 20 hours at room temperature (at which time 0.95 molar equivalent of perbenzoic acid had been consumed) additional benzene was added and the mixture extracted with 5% sodium carbonate solution, water and dried over sodium sulfate. The residue (1.68 g. of crude 17(20)-oxide) after removal of solvent was dissolved in 70 ml. of ethanol and 1.20 g. of sodium hydroxide in 35 ml. of water was added. The mixture was stirred at room temperature for 2 hours, 4 ml. of acetic acid was added and the ethanol removed *in vacuo*. Saturated sodium chloride solution was added and the mixture was extracted with chloroform. The chloroform extract was washed with aqueous potassium bicarbonate, saturated sodium chloride solution and dried over magnesium sulfate. Removal of the solvent left an amorphous residue (1.26 g.) which on crystallization from ether and repeated crystallization from benzene–ethyl acetate gave 225 mg. of **3α,17α-dihydroxy-16β-methylpregnane-11,20-dione** (6), m.p. 192–197°, $[\alpha]^{chf}D$ + 67°; $[\lambda]^{chf}_{max}$ 2.75, 2.90, 5.85 μ.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 72.97; H, 9.25.

The 16β-methylpregnane 6 was slightly more polar than its 16α-methyl analog[18] on paper (benzene–formamide system) and gave a noticeably weaker Zimmerman test.[52] Chromatography of the mother liquors on neutral alumina gave from the 50% benzene–chloroform eluates small additional quantities of 6. The 100% chloroform eluates gave a new substance, **3α,17α-dihydroxy-16β,17β-dimethyl-D-homo-5β-androstane-11,17a-dione** (7a), m.p. 213–215°, $[\alpha]^{chf}D$ +35°; λ^{chf}_{max} 2.87, 5.86 μ–very different from 6 in the fingerprint region.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 72.72; H, 9.40.

The D-homo compound 7a was converted into the corresponding 3-monoacetate 7b, m.p. 202–207°, $[\alpha]^{chf}_D$ + 66°, by treatment with acetic anhydride and pyridine at room temperature for 16 hours.

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97. Found: C, 71.13; H, 9.21.

Thermal D-Homoannulation of the 16β-Methyl Ketol 6.—The 16β-methyl ketol 6 (100 mg.) was heated to 200° for 5 minutes, cooled and crystallized from ether to give 50 mg. of the D-homo compound 7a, m.p. 200–210°, of identical infrared spectrum and mobility on paper as authentic material.

16α,17α,Oxido-16β-methylpregnane-3α-ol-11,20-dione (8a).—The 16-methyl-16-pregnene (3b, 20.00 g.) was dissolved in 600 ml. of methanol, the solution was cooled to 18°, and 80 ml. of 30% hydrogen peroxide followed by 80 ml. of 2.5 *N* sodium hydroxide were added. Considerable material precipitated from solution, but all re-dissolved on stirring the reaction mixture at 25–30° for 40 minutes. The solution was kept at 15–20° for 18 hours at which time the ultraviolet maximum at 249 had disappeared completely. Saturated salt water (600 ml.) was added slowly, the crystalline precipitate was filtered, washed with water and dried in air and in vacuum; yield 17.36 g. (93%), m.p. 176–177°; hexagonal prisms, m.p. 178–180°, from acetone–ether; λ^{chf}_{max} 2.9–3.0, 5.86 μ.

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.23; H, 9.12.

Room temperature acetylation in acetic anhydride–pyridine gave **3α-acetoxy-16α,17α-oxido-16β-methylpregnane-11,20-dione** (8b), m.p. 162–164°, $[\alpha]^{chf}D$ +115°; λ^{chf}_{max} 5.80, 5.85, 7.99 μ.

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.65; H, 8.51. Found: C, 71.65; H, 8.25.

3α-Acetoxy-16-methylenepregnane-17α-ol-11,20-dione (11b) **and 3α-Acetoxy-15α-bromo-16-methyl-16-pregnene-11,20-dione** (19).—To a stirred solution of 5.05 g. of 3α-acetoxy-16α,17α-oxido-16β-methylpregnane-11,20-dione (8b) in 75 ml. of acetic acid maintained at 10–15° was added 25 ml. of cold 15% hydrogen bromide in acetic acid. After 35 minutes the mixture was concentrated to dryness *in vacuo* (t = 15°) and the residue chromatographed on 200 g. of neutral alumina. From the 60:40 petroleum ether–benzene eluates (2.10 g.) there was obtained 3α-acetoxy-15α-bromo-16-methyl-16-pregnene-11,20-dione (19); rectangular prisms from ether–acetone, m.p. 138–148° dec., $[\alpha]^{chf}D$ +43°; λ^{MeOH}_{max} 250 mμ (9,000); λ^{chf}_{max} 5.80, 5.84, 6.00, 6.20, 7.99 μ.

Anal. Calcd. for $C_{24}H_{33}O_4Br$: C, 61.93; H, 7.14; Br, 17.17. Found: C, 61.83; H, 6.99; Br, 16.89.

From the 50:50 petroleum ether–benzene to 100% benzene eluates (1.60 g.) there was obtained 3α-acetoxy-16-methylene-pregnane-17α-ol-11,20-dione (11b); prisms from ether–acetone, m.p. 198–200°, $[\alpha]^{chf}D$ −5.2°; λ^{chf}_{max} 2.92 (broad), 5.80, 5.85, 6.05, 7.98 μ.

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.51; H, 8.07.

Methanolic potassium hydroxide hydrolysis (30 minutes at 25°) of 11b gave the corresponding 3-alcohol, **16-methylenepregnane-3α,17α-diol-11,20-dione** (11a), m.p. 157–159°; λ^{chf}_{max} 2.71, 2.85, 5.85, 6.03 μ.

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.29; H, 8.94. Found: C, 72.76; H, 8.80.

16-Methylene-5β-androstane-3α,11β-diol-17-one (13).—A solution of 200 mg. of 3α-acetoxy-16-methylenepregnane-17α-ol-11,20-dione (11b) in 20 ml. of dry tetrahydrofuran was added to a stirred mixture of 200 mg. of lithium aluminum hydride in 20 ml. of tetrahydrofuran. The reaction mixture was refluxed 75 minutes, cooled and worked up[53] by cautious addition of 5 ml. of ethyl acetate followed by 10 ml. of saturated aqueous sodium sulfate and 10 g. of anhydrous magnesium sulfate. The inorganic salts were filtered, washed with ethyl acetate and the solvent removed *in vacuo*. The residue [principally 16-methylenepregnane-3α,11β-17α,20β-tetrol; λ^{chf}_{max} 3.0 μ strong (OH), 6.09 μ weak (C=CH_2)] was dissolved in 20 ml. of methanol and 250 mg. of sodium metaperiodate in 9 ml. of water was added. After 18 hours at 25° the precipitated sodium iodate was filtered, washed with ethyl acetate and the filtrate concentrated to a small volume. Water was added and the mixture extracted with ethyl acetate. The latter extract was dried over magnesium sulfate and the solvent removed *in vacuo*. Crystallization of the residue from acetone–ether yielded hexagonal plates of 16-methylene-5β-androstane-3α,11β-diol-17-one (13), m.p. 163–166°,; λ^{MeOH}_{max} 227 mμ (8,700); λ^{chf}_{max} 2.75, 2.82–2.92, 5.79 strong, 6.07 strong μ.

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.44; H, 9.50. Found: C, 75.68; H, 9.50.

The 16-methylene-17-one 13 was synthesized independently by application of the Mannich reaction to 3α,11β-dihydroxy-5β-androstane-17-one 17 as follows: a solution of 5.00 g. of 3α-11β-dihydroxy-5β-androstane-17-one (prepared from 3α,17α-dihydroxypregnane-11,20-dione by the $LiAlH_4$–$NaIO_4$ sequence), 2.5 g. of paraformaldehyde, 9.0 g. of dimethylamine hydrochloride in 40 ml. of isoamyl alcohol was treated by the procedures of reference 15 to give 1.1 g. of crude neutral material. Chromatography of the latter on 50 g. of neutral alumina and recrystallization of the crystalline residues of the chloroform–1% ethyl acetate–chloroform eluates from acetone–ether gave the 16-methylene-17-one 13 identical with the material prepared above by mixed m.p., ultraviolet and infrared criteria.

16β-Methyl-5β-androstane-3α,11β-diol-17-one (15).—A solution of 55 mg. of 16 methylene-5β-androstane-3α,-11β-diol-17-one (13) in 10 ml. of ethanol was hydrogenated at atmospheric pressure over 20 mg. of 5% palladium-on-charcoal catalyst. Hydrogen uptake was complete within 10 minutes. The catalyst was removed by filtration and the filtrate was concentrated to dryness. Two crystallizations of the residue from ether–acetone yielded the 16β-methyl-17-ketone 15, m.p. 183–185°; λ^{chf}_{max} 2.74, 2.86–2.91, 5.76 μ, identical by mixed m.p. and infrared comparisons with an authentic sample[17] prepared from 16β-methylpregnane-3α,-17α-diol-11,20-dione (6) by the lithium aluminum hydride–sodium periodate sequence.

16α-Methyl-5β-androstane-3α,11β-diol-17-one (16) was prepared from 16α-methylpregnane-3α,17α-diol-11,20-dione[18] by successive treatment with lithium aluminum hydride and sodium periodate as described above; prismatic needles from ether, m.p. 180–183°; λ^{chf}_{max} 2.78, 2.92 broad, 5.78 μ.

(52) Procedure of K. Savard, *J. Biol. Chem.*, **202**, 457 (1953).

(53) Procedure of R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, THIS JOURNAL, **74**, 4223 (1952).

Anal. Calcd. for $C_{20}H_{32}O_3$: C, 74.95; H, 10.07. Found: C, 75.01; H, 9.93.

3α-Acetoxy-11-ketoetiobilianic Acid Anhydride (17).—A solution of 150 mg. of 3α-acetoxy-16-methylenepregnane-17α-ol-11,20-dione (11b) in 10 ml. of acetone was treated with 300 mg. of potassium permanganate in 30 ml. of acetone as described in reference 12a. The acidic fraction of the product was acetylated (acetic anhydride–pyridine 25° over-night) to give the anhydride 17, m.p. 205–211°, undepressed with an authentic sample of m.p. 206–213°.[12a]

Treatment of the 16β-Methyl-16α,17α-oxide 8b with Lithium Aluminum Hydride.—Reduction of the 16β-methyl-16α,17α-oxide 8b with lithium aluminum hydride in refluxing tetrahydrofuran gave a polar compound, m.p. 216–220°; λ_{max}^{Nujol} 2.90, 3.00–3.05 μ (OH)—no carbonyl absorption. Since this substance was recovered unchanged on attempted cleavage with sodium periodate, it is concluded that the oxide function was not reduced and the lithium aluminum hydride product is formulated as 3α,11β,20-trihydroxy-16α,-17α-oxido-16β-methylpregnane.

Catalytic Hydrogenation of 3α-Acetoxy-15α-bromo-16-methyl-16-pregnene-11,20-dione (19).—A solution of 200 mg. of 19 in 20 ml. of methanol was reduced at atmospheric pressure over 300 mg. of 25% Pd-on-$CaCO_3$ catalyst. When hydrogen uptake ceased the catalyst was removed by filtration and the filtrate concentrated to dryness. Crystallization of the residue from ether-petroleum ether gave 150 mg. of 3α-acetoxy-16β-methylpregnane-11,20-dione (4), m.p. 160–162°, identical with authentic material (see above) by mixed m.p. and infrared criteria.

Zinc Debromination of 3α-Acetoxy-15α-bromo-16-methyl-16-pregnene-11,20-dione (19).—To a stirred solution of 100 mg. of 19 in 5 ml. of acetic acid was added 50 mg. of zinc dust. After 10 minutes another 50 mg. of zinc dust was put in and after 30 minutes more still another 50 mg. of zinc dust was added. The mixture was stirred another 45 minutes, the zinc was removed by filtration and washed with a little acetic acid. Addition of water to the filtrate precipitated 3α-acetoxy-16-methyl-16-pregnene-11,20-dione (3), m.p. 157–162°, identical with authentic material (see above) by mixed m.p. and infrared comparisons.

3α-Acetoxy-16-methyl-14,16-pregnadiene-11,20-dione (20).—A solution of 200 mg. of 3α-acetoxy-15α-bromo-16-methyl-16-pregnene-11,20-dione (19) in 4 ml. of pyridine was kept 18 hours at 25°. The yellow solution then was concentrated to dryness (bath temperature 25°), water was added and the mixture was extracted with chloroform. The chloroform extract was washed with dilute nitric acid, dilute potassium bicarbonate solution and water. It was dried over magnesium sulfate and concentrated to dryness. The residue was crystallized twice from acetone–ether to give the 16-methyl-14,16-pregnadiene (20), m.p. 187–189°, $[\alpha]^{chf}D$ +341°, λ_{max}^{MeOH} 304 mμ (10,000).

Anal. Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found: C, 74.99; H, 8.35.

Reaction of 3α-Acetoxy-16α,17α-oxido-16β-methylpregnane-11,20-dione (8b) with Hydrogen Chloride in Acetic Acid.—To a solution of 5.00 g. of the oxide 8b in 25 ml. of acetic acid was added 25 ml. of 5% hydrogen chloride in acetic acid. After 4 hours at 25° the solution was concentrated to dryness *in vacuo* and flushed several times with benzene. The residue had λ_{max}^{MeOH} 244 mμ, $E_{cm}^{1\%}$ 56; 304 mμ, $E_{cm}^{1\%}$ 52.

Chromatography of the residue on neutral alumina gave the following results: From the 50–65% benzene–petroleum ether eluates were obtained mixtures of 3α-acetoxy-15α-chloro-16-methyl-16-pregnene-11,20-dione (23) and 3α-acetoxy-16-methyl-14,16-pregnadiene-11,20-dione (20) (1.42 g.) from which it was possible to separate 23 by fractional crystallization from acetone–ether; m.p. 180–188°, $[\alpha]^{chf}D$ +128°, λ_{max}^{MeOH} 245 mμ (9,100); λ_{max}^{chf} 5.80sh, 5.85, 6.00, 6.20, 8.1 μ. The n.m.r. spectrum of 23 showed the presence of the intact C-16 and side chain methyl groups.

Anal. Calcd. for $C_{24}H_{33}O_4Cl$: C, 68.47; H, 7.90; Cl, 8.42. Found: C, 68.27; H, 8.04; Cl, 8.58.

Although the 16-methyl-14,16-dienone 20 was not isolated, its presence in the crystallization mother liquors was demonstrated by characteristic ultraviolet absorption at 304 mμ and paper chromatographic mobility.

From the 75% benzene–petroleum ether to 50% chloroform–benzene eluates (2.31 g.) there was obtained 1.23 g. of 3α-acetoxy-16-methylenepregnane-17α-ol-11,20-dione (11b), m.p. 195–200°, identical with an authentic sample.

Fractional crystallization of these mother liquors following removal of most of the 16-methylenepregnane 11b gave a new substance which paper chromatography showed was not present before the alumina chromatography. This material which was also obtained from the 100% chloroform eluates is formulated as **3α-acetoxy-17α-hydroxy-16-methylene-17β-methyl-D-homo-5β-androstane-11,17a-dione (48)**, m.p. 218–221°, $[\alpha]^{chf}D$ + 57°; λ_{max}^{chf} 2.89, 5.80sh, 5.84, 6.04(weak), 7.95 μ.

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.96; H, 8.61.

Dehydrochlorination of 3α-Acetoxy-15α-chloro-16-methyl-16-pregnene-11,20-dione (23).—To a solution of 100 mg. of the 15α-chloro compound 23 in 4 ml. of dimethylformamide was added 110 mg. of lithium chloride and 100 mg. of lithium carbonate and the mixture refluxed under nitrogen for 90 minutes[54]; after cooling water was added and the precipitated product was filtered, washed with water and dried in air. Crystallization from acetone–ether gave 3α-acetoxy-16-methyl-14,16-pregnadiene-11,20-dione (20) identical with material prepared by pyridine dehydrobromination of the 15α-bromo-16-methyl-16-pregnene 19 by mixed m.p., ultraviolet, infrared and paper chromatographic criteria. Attempted dehydrochlorination of 23 in pyridine failed at 25° and occurred to about 50% on refluxing for 3.5 hours. Partial dehydrochlorination also occurred on heating 23 to 200° in nitrogen at atmospheric pressure.

Hydrogenation of 3α-Acetoxy-17α-hydroxy-16-methylene-D-homo-5β-androstane-11,17a-dione (48).—A solution of 100 mg. of the 16-methylene-D-homo compound 48 in 25 ml. of methanol was hydrogenated over 100 mg. of 25% palladium on-calcium carbonate catalyst. Hydrogen uptake was complete in 20 minutes, the catalyst was removed by filtration and washed with methanol. Removal of the solvent from the combined filtrate and washing and crystallization of the residue from ether–petroleum ether gave 60 mg. of 3α-acetoxy-17α-hydroxy-16α,17β-dimethyl-D-homo-5β-androstane-11,17a-dione (49), m.p. 150–153°, identical by mixed m.p., paper chromatographic and infrared criteria with an authentic sample and distinctly different from the corresponding 16β-methyl-D-homo compound 7b. The mother liquors contained 49 and a trace of 48 as indicated by paper chromatography (ligroin–formamide system).

3α-Acetoxy-17α-hydroxy-16α,17β-dimethyl-D-homo-5β-androstane-11,17a-dione (49).—D-Homoannulation of 3α,-17α-dihydroxy-16α-methylpregnane-11,20-dione (24) by the BF_3-etherate–acetic anhydride–acetic acid procedure[12] followed by saponification and acetylation at C-3 gave the 16α-methyl-D-homo compound 49, m.p. 156–159°, $[\alpha]^{chf}D$ + 55°; λ_{max}^{chf} 2.88, 5.80, 5.86, 8.0 μ.

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97. Found: C, 71.49; H, 8.83.

Reaction of 3α-Acetoxy-16α,17α-oxido-16β-methylpregnane-11,20-dione (8b) with Hydrogen Fluoride in Tetrahydrofuran.—To a solution of 4.44 g. of the oxide 8b in 16.5 ml. of tetrahydrofuran in a polyethylene bottle cooled to −60° was added 23 ml. of a 2:1 (by weight) hydrogen fluoride–tetrahydrofuran mixture. The reaction mixture was kept at 10–20° for 2.5 hours, poured into excess cold 5% aqueous sodium carbonate solution and extracted with chloroform. The chloroform extract was washed with water, saturated aqueous sodium chloride and dried over magnesium sulfate. The residue had λ_{max}^{MeOH} 240 mμ, $E_{cm}^{1\%}$ 36; 304 mμ, $E_{cm}^{1\%}$ 60. Chromatography on neutral alumina gave 1.30 g. of the 16-methylenepregnane 11b, 265 mg. of the 16-methylene-D-homo-5β-androstane 48 as well as an unresolved mixture (1.10 g.) of the 14,16-pregnadiene 20 and a new fluorine-containing substance formulated as **3α-acetoxy-15α-fluoro-16-methyl-16-pregnene-11,20-dione**. This mixture contained 1.0% fluorine.

Reaction of 3α-Hydroxy-16α,17α-oxido-16β-methylpregnane-11,20-dione (8a) with Perchloric Acid. Mixture of Olefins 11a and 21a.—To a solution of 2.69 g. of the oxide 8a in 55 ml. of dioxane was added 27 ml. of 2 *M* aqueous perchloric acid. The clear solution was kept at 25–30° for 65 hours (24 hours resulted in incomplete reaction). Cold water (175 ml.) was added, the slurry was chilled to 8° and filtered after 30 minutes. The precipitate was washed with water and dried *in vacuo* at 50° to give 2.27 g. (95%) of a

(54) *Cf.* R. Joly, J. Warnant, G. Nominé and D. Berlin, *Bull. soc. chim.*, 366 (1958).

mixture of 3α,17α-dihydroxy-16-methylenepregnane-11,20-dione (11a) and 3α,17α-dihydroxy-16-methyl-15-pregnene-11,20-dione (21a), m.p. 158–167°. Paper chromatography (benzene–formamide) failed to resolve the mixture. However, partition chromatography on Supercel utilizing ethylene glycol as the stationary phase and 30% ethylene dichloride in isoöctane as the mobile phase[55] separated the two substances. The 16-methylenepregnane 11a was identical with material obtained as described above. The 16-methyl-15-pregnene (21a) had m.p. 182–187°, $[\alpha]^{chf}D$ −26°; λ_{max}^{chf} 2.73, 2.85, 5.86 μ.

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.29; H, 8.94. Found: C, 72.70; H, 8.91.

Room temperature acetylation gave the corresponding 3α-acetate 21b, m.p. 221–228°, $[\alpha]^{chf}D$ +6.3.°

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.39; H, 8.58.

The relative proportions of 11a and 21a in the perchloric acid product are of the order of 1:1, as estimated by the partition chromatography and by lithium aluminum hydride–sodium periodate degradation of an aliquot. The product of this sequence was a 1:1 mixture of 16-methylene-5β-androstane-3α,11β-diol-17-one (13) and 16-methyl-Δ^{15}-5β-androstene-3α,11β-diol-17-one (14) (see below). The mixture had m.p. 166–171°, λ_{max}^{MeOH} 232.5 (6,400); λ_{max}^{chf} 2.75, 2.80–2.90, 5.79, 5.87, 6.07, 6.21 μ. The conjugate carbonyl peaks at 5.79 and 5.87 μ were of equal intensity.

Reaction of Above Mixture of 13 and 14 with Diazomethane.—A solution of 400 mg. of the above mixture of isomeric unsaturated ketones 13 and 14 in *ca.* 5 ml. of tetrahydrofuran and 45 ml. of ether was saturated with diazomethane and kept at 15° for 18 hours. The yellow solution was concentrated *in vacuo* to a foam and chromatographed on 25 g. of neutral alumina. From the chloroform eluates there was obtained, after crystallization from acetone–ether, **16-methyl-Δ^{15}-5β-androstene-3α,11β-diol-17-one** (14), m.p. 187–190°, λ_{max}^{MeOH} 243 mμ (6850); λ_{max}^{chf} 2.74, 2.90, 5.80sh, 5.88, 6.22 μ. The absence of 6.07 μ absorption shows the absence of the 16-methylene-17-ketone 13.

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.44; H, 9.50. Found: C, 75.37; H, 9.55.

Application of the $LiAlH_4$–$NaIO_4$ sequence to the 16-methyl-15-pregnene 21a led directly to 14 identical with the above sample by mixed m.p. and infrared criteria.

From the 2% ethyl acetate–chloroform eluates there was obtained the pyrazoline 22, m.p. 222–226° dec.; λ_{max}^{chf} 2.74, 2.89, 5.74, 6.43 μ.

Anal. Calcd. for $C_{21}H_{32}O_3N_2$: C, 69.97; H, 8.95; N, 7.77. Found: C, 70.32; H, 9.24; N, 7.58.

Treatment of the pure exomethylene ketone 13 with diazomethane led to the identical pyrazoline.

Reaction of Unsaturated Ketols 11b and 21b with Hydrogen Bromide in Acetic Acid. (A) Exocyclic Ketol 3α-Acetate 11b.—A solution of 17 mg. of the exocyclic ketol 3α-acetate 11b in 1 ml. of acetic acid and 0.3 ml. of 15% hydrogen bromide in acetic acid was kept at 15° for one hour. Benzene was added and the mixture concentrated to dryness *in vacuo*. Crystallization of the residue gave only starting material 11b, m.p. 192–196°, confirmed by mixed melting point. Paper chromatography (benzene–cyclohexane 1:3–formamide) of the total product revealed no other component.

(B) Endocyclic Ketol 3α-Acetate 21b.—A solution of 20 mg. of 21b similarly treated with hydrogen bromide in acetic acid for 30 minutes gave a mixture of the 15-bromo compound 19 (60–65%) and the 14,16-diene-20-one 20 (30–35%); λ_{max}^{MeOH} 249 mμ (6,100), 304 mμ (3,400). When the time of reaction was cut to 10 minutes the 15-bromo compound 19 was essentially the only product.

(C) Perchloric Acid Product 3α-Acetate 11b + 21b.—Fifty mg. of the acetylated perchloric acid product was subjected to the above conditions for 30 minutes. Paper chromatography showed the product to consist primarily of the exocyclic ketol 3α-acetate 11b and the 15-bromo compound 19.

16α-Methylpregnane-3α,17α-diol-11,20-dione (24) and 16β-Methylpregnane-3α,17α-diol-11,20-dione (6). (A) Catalytic Reduction of the Perchloric Acid Product.—A solution of 3.05 g. of the perchloric acid-produced olefin mixture (11a and 21a) was reduced in hydrogen at 1 atmosphere and 25° in the presence of 2.0 g. of 25% Pd–$CaCO_3$ catalyst. Hydrogen uptake was complete in 45 minutes. The mixture was filtered, the filtrate was taken to dryness and the residue crystallized from ether, m.p. 160–170°. The infrared spectrum, when compared with spectra of synthetic mixtures of the 16α-methylpregnane 24 and the 16β-methylpregnane 6, showed the product to be a 7:3 mixture of 24 and 6. Chromatography of 1 g. of the product on 100 g. of Florisil resolved the mixture into the 16α-methylpregnane-3α,17α-diol-11,20-dione (24), m.p. 188–192°, identical with an authentic sample by mixed melting point, paper chromatographic and infrared spectral criteria as the more mobile component, and 16β-methylpregnane-3α,17α-diol-11,20-dione (6), m.p. 190–195°, similarly identical with authentic material prepared by the peracid route (see above) as the more polar component.

(B) Hydrogenolysis of 16α,17α-Oxido-16β-methylpregnane-3α-ol-11,20-dione (8a).—A solution of 10.0 g. of the oxide 8a in 180 ml. of dioxane and 112 ml. of 2 *M* aqueous perchloric acid was stirred in hydrogen at 1 atmosphere and 25° over 4.4 g. of 5% palladium-on-charcoal catalyst. Hydrogen uptake ceased after 25 hours. The reaction mixture was filtered and the product precipitated by adding 300 ml. of 2:1 saturated sodium chloride solution and water. The mixture was chilled, filtered, the precipitate washed with water and dried *in vacuo* at 50° to yield 9.26 g., m.p. 155–165°. Infrared analysis indicated the product to consist of 70 ± 2% of 24 and 30 ± 2% of 6. Solubility analysis confirmed this result and chromatography on Florisil as described under (A) gave the pure components 24 and 6.

21-Bromo-16β-methylpregnane-3α,17α-diol-11,20-dione (25).—To a stirred solution of 15.7 g. of 16β-methylpregnane-3α,17α-diol-11,20-dione (6) in 220 ml. of chloroform and 0.72 ml. of methanol was added 7.88 g. of bromine in 143 ml. of chloroform. The rate of dropwise addition was such that the color of the mixture was not darker than yellow-orange. The temperature was kept between 22 and 25°; time of addition, 3–4 hours. The reaction mixture was poured into 1580 ml. of ether, washed successively with 400 ml. of water containing a little sodium sulfite, twice with 200 ml. of saturated sodium bicarbonate solution and twice with 200 ml. of water. The extract was dried over sodium sulfate and the solvents removed *in vacuo*. The partly crystalline residue was triturated with 75–100 ml. of warm benzene, kept at ~5–10° for 3 hours, filtered and washed with cold benzene; yield 18.6 g., m.p. 165–175° dec.

Anal. Calcd. for $C_{22}H_{33}O_4Br$: C, 59.85; H, 7.53; Br, 18.10. Found: C, 59.34; H, 7.55; Br, 18.14.

16β-Methylpregnane-3α,17α,21-triol-11,20-dione 21-Acetate (26).—A mixture of 18.5 g. of 16β-methyl-21-bromide 25, 19.6 g. of potassium acetate, 15.8 g. of potassium iodide, 0.2 ml. of acetic acid and 400 ml. of acetone was refluxed with stirring for 17 hours. The reaction mixture was cooled to 25°, filtered from inorganic salts and the precipitate washed with acetone. The combined washes and filtrate were concentrated to dryness under vacuum. Three hundred ml. of water was added to the residue, the crystalline slurry was kept at 15–20° several hours, filtered, washed with water and dried in air; yield 14.3 g. Crystallization from acetone–ether gave prisms, m.p. 222–228°, $[\alpha]^{chf}D$ +124.5°; λ_{max}^{Nujol} 2.87–2.95, 5.73sh, 5.80, 5.83 μ.

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 68.56; H, 8.63. Found: C, 68.79; H, 8.44.

16β-Methylpregnane-17α,21-diol-3,11,20-trione 21-Acetate (27).—To a solution of 14.2 g. of 16β-methylpregnane-3α,17α,21-triol-11,20-dione 21-acetate in 355 ml. of *t*-butyl alcohol and 71 ml. of water cooled to 15° was added 12.4 g. of N-bromosuccinimide. The slurry was stirred at 15° for 2 hours, kept at 0° for 18 hours and stirred at 15° for 2 hours. Bromine color was discharged by adding aqueous sodium bisulfite and the *t*-butyl alcohol removed *in vacuo*, resulting in a crystalline slurry. Additional water (250 ml.) was put in and the mixture aged at 15°. The product was filtered, washed with water and dried in air; yield 13.6 g., m.p. 198–204°. Crystallization from acetone–ether gave rectangular plates, m.p. 210–212°, $[\alpha]^{chf}D$ +130°; λ_{max}^{chf} 2.88–2.99, 5.75, 5.80, 5.86 μ.

Anal. Calcd. for $C_{24}H_{34}O_6$: C, 68.88; H, 8.19. Found: C, 68.81; H, 7.91.

(55) Procedure of N. L. Trenner. We are very grateful to Dr. N. L. Trenner and Mr. J. Beck of these laboratories for performing this chromatogram.

2,4 - Dibromo - 16β - methylpregnane - 17α,21 - diol - 3,-11,20-trione 21-Acetate.—A stirred solution of 3.70 g. of 16β-methylpregnane-17α,21-diol-3,11,20-trione 21-acetate (27) in 74 ml. of chloroform and 0.8 ml. of acetic acid was cooled to 0°. A few drops of a solution of 2.92 g. of bromine in 10 ml. of acetic acid was added at 0°. When decolorization occurred the mixture was cooled to −15°, and one-half (5 ml.) of the bromine solution added dropwise. The mixture was warmed to 5 to 10° and the remainder of the bromine added. After 3 minutes, 1.7 g. of sodium acetate in 7 ml. of water was added followed by a few drops of aqueous sodium bisulfite to destroy any remaining free bromine. Water was added to the colorless solution and the mixture extracted with chloroform. The chloroform extract was washed successively with potassium bicarbonate, water and dried over magnesium sulfate. The 2,4-dibromo-16β-methylpregnane was obtained as the residue (5.2 g.) and was utilized without purification.

16β-Methylprednisone Acetate (29b).—The crude 2,4-dibromide (5.1 g.) was dissolved in 25 ml. of dimethylformamide, 1 g. of sodium bromide was added and the mixture stirred at 25° for one hour under nitrogen. Dimethylaniline (5 ml.) was added and the solution kept at 135° for 2.5 hours under nitrogen. The mixture was cooled to 10° and added slowly to a stirred mixture of 3.5 ml. of concentrated hydrochloric acid and 147 ml. of water. The light brown precipitate was aged several hours at 10°, filtered, washed with water until neutral and dried in air.

An ethyl acetate solution of the crude product was treated with decolorizing charcoal. Concentration of the filtrate gave 16β-methylprednisone acetate (29b) in 2 crops; yield 2.21 g., m.p. 225–230° or better. Further crystallization from acetone raised the m.p. to 230–233°, $[\alpha]^{chf}D$ +216°, λ_{max}^{MeOH} 238 mμ (15,100); λ_{max}^{chf} 2.9–3.0, 5.75, 5.79, 5.84, 6.01, 6.16, 6.19, 8.00, 11.20 μ.

Anal. Calcd. for $C_{24}H_{30}O_6$: C, 69.55; H, 7.30. Found: C, 69.25; H, 7.25.

16β-Methylprednisone (29a).—To a stirred refluxing suspension under nitrogen of 5.00 g. of 16β-methylprednisone acetate in 150 ml. of methanol was added 5.00 g. of potassium bicarbonate in 50 ml. of water. The pale yellow solution was refluxed 12 minutes under nitrogen, cooled to 20° and 5.0 ml. of acetic acid in 38 ml. of water added. Additional water was put in and the mixture extracted with ethyl acetate. The ethyl acetate extract was washed with potassium bicarbonate solution, saturated sodium chloride and dried over magnesium sulfate. Removal of the solvent left a crystalline residue of 16β-methylprednisone (4.5 g.) which gave analytically pure material on crystallization from ethyl acetate; m.p. 195–200°, $[\alpha]^{chf}D$ +205°, λ_{max}^{MeOH} 238 mμ (14,900); λ_{max}^{chf} 2.90, 5.82, 6.00, 6.14, 6.19, 11.21 μ.

Anal. Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H, 7.57. Found: C, 70.91; H, 7.57.

4 - Bromo - 16β - methylpregnane - 17α,21 - diol - 3,11,20-trione 21-Acetate.—To a stirred solution of 24.0 g. of 16β-methylpregnane-17α,21-diol-3,11,20-trione-21-acetate in 336 ml. of chloroform and 408 ml. of glacial acetic acid at −10° was added 9.27 g. of bromine in 240 ml. of chloroform at such a rate that the reaction mixture retained a pale yellow color. A few drops of 15% HBr in acetic acid was added initially. Upon completion of the addition a cold solution of 55.5 g. of sodium acetate in 330 ml. of water was added. The layers were separated and the aqueous layer extracted with 3 × 100 ml. of chloroform. The combined chloroform solution was washed with water, dilute aqueous potassium bicarbonate, saturated salt solution, dried over sodium sulfate and taken to dryness *in vacuo*. The residual tan foam crystallized when triturated with ether; yield 25.5 g., m.p. 159–164° dec. Recrystallization of a sample from acetone–ether did not change the m.p.; λ_{max}^{chf} 2.85–2.92, 5.74, 5.79, 5.85 μ.

Anal. Calcd. for $C_{24}H_{33}O_6Br$: C, 57.94; H, 6.68; Br, 16.06. Found: C, 58.44; H, 6.75; Br, 15.58.

16β-Methylcortisone Acetate (28b).—A solution of 25.3 g. of 4-bromo-16β-methylpregnane-17α,21-diol-3,11,20-trione 21-acetate and 12.7 g. of lithium chloride in 510 ml. of dimethylformamide, under nitrogen, was stirred for 4 hours at 100°. The reaction mixture then was cooled to 10° and 255 ml. of water added, with stirring, such that the internal temperature was kept below 30°. The mixture was stirred in an ice-bath until crystallization occurred and aged overnight at 0°. The crystalline product was filtered, washed with cold 50% aqueous DMF, then by water and dried *in vacuo* at 50°; yield 14.90 g., 70.5%, m.p. 208–224°. Treatment with decolorizing charcoal in acetone followed by crystallization from that solvent gave the analytical sample, m.p. 230–236°, $[\alpha]^{chf}D$ +252°, λ_{max}^{MeOH} 238 mμ (15,800); λ_{max}^{chf} 2.85–2.98, 5.73, 5.79, 5.85, 6.00, 6.18 μ.

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.75. Found: C, 69.24; H, 7.58.

The original aqueous mother liquors on further dilution with water deposited solid material with λ_{max}^{MeOH} 225 mμ (7,000), which paper chromatography (benzene–formamide) indicated to contain, in addition to 28b, a slightly more mobile ultraviolet-absorbing substance, probably the Δ^1-isomer of 28b, and a more mobile non-ultraviolet-absorbing species, probably the saturated compound 27.

16β - Methylcortisone (28a).—To a stirred, refluxing slurry of 2.00 g. of 16β-methylcortisone acetate (28b) in 60 ml. of methanol under nitrogen was added a solution of 2.00 g. of potassium bicarbonate in 20 ml. of water. The reaction mixture was refluxed for 10 minutes, cooled to room temperature and neutralized by addition of a solution of 2 ml. of glacial acetic acid in 15 ml. of water. The product crystallized out upon stirring in an ice-bath. It was filtered, washed with water and dried; yield 1.51 g. (84%), m.p. 202–212°. Crystallization from acetone–ether gave material with the same m.p., $[\alpha]^{chf}D$ +237°, λ_{max}^{MeOH} 238 mμ (16,200); λ_{max}^{chf} 2.87, 5.85, 6.00, 6.15 μ.

Anal. Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.09. Found: C, 70.84; H, 8.40.

16β-Methylcortisone BMD (34).—To a stirred solution of 7.43 g. of 16β-methylcortisone alcohol (28a) in 365 ml. of methylene chloride at room temperature was added a solution (premixed at 0°) of 110 ml. of 37% aqueous formaldehyde solution and 110 ml. of concentrated hydrochloric acid. The reaction mixture was stirred at room temperature for 20 hours, then diluted with 960 ml. of water. The layers were separated and the aqueous layer extracted with methylene chloride. The combined methylene chloride layer and extracts were washed with water, excess dilute aqueous potassium bicarbonate, water, dried over magnesium sulfate and taken to dryness *in vacuo* (flushed with benzene). The residue was triturated with ether to yield 6.69 g. of crystalline 34 (81% yield), m.p. 220–247° dec. Recrystallization from ether gave the analytical sample, m.p. 248–256° dec., $[\alpha]^{chf}D$ +88°, λ_{max}^{MeOH} 238 mμ (15,000); λ_{max}^{chf} 5.85, 6.00, 6.15, 9.1–9.2 μ.

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.75. Found: C, 69.17; H, 7.52.

Δ^4-16β-Methylpregnene-3,11,21-triol-20-one BMD (35).—To a stirred solution of 2.00 g. of lithium aluminum hydride in 200 ml. of dry tetrahydrofuran, under nitrogen, was added a solution of 2.00 g. of 16β-methylcortisone BMD in 100 ml. of tetrahydrofuran. Addition was complete in 10 minutes and the reaction mixture was stirred and refluxed for 100 minutes. The reaction mixture was cooled to 5° and quenched by cautious addition of 50 ml. of ethyl acetate, followed by the addition of 100 ml. of saturated sodium sulfate solution, and then 100 g. of anhydrous magnesium sulfate. The resulting mixture was filtered, the inorganic precipitate washed thoroughly with ethyl acetate and the combined washings and filtrate taken to dryness *in vacuo* to yield 1.9 g. (94%) of 35. Infrared analysis indicated only a trace amount of carbonyl absorption remained. Paper chromatography (benzene–chloroform 1:1–formamide) showed the presence of a minor amount of a more polar component, presumably the 11α-hydroxy epimer.

16β-Methylhydrocortisone BMD (36).—To a solution of 1.9 g. of the above crude triol-one BMD (35) in 200 ml. of acetone and 100 ml. of benzene was added 30 g. of manganese dioxide. The reaction mixture was stirred at room temperature overnight, filtered through Celite, the filter pad washed thoroughly with acetone, and the combined filtrate and washings taken to dryness *in vacuo*. The residue on trituration with ether yielded crystalline 36, 1.23 g. (65%), m.p. 220–240°, λ_{max}^{MeOH} 242 mμ (14,700). Recrystallization yielded material with m.p. 226–242°, $[\alpha]^{chf}D$ +33°, λ_{max}^{MeOH} 242 mμ (15,500); λ_{max}^{chf} 6.00, 6.14, 9.1–9.2 μ.

Anal. Calcd. for $C_{24}H_{34}O_6$: C, 68.87; H, 8.18. Found: C, 68.82; H, 8.10.

16β-Methylhydrocortisone (30a).—To a solution of 700 mg. of 16β-methylhydrocortisone BMD (36) in 35 ml. of glacial acetic acid was added 35 ml. of water. The system was purged with nitrogen and heated on the steam-bath for 4 hours. The reaction mixture then was concentrated to dryness *in vacuo* and flushed twice with benzene to yield 680 mg. (108%) of crude product. Pure material was obtained by repeated crystallization from ethyl acetate; m.p. 207–214°, $[\alpha]^{acetone}_D$ +156°, λ^{MeOH}_{max} 242 mμ (16,300); λ^{chf}_{max} 2.90, 5.84, 6.01, 6.16 μ.

Anal. Calcd. for $C_{22}H_{32}O_5$: C, 70.12; H, 8.56. Found: C, 70.49; H, 8.49.

16β-Methylhydrocortisone 21-Acetate (30b).—Acetylation of 500 mg. of 16β-methylhydrocortisone (30a) with 2 ml. of acetic anhydride and 7 ml. of pyridine overnight at 25° gave 16β-methylhydrocortisone acetate (30b), which was purified by crystallization from acetone–ether; m.p. 220–223°, $[\alpha]^{chf}_D$ +187°, λ^{MeOH}_{max} 242 mμ (15,800); λ^{chf}_{max} 2.87, 5.73, 5.77, 6.15, 8.10 μ.

Anal. Calcd. for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19. Found: C, 69.10; H, 7.89.

The over-all yield of 16β-methylhydrocortisone acetate (30b) from 16β-methylcortisone acetate (28b) by this route was about 25%.

16β-Methylprednisone BMD (37a).—To a stirred solution of 16β-methylprednisone (29a) in 200 ml. of methylene chloride at 25° was added a solution (premixed at 0°) of 56 ml. of 37% aqueous formaldehyde and 56 ml. of concentrated hydrochloric acid. The reaction mixture was stirred at 25° for 20 hours. Addition of water followed by extraction with methylene chloride gave a residue of 16β-methylprednisone BMD (37a) purified by crystallization from benzene and acetone–ether; 3.64 g., m.p. 203–206°; λ^{MeOH}_{max} 238 mμ (15,400); λ^{chl}_{max} 5.84, 6.00, 6.13, 6.19, 9.15 μ.

Anal. Calcd. for $C_{24}H_{30}O_6$: C, 69.55; H, 7.30. Found: C, 69.14; H, 7.34.

An additional 0.40 g. of good 37a was obtained from the mother liquors.

16β-Methylprednisone BMD 3-Semicarbazone (37b).—To a solution of 3.64 g. of 16β-methylprednisone BMD (37a) in 73 ml. of methanol and 44 ml. of dimethylformamide were added 2.91 g. of semicarbazide free base and 0.73 g. of semicarbazide hydrochloride. The mixture was stirred at room temperature for 18 hours during which time the product partially precipitated. Water was added with stirring, the precipitate aged at 0° for 1 hour, filtered, washed with water and dried in air; 4.0 g., m.p. 200–211°; λ^{MeOH}_{max} 294 mμ (23,400), 244 mμ (11,600).

16β-Methylprednisolone BMD 3-Semicarbazone (38).—To 5.4 g. of 16β-methylprednisone BMD 3-semicarbazone (37b) in 200 ml. of tetrahydrofuran was added 6 g. of sodium borohydride in 20 ml. of tetrahydrofuran and 20 ml. of water and the mixture refluxed with stirring. After 8 hours, 600 mg. of $NaBH_4$ was added, and then after 18 hours, 2 g. of $NaBH_4$. After a total of 25 hours, the mixture was cooled and 12.5 ml. of acetic acid in 50 ml. of water was added cautiously. Most of the tetrahydrofuran was removed *in vacuo*, additional water was put in and the granular precipitate filtered, washed with water and dried in air; yield 5.36 g., λ^{MeOH}_{max} 293 μ (19,000), 243 μ (9,200), which was utilized without further purification.

16β-Methylprednisolone (33a).—A solution of the product of the reduction step (5.36 g.) in 150 ml. of acetic acid and 150 ml. of water was heated on the steam-bath (temp. ~95–100°) for 4 hours. The mixture then was concentrated *in vacuo* nearly to dryness and extracted with ethyl acetate. The ethyl acetate extract was washed with aqueous potassium bicarbonate and saturated salt solution, dried over magnesium sulfate and taken to dryness (4.1 g.). Paper chromatography showed the crystalline residue to consist largely of 16β-methylprednisolone. Crystallization of a sample from acetone–ether gave pure 33a, m.p. 205–210°, $[\alpha]^{chf}_D$ +145°, λ^{MeOH}_{max} 243 mμ (14,600); λ^{chf}_{max} 2.9–3.0, 5.85, 6.02, 6.16, 6.21, 11.24 μ.

Anal. Calcd. for $C_{22}H_{30}O_5$: C, 70.57; H, 8.08. Found: C, 70.81; H, 8.07.

16β-Methylprednisolone 21-Acetate (33b).—The product of the previous step was dissolved in 20 ml. of pyridine and 4 ml. of acetic anhydride. After 18 hours at 20°, the solution was pumped to dryness *in vacuo*, last traces of reagents being removed by flushing several times with benzene. Crystallization of the residue from acetone–ether gave 1.06 g. of 16β-methylprednisolone acetate (33b), m.p. 206–210°. A second crystallization from acetone–ether raised the m.p. to 210–214°, $[\alpha]^{chf}_D$ +129°, λ^{MeOH}_{max} 243 mμ (15,000); λ^{chf}_{max} 2.9, 5.75, 5.79, 6.01, 6.15, 6.20, 8.05, 11.20 μ.

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.75. Found: C, 68.76; H, 7.71.

Chromatography of the mother liquors on neutral alumina gave from the 50–75% benzene–chloroform eluates 700 mg. of single spot (benzene–formamide system) 33b; total yield 1.76 g., 30–35% from 16β-methylprednisone acetate.

From the 20–30% benzene–chloroform eluates was obtained 400 mg. of single spot mobile material which did not give a positive tetrazolium test and was identified as **16β-methylprednisolone BMD.** Crystallization from acetone–ether gave analytically pure material, m.p. 245–249°, λ^{MeOH}_{max} 243 mμ (14,800); λ^{chf}_{max} 2.80, 6.00, 6.13, 6.20, 9.1–9.2, 11.21 μ.

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.75. Found: C, 69.09; H, 7.59.

16β-Methylprednisolone 21-acetate 33b also was obtained by selenium dioxide dehydrogenation of 16β-methylhydrocortisone 21-acetate (30b) in refluxing *t*-amyl alcohol for 18 hours[35] and then chromatography on neutral alumina (yield 25–30%).

The 17α,21-Dihydroxy-16β-methyl-1,4,9(11)-pregnatriene-3,20-dione 21-Acetate (39).—To a stirred solution at 0° of 1.26 g. of 16β-methylprednisolone acetate (33b) in 7.5 ml. of dry dimethylformamide and 1.26 ml. of dry pyridine was added 1.26 ml. of cold methyl chlorosulfite. The mixture was kept at 8–10° for 90 minutes, then cooled to 0° and 50 ml. of cold water was added dropwise with stirring. The precipitate was aged at 0° for 1 hour, filtered, washed with water and dried in air; yield 1.18 g., m.p. 182–197°. The total product was chromatographed on 45 g. of neutral alumina to give 760 mg. (64%) of 9(11)-olefin 39 (after combination of fractions and crystallization from acetone–ether) as plates, m.p. 208–212°, $[\alpha]^{chf}_D$ +75°, λ^{MeOH}_{max} 238 mμ (15,200); λ^{chf}_{max} 2.85, 5.73, 5.77, 5.99, 6.12, 6.19, 8.1, 11.22 μ.

Anal. Calcd. for $C_{24}H_{30}O_5$: C, 72.33; H, 7.59. Found: C, 72.30; H, 7.60.

Two additional substances also were isolated from the chromatogram in minor amounts. From the early fractions there was obtained **21-hydroxy-16β-methyl-1,4,9(11),16-pregnatetraene-3,20-dione 21-acetate,** m.p. 173–176°, λ^{MeOH}_{max} 243 mμ (21,600); λ^{chf}_{max} 5.73, 6.00, 6.12, 6.18, 8.00, 11.2 μ.

Anal. Calcd. for $C_{24}H_{28}O_4$: C, 75.76; H, 7.41. Found: C, 75.83; H, 7.52.

From the later fractions there was obtained the **11β-methyl sulfite ester of 16β-methylprednisolone 21-acetate,** m.p. 202–205, λ^{MeOH}_{max} 241 mμ (14,900); λ^{chf}_{max} 2.9, 5.74, 5.78, 6.01, 6.15, 6.20, 8.10, 8.4–8.5, 11.22 μ.

Anal. Calcd. for $C_{25}H_{34}O_8S$: C, 60.71; H, 6.93; S, 6.15. Found: C, 59.96; H, 6.83; S, 6.47.

9α-Bromo-16β-methylprednisolone 21-Acetate (40).—To a stirred slurry of 703 mg. of the 1,4,9(11)-pregnatriene (39) in 12 ml. of acetone maintained at 0° was added 460 mg. of N-bromosuccinimide, followed by 1.5 ml. of cold 0.20 *N* aqueous perchloric acid, added dropwise. The slurry was stirred at 0°. After 17 minutes all the solid dissolved to give a deep yellow solution. After 75 minutes, the product began to precipitate. After a total time of 4 hours, several drops of aqueous sodium bisulfite was added to destroy excess N-bromosuccinimide and then 40 ml. of cold water, added slowly. The precipitate was stirred one hour at 0°, filtered, washed with water and dried in air; yield 820 mg. (94%) of white powder, m.p. 140–142° dec. A probe crystallized from acetone–ether as prisms, m.p. 140–142° dec., λ^{MeOH}_{max} 242 mμ (13,200); λ^{chf}_{max} 2.71, 2.98, 5.71, 5.77, 5.99, 6.15, 6.21, 8.05, 11.22 μ.

The 9β,11β-Oxido-17α,21-dihydroxy-16β-methyl-1,4-pregnadiene-3,20-dione 21-Acetate (41b).—To a stirred slurry of 740 mg. of the bromohydrin 40 in 14 ml. of methanol at 23° under nitrogen was added 1.80 ml. of 1.07 *N* methanolic sodium methoxide under nitrogen. After 7 minutes all the steroid had dissolved (red solution) and after an additional 7 minutes aqueous acetic acid was added dropwise until the color of the solution changed from red

to yellow. The solution was concentrated on the water-pump until the volume was 5–6 ml. (product partly precipitated) and 40 ml. of cold water was added with stirring. After aging, the precipitate was filtered, washed with water and dried in air to give 446 mg. of **9β,11β-oxido-17α,21-dihydroxy-16β-methyl-1,4-pregnadiene-3,20-dione** (**41a**), m.p. 210–220°, λ_{max}^{MeOH} 249 mμ[56] (14,500); λ_{max}^{Nujol} 2.82, 2.96, 5.82, 6.01, 6.16, 11.24 μ. An additional 120 mg. of comparable oxide was obtained by salting the aqueous mother liquors and extraction with ethyl acetate.

Acetylation of 550 mg. of the combined product in 6 ml. of pyridine and 1.5 ml. of acetic anhydride for 16 hours at 25° gave the oxide-acetate 41b, 560 mg., single spot with benzene–cyclohexane 2:1. Crystallization from acetone–ether gave prismatic needles, m.p. 225–228°, $[\alpha]_D^{chf}$ +100°, λ_{max}^{MeOH} 249 mμ (15,000).

Anal. Calcd. for $C_{24}H_{30}O_6$: C, 69.55; H, 7.30. Found: C, 69.68; H, 7.26.

16β-Methyl-9α-fluoroprednisolone 21-Acetate (**42b**).—A solution made up of 500 mg. of the oxide acetate 41b in 7 ml. of chloroform cooled to −70° (acetone–Dry Ice-bath) was added to a mixture of 3.5 ml. of hydrogen fluoride–tetrahydrofuran reagent 2:1 by weight and 2.45 ml. of additional tetrahydrofuran kept at −70° in a polyethylene bottle. The reaction mixture was kept at minus 5° for 4.5 hours and then transferred by means of a polyethylene pipet to a stirred mixture of 40 ml. of chloroform and 50 ml. of 30% aqueous potassium carbonate precooled to 0°. The layers were separated and the aqueous layer extracted with additional chloroform. The combined chloroform layer was washed with saturated salt solution and dried over magnesium sulfate. Crystallization of the residue from acetone–ether (charcoal treatment) gave 210 mg. of hexagonal prisms, m.p. 205–208°, $[\alpha]_D^{chf}$ +140°, λ_{max}^{MeOH} 238 mμ (14,800); λ_{max}^{chf} 2.87, 5.73, 5.76, 5.98, 6.13, 6.19, 8.05, 11.21 μ.

Anal. Calcd. for $C_{24}H_{31}O_6F$: C, 66.35; H, 7.19. Found: C, 65.99; H, 7.37.

Chromatography of the mother liquors on neutral alumina and crystallization of the enriched fractions gave an additional 102 mg. of product, m.p. 203–207°; total 312 mg. + additional low melting material.

16β-Methyl-9α-fluoroprednisolone (**42a**).—To a stirred suspension prepared from 260 mg. of 16β-methyl-9α-fluoroprednisolone acetate 42b in 7 ml. of methanol under nitrogen (temperature 20–25°) was added 0.60 ml. of 1.07 *N* methanolic sodium methoxide. Within 2 minutes all the steroid had dissolved. After an additional 10 minutes, a slight excess of 50% aqueous acetic acid was added dropwise and most of the methanol removed *in vacuo*. Ethyl acetate and saturated NaCl were added and the mixture extracted several times with ethyl acetate. The ethyl acetate extract was washed with aqueous potassium bicarbonate, saturated sodium chloride and dried over magnesium sulfate. Concentration of the ethyl acetate extract to a small volume gave 16β-methyl-9α-fluoroprednisolone (42a), 115 mg., m.p. 231–234° dec., $[\alpha]_D^{acetone}$ +108°, λ_{max}^{MeOH} 238 mμ (15,200); λ_{max}^{Nujol} 2.90, 2.95, 5.88, 6.10, 6.15, 6.20, 11.25 μ.

Anal. Calcd. for $C_{29}H_{39}O_5F$: C, 67.33; H, 7.45; F, 4.84. Found: C, 67.29; H, 7.48; F, 4.30.

Concentration of the filtrate gave 47 mg. of excellent quality product, m.p. 231–234°, with additional good material in the mother liquors.

The 16β-Methyl-4,9(11)-pregnadiene-17α,21-diol-3,20-dione 21-Acetate (**43**).—To a stirred solution of 2.20 g. of 16β-methylhydrocortisone acetate (30b) in 13.2 ml. of dimethylformamide and 2.2 ml. of pyridine at 0° was added 2.2 ml. of methyl chlorosulfite (all under nitrogen). The resulting reaction mixture was stirred at 8–10° for 1.5 hours. The mixture then was cooled to 0°, diluted, cautiously, with 75 ml. of ice-water, and aged at 0° for 0.5 hour. The crystalline product was filtered, washed with water and dried. The yield was 2.095 g. (100%) of material with m.p. 176–203°. The crude product was chromatographed on neutral alumina and gave 1.05 g. (50%) of the 16β-methylpregnadiene 43 from the 10–30% benzene–chloroform eluates; plates from acetone–ether, m.p. 210–215°, $[\alpha]_D^{chf}$ +145°, λ_{max}^{MeOH} 238 mμ (17,000); λ_{max}^{chf} 2.74, 2.85–2.90, 5.75, 5.78, 6.00, 6.16 μ.

Anal. Calcd. for $C_{24}H_{32}O_5$: C, 71.87; H, 8.06. Found: C, 71.75; H, 8.30.

Fractional crystallization of the mother liquors from acetone–ether gave an additional small crop of 43 and then a new substance, evidently the alumina-catalyzed D-homo rearrangement product of 43, namely, **16β-methyl-4,9(11)-D-homoandrostadiene-17β-acetoxymethyl-17α-ol-17a-one** (**47**), m.p. 154–157°, $[\alpha]_D^{chf}$ +95°, λ_{max}^{MeOH} 238 mμ (17,600); λ_{max}^{chf} 2.79, 5.75, 5.84, 5.97, 6.13 μ.

Anal. Calcd. for $C_{24}H_{32}O_5$: C, 71.87; H, 8.06. Found: C, 71.97; H, 8.05.

The 16β-methylpregnadiene 43 was converted in part to the D-homoandrostadiene 47 by slurrying with neutral alumina in 10% chloroform–benzene or by heating to 215° as evidenced by paper chromatography (benzene–cyclohexane 1:1–formamide).

16β-Methyl-9α-bromohydrocortisone 21-Acetate (**44**).—To a stirred solution composed of 535 mg. of 16β-methyl-4,9(11)-pregnadiene-17α,21-diol-3,20-dione 21-acetate (43) in 20 ml. of acetone at 0° were added 364 mg. of N-bromosuccinimide and 1.19 ml. of 0.2 *M* perchloric acid. The reaction mixture was stirred at 20° for 2 hours. The mixture then was chilled to 0° and 2 ml. of sodium bisulfite solution added. This mixture now was concentrated *in vacuo* at room temperature to remove most of the acetone; the residue was chilled, diluted with 40 ml. of water, aged at 0° for 20 minutes and the product filtered, washed with water and dried thoroughly. The yield was 610 mg., or 92%. Recrystallization of a sample from acetone–ether yielded material with m.p. 127–130° dec., $[\alpha]_D^{chf}$ +166°, λ_{max}^{MeOH} 243 mμ (15,400); λ_{max}^{chf} 2.75, 2.85–2.95, 5.75, 5.79, 6.01, 6.15 μ.

16β-Methyl-4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione (**45a**).—To a stirred solution of 510 mg. of 16β-methyl-9α-bromohydrocortisone acetate (44) in 10.5 ml. of methanol under nitrogen was added, at 25°, 1.22 ml. of 1.12 *M* sodium methoxide. The mixture was stirred at 25° for 8 minutes, then quenched by addition of aqueous acetic acid until the color changed from red to light yellow. The methanol was removed under nitrogen at room temperature. To the residue was added 30 ml. of 65% saturated salt solution. The resulting crystals were aged at 0° for 1 hour, filtered, washed with cold water, and dried to yield 330 mg. (86%) of single spot material, m.p. 170–180°, λ_{max}^{MeOH} 243 mμ (14,100); λ_{max}^{Nujol} 2.9–3.0, 5.82, 6.05, 6.19 μ. Paper chromatography of the mother liquors indicated the presence of additional usable material.

16β-Methyl-4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-Acetate (**45b**).—16β-Methyl-4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione (45a, 320 mg.) was acetylated in 6 ml. of dry pyridine and 0.3 ml. of acetic anhydride overnight at room temperature. The reaction mixture was taken to dryness *in vacuo* and flushed several times with benzene. The residue (single spot) was used directly for the HF addition step. Crystallization from acetone–ether yielded the analytical sample, m.p. 212–216°, $[\alpha]_D^{chf}$ +58°, λ_{max}^{MeOH} 244 mμ (14,400), λ_{max}^{chf} 2.75, 2.85–2.93, 5.75sh, 5.78, 6.00, 6.15 μ.

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.20; H, 7.71. Found: C, 68.87; H, 7.64.

The 16β-Methyl-9α-fluoro-hydrocortisone 21-Acetate (**46b**).—Three hundred mg. of 16β-methyl-4-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate (45b) was dissolved in 3 ml. of chloroform and chilled to −70° in a small polyethylene bottle. In a separate polyethylene bottle (at −70°) 1.5 ml. of a 2:1 (by wt.) hydrogen fluoride–tetrahydrofuran reagent was added to 1.6 ml. of chilled (−70°) tetrahydrofuran. To the resulting mixture was added the cold chloroform solution of oxide-acetate 45b, rinsed in with 2 ml. of additional chloroform. The reaction mixture was maintained at −30° for 4 hours, then chilled to −70° and added, dropwise, to a cold (0°) mixture of 25 ml. of chloroform, 40 ml. of water and 10 g. of potassium carbonate. The layers were separated and the basic aqueous layer extracted with chloroform. The combined chloroform solution was washed with 20 ml. of saturated salt solution, dried over magnesium sulfate, and taken to dryness *in vacuo*. The crude residue crystallized from ether to yield 220 mg. (70% yield from the oxide-alcohol

(56) The very pronounced bathochromic shift (+6 mμ) produced by the 9β,11β-oxide function on the ultraviolet maximum of 1,4-diene-3-ones is noteworthy.

45a) of essentially single spot material, m.p. 206–215°. Recrystallization from acetone–ether afforded material, m.p. 215–225°, $[\alpha]^{chf}D$ +170°, λ_{max}^{MeOH} 238 mμ (16,800); λ_{max}^{Nujol} 2.76, 2.84, 5.74, 5.78, 6.0, 6.1 μ.

Anal. Calcd. for $C_{24}H_{33}O_6F$: C, 66.03; H, 7.61; F, 4.35. Found: C, 66.12; H, 7.77; F, 4.22.

16β-Methyl-9α-fluorohydrocortisone (46a).—To a stirred solution of 135 mg. of 16β-methyl-9α-fluorohydrocortisone acetate (46b) in 5 ml. of methanol, under nitrogen, was added 0.3 ml. of 1.0 *M* sodium methoxide. The reaction mixture was stirred at 24° for 10 minutes, then quenched by the addition of aqueous acetic acid (dropwise, until just acid) and blown down to one-half volume under nitrogen. The residue was diluted with 25 ml. of 75% saturated salt solution and extracted with ethyl acetate. The ethyl acetate extract was washed with saturated salt solution, dried over magnesium sulfate and concentrated, *in vacuo*, until crystallization occurred. After aging at 0°, the crystals were filtered, washed with ether and dried to yield 87 mg. (68%) of needles, m.p. 220–224°, $[\alpha]^{acetone}D$ +152°, λ_{max}^{MeOH} 239 mμ (16700); λ_{max}^{Nujol} 2.85–2.90, 5.83, 6.06, 6.15 μ.

Anal. Calcd. for $C_{22}H_{31}O_5F$: C, 66.98; H, 7.92; F, 4.81. Found: C, 67.12; H, 8.19; F, 4.76.

Additional good 46a was obtained on concentration of the mother liquors.

RAHWAY, N. J.

[CONTRIBUTION FROM THE BIOLOGICAL AND CHEMICAL RESEARCH DIVISIONS OF G. D. SEARLE & CO.]

Microbiological Transformations. V. 1α- and 2β-Hydroxylations of C_{19}-Steroids

BY R. M. DODSON, ARTHUR H. GOLDKAMP AND R. D. MUIR

RECEIVED FEBRUARY 8, 1960

Microbiological hydroxylations of steroids at positions 1α and 2β are recorded. Proofs for both the positions and configurations of the new hydroxyl groups are presented in detail. The 1α-hydroxylated products have been correlated through suitable chemical transformations with a 1β-hydroxysteroid obtained by degradation of the steroidal sapogenin, ruscogenin.

We have reported recently the microbiological hydroxylations of typical C_{19}-steroids at positions C-1 and C-2 by use of a species of *Penicillium*.[1] Of a number of steroids incubated with *Penicillium* sp. A.T.C.C. 12,556, only three—all C_{19}-steroids—were found to hydroxylate to a significant extent at position 1. These were 4-androstene-3,17-dione (I), androstane-3,17-dione (V) and dehydroisoandrosterone (VII). One of the substrates, 4-androstene-3,17-dione (I), also was found to hydroxylate at position 2. With other C_{19}- and C_{21}-steroids, hydroxylations at positions 2, 6, 7 and 15 also have been observed with this organism and will be reported at a later date.[2] In view of the novelty of 1- and 2-hydroxylations,[1,3] we wish to restrict ourselves here to recording in full our examinations of these reactions and of those chemical transformations which are significant in establishing and confirming our earlier structural assignments.[1]

Structures of the 1-Oxygenated Products. A. Positional Assignments.—Incubation of 4-androstene-3,17-dione (I) with *Penicillium* sp. A.T.C.C. 12,556 and subsequent chromatography gave rise to four new trioxygenated C_{19}- steroids (II, III, IVa, Chart I; and XII, Chart II). The position of the new hydroxyl group in II was based on the very facile elimination of acetic acid from its acetate to give 1,4-androstadiene-3,17-dione (IX), identified by comparison with authentic[4] material. Since both 2-acetoxyandrostenediones were known to us and were different from II acetate, and since these should not be converted readily to androstadienedione (IX), only C-1 remained for the position of the newly introduced hydroxyl group.

Initial assignment of position in the other two 1-oxygenated products III and IVa depended primarily on relating them through simple chemical transformations to the major product VIII obtained by incubation of dehydroisoandrosterone (VII) with this same organism. Oppenauer oxidation of VIII gave 1,4-androstadiene-3,17-dione. This result was best interpreted as a combination of the usual Oppenauer oxidation of a 3-hydroxy-Δ^5- to a 3-keto-Δ^4-system[5] and subsequent β-elimination of the second hydroxyl group. This placed the new hydroxyl function at position 1. Stepwise chemical reduction of VIII to IVa and VI, also obtained from I and III, respectively, served to correlate all of the 1-hydroxylated fermentation products with respect to positional assignment. Furthermore, the configurations of the new hydroxyl functions in III, IVa and VIII, though without stereochemical designation at this point, were thus shown to be identical. The remaining possible exception, though an unlikely one by analogy with the other fermentation products, was that of II.

Proof that II belonged to the same configurational series as the other 1-oxygenated products was obtained by correlation of II and VIII through a common transformation product. This is illustrated in Chart II, which shows the conversion of both II and VIII to 1,17β-diacetoxy-4-androsten-

(1) A preliminary communication of these results appeared in THIS JOURNAL, **79**, 3921 (1957). This organism has been designated as *Penicillium* sp., A.T.C.C. 12,556; G. D. Searle & Co. collection number M31-277.

(2) R. M. Dodson, Robert C. Tweit and R. D. Muir, unpublished results.

(3) (a) H. L. Herzog, M. J. Gentles, E. B. Hershberg, F. Carvajal, D. Sutter, W. Charney and C. P. Schaffner, THIS JOURNAL, **79**, 3921 (1957); (b) G. Greenspan, C. P. Schaffner, W. Charney, H. L. Herzog and E. B. Hershberg, *ibid.*, **79**, 3922 (1957); (c) W. J. McAleer, M. A. Kozlowski, T. H. Stoudt and J. M. Chemerda, *J. Org. Chem.*, **23**, 508 (1958); (d) M. Shirasaka, M. Tsuruta and M. Nakamura, *Bull. Agr. Chem. Soc. Japan*, **22**, 273 (1958); (e) M. Shirasaka, R. Takasaki, R. Hayashi and M. Tsuruta, *Bull. Agr. Chem. Soc. Japan*, **23**, 245 (1959).

(4) (a) H. H. Inhoffen, G. Zühlsdorff and Huang-Minlon, *Ber.*, **73B**, 451 (1940); (b) C. Djerassi and C. R. Scholz, *J. Org. Chem.*, **13**, 697 (1948).

(5) (a) R. V. Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937); (b) C. Djerassi, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 207.