Synthesis of 6α, 16α-Dimethylprogesterone

SEYMOUR BERNSTEIN, E. W. CANTRALL, AND J. P. DUSZA

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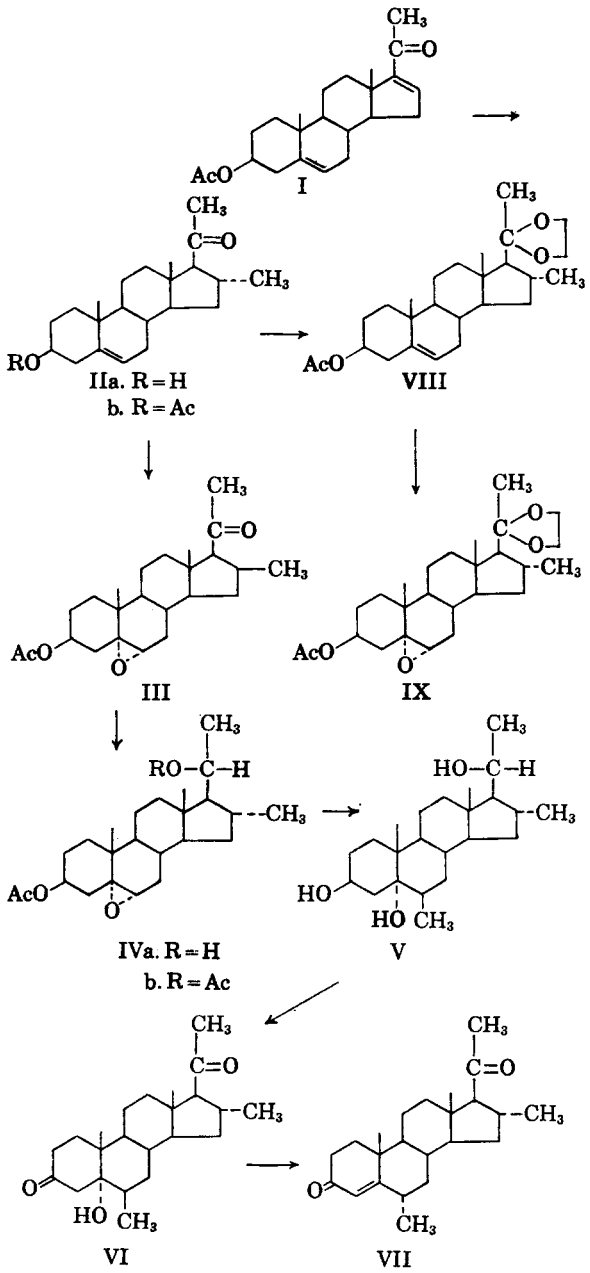
A C_6-methyl group is known to enhance both progestational[1] and anti-inflammatory[2] activities of steroid hormones. A C_{16}-methyl group markedly enhances anti-inflammatory activity.[3]

In view of these findings, it was of interest to examine the combined effect of C_6 and C_{16} methyl groups on the biological activities of steroid hormones. Initially, for this purpose we have synthesized 6α,16α-dimethylprogesterone (VII) details of which will be reported here.

The conjugate addition of methyl Grignard reagent to the Δ^{16}-20-one grouping of 3β-acetoxy-5, 16-pregnadien-20-one (I) gave the known 3β-hydroxy-16α-methyl-5-pregnen-20-one (IIa).[4] The product was not isolated as such but was converted directly into its acetate IIb, as the latter was found to be more readily purified than the free steroid. Treatment of IIb in methylene chloride at −5° with 1.2 equivalents of perbenzoic acid gave the corresponding 5α,6α-epoxide III in 63% yield. Reduction of III in methanol with sodium borohydride afforded 3β-acetoxy-5α,6α-epoxy-16α-methylpregnan-20β-ol (IVa).[5] Reaction of IVa with methylmagnesium bromide[6] gave 6β,16α-dimethylpregnane-3β,5α,20β-triol (V) in 72% yield. Oxidation of the latter with 8*N* chromic acid–sulfuric acid reagent[7] in acetone at 0° gave 5α-hydroxy-6β,16α-dimethylpregnane-3,20-dione (VI) in 85% yield. Dehydration under alkaline conditions[8] was accompanied by epimerization[1a,b]



of the 6β-(axial)-methyl group, and the desired product, 6α,16α-dimethylprogesterone (VII) was obtained in 87% yield.

Prior to the elaboration of the above pathway to VII, protection of the C_{20}-carbonyl group by ethylene ketal formation was considered. Accordingly, 3β-acetoxy-16α-methyl-5-pregnen-20-one (IIb) in benzene was converted to a ketal in the usual

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(2) G. B. Spero, J. L. Thompson, B. J. Magerlein, A. R. Hanze, H. C. Murray, O. K. Sebek, and J. A. Hogg, *J. Am. Chem. Soc.*, 78, 6213 (1956).

(3) (a) G. E. Arth, John Fried, D. B. R. Johnston, D. R. Hoff, L. H. Sarett, R. H. Silber, A. C. Stoerk, and C. A. Winter, *J. Am. Chem. Soc.*, 80, 3161 (1958); (b) E. P. Oliveto, R. Rausser, H. L. Herzog, E. B. Hershberg, S. Tolksdorf, M. Eisler, P. L. Perlman, and M. M. Pechet, *J. Am. Chem. Soc.*, 80, 6687 (1958).

(4) R. E. Marker and H. M. Crooks, Jr., *J. Am. Chem. Soc.*, 64, 1280 (1942).

(5) The newly formed hydroxyl group has been assigned the 20β-configuration based on the positive change in molecular rotation observed upon acetylation of IV_a [$M_D(IV_b)$-$M_D(IV_a)$ = +122°]. This assignment is in agreement with the literature values for the change in molecular rotation observed upon acetylation of 20β-hydroxypregnanes both substituted and unsubstituted at C-16 [R. H. Mazur and J. A. Cella, *Tetrahedron*, 7, 130 (1959)].

(6) See among others, E. Velarde, J. Iriarte, H. J. Ringold, and C. Djerassi, *J. Org. Chem.*, 24, 311 (1959).

(7) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946); and, J. Iriarte, C. Djerassi, and H. J. Ringold, *J. Am. Chem. Soc.*, 81, 436 (1959).

(8) A. H. Bowers and H. J. Ringold, *J. Am. Chem. Soc.*, 80, 3091 (1958).

manner[9] with ethylene glycol and *p*-toluenesulfonic acid to the 20-ethylene ketal VIII. Conversion to the 5α,6α-epoxide IX was readily accomplished with perbenzoic acid. It was then planned to treat IX with methylmagnesium bromide, followed by ketal removal and dehydration of the 5α-hydroxyl group. At this point additional epoxide was required, but unfortunately the ketal reaction was for some inexplicable reason non-reproducible. Consequently this synthetic scheme was set aside.

In the Clauberg progestational assay (subcutaneous route) 6α,16α-dimethylprogesterone (VII) displayed an activity *at least* comparable to that of progesterone.[10]

EXPERIMENTAL

All melting points are uncorrected and unless noted otherwise were determined in open soft-glass capillaries. All rotations were measured in chloroform at 25°. The infrared spectra were determined in pressed discs of potassium bromide. The ultraviolet spectra were determined in methanol. The *petroleum ether* used boiled at 60–70° (Skellysolve B).

3β-Acetoxy-16α-methyl-5-pregnen-20-one (IIb). Nitrogen was bubbled through 1320 ml. of a *ca.* 3.0*M* solution of methylmagnesium bromide in diethyl ether containing 20.0 g. of cuprous chloride. A solution containing 200 g. of 16-dehydropregnenolone acetate (I) in 3 l. of tetrahydrofuran (freshly distilled from lithium aluminum hydride) was added to the Grignard solution with stirring over 40 min. An additional 2 l. of tetrahydrofuran was added, and 1.1 l. of solution was distilled (reflux temperature 61°). Reflux under nitrogen was continued with stirring for 7.5 hr. The mixture was left to stand overnight at room temperature and was then poured into 12 l. of water containing 300 g. of ammonium chloride. The product was extracted with two 2-l. portions of methylene chloride. The combined extracts were washed with water and saturated sodium chloride solution and evaporated to a semicrystalline mass *in vacuo* on the steam bath. The crude product was heated on a steam bath for 1.75 hr. with a mixture of 1 l. of pyridine and 500 ml. of acetic anhydride. The excess acetic anhydride was decomposed with methanol, and the mixture was evaporated to a crystalline mass. The crude product was dissolved in 2 l. of ether: methylene chloride (3:1) and the solution was washed successively with 10% hydrochloric acid, water, 10% potassium bicarbonate, water, and saturated sodium chloride solutions. The solution was evaporated, and the residue was crystallized from methylene chloride–methanol to give 122.6 g. (58.6%) of colorless prisms, m.p. 176–180° (lit.[4] m.p. 173–175°). An additional 13.7 g. (6.5%, m.p. 175–179°) was obtained on working up the mother liquors.

3β-Acetoxy-5α,6α-epoxy-16α-methylpregnan-20-one (III). A solution containing 5.0 g. of 16α-methylpregnenolone acetate (IIb) in 100 ml. of methylene chloride was cooled to −5°, and 42 ml. of a 0.386*M* solution of perbenzoic acid in benzene was added dropwise with stirring over 10 min. The reaction mixture was kept at −5° for *ca.* 24 hr., following which it was washed successively with ice cold 5% sodium hydroxide solution, water, and saturated sodium chloride solution. The colorless solution was dried over anhydrous sodium sulfate and evaporated *in vacuo* to a colorless crystalline mass. On crystallization from acetone–petroleum ether, the crude product afforded 3.29 g. (63%) of colorless needles, m.p. 157.5–162°. An analytical sample had m.p. 167.5–168.5°; $[\alpha]_D$ −10°; ν_{max}^{KBr} 1745, 1710, 1370, 1250 and 1039 cm.$^{-1}$

Anal. Calcd. for $C_{24}H_{36}O_4$ (388.53): C, 74.19; H, 9.34. Found: C, 73.76; H, 9.33.

3β-Acetoxy-5α,6α-epoxy-16α-methylpregnan-20β-ol (IVa). A solution containing 512 mg. of sodium borohydride in 17 ml. of methanol was added dropwise, with stirring, over a period of 5 min. to a suspension of 1.30 g. of 3β-acetoxy-5α,6α-epoxy-16α-methylpregnan-20-one (III) in 35 ml. of methanol (most of the steroid dissolved before the addition of the reducing agent; all of it dissolved within *ca.* 2 min. after the first of the borohydride solution was added). The resulting solution was stirred for 10 min. at room temperature, and the excess borohydride was decomposed with a few drops of glacial acetic acid. The reaction mixture was evaporated *in vacuo* on a steam bath to a solid mass. The latter was taken up with *ca.* 75 ml. of ether–methylene chloride (2:1) and the extract was washed successively with water and saturated sodium bicarbonate solution. Concentration of the extract *in vacuo* afforded a solid mass, which on crystallization from acetone–petroleum ether gave 967 mg. (74%) of colorless blades, m.p. 178.5–180.5°.

A sample for analysis had m.p. 177–179.5°; $[\alpha]_D$ −76°; ν_{max}^{KBr} 1730, 1705, 1268, and 1042 cm.$^{-1}$

Anal. Calcd. for $C_{24}H_{38}O_4$ (390.54): C, 73.80; H, 9.81. Found: C, 73.92; H, 9.97.

5α,6α-Epoxy-16α-methylpregnan-3β,20β-dioldiacetate (IVb). Acetylation of 202 mg. of 3β-acetoxy-5α,6α-epoxy-16α-methylpregnan-20β-ol (IVa) with a mixture of 3 ml. of acetic anhydride and 3 ml. of pyridine and crystallization of the crude product IVb from aqueous acetone afforded 173 mg. of colorless needles, m.p. 146–147°. A sample for analysis was recrystallized from the same solvents, m.p. 147–148°; $[\alpha]_D$ −40°.

Anal. Calcd. for $C_{26}H_{40}O_5$ (432.58): C, 72.19; H, 9.32. Found: C, 71.80, 72.09; H, 9.54, 9.51.

6β,16α-Dimethylpregnan-3β,5α,20β-triol (V). To a solution containing 696 mg. of 3β-acetoxy-5α,6α-epoxy-16α-methylpregnan-20β-ol (IVa) in 30 ml. of benzene through which a slow stream of nitrogen was being bubbled was added 10 ml. of a *ca.* 3.0*M* solution of methylmagnesium bromide in diethyl ether. A grey solid precipitated, and the mixture was distilled until the reflux temperature had reached 65°. Reflux with stirring under a nitrogen atmosphere was continued for 3 hr. The mixture was treated with 10 ml. of saturated ammonium chloride solution, diluted with *ca.* 200 ml. of ether:benzene (3:1), and acidified with 10% sulfuric acid. The organic phase was washed successively with 5% sodium hydroxide, water, and saturated sodium chloride solutions. Evaporation *in vacuo* afforded a semicrystalline solid which crystallized from ethyl acetate–benzene to give 468 mg. (72%) of colorless needles m.p. 220–228° dec. An analytical specimen melted at 221–228° $[\alpha]_D$ −40°; ν_{max}^{KBr} 3430 and 1040 cm.$^{-1}$

Anal. Calcd. for $C_{23}H_{40}O_3$ (364.55): C, 75.77; H, 11.06. Found: C, 75.28; H, 11.01.

5α-Hydroxy-6β,16α-dimethylpregnan-3,20-dione (VI). A solution containing 5.0 g. of 6β,16α-dimethylpregnan-3β,5α,20β-triol (V) in 1 l. of acetone was cooled to −4°, and 15.0 ml. of an 8*N* solution of chromium trioxide in sulfuric acid was added dropwise over 25 min. The mixture was poured into a separatory funnel containing 3 l. of water, and the product was extracted with 1.5 l. of ether–methylene chloride (2:1) and twice with 500 ml. of ether. The combined extracts were washed with water and saturated sodium bicarbonate solution. Evaporation of the colorless extract *in vacuo* afforded a solid which crystallized from methylene chloride–methanol to give 3.58 g. (72%) of short needles, m.p. 260.5–262° dec. An additional 622 mg. (13%), m.p. 260–263° dec., was obtained on concentration of the mother liquors. The analytical specimen melted at 262.5–265° dec.; $[\alpha]_D$ +54°; ν_{max}^{KBr} 1705 cm.$^{-1}$

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(10) This assay was carried out by the Endocrine Laboratories, Madison, Wis.

Anal. Calcd. for $C_{22}H_{36}O_3$ (360.51): C, 76.62; H, 10.07. Found: C, 76.35, 76.10; H, 10.27, 10.41.

6α-16α-Dimethylprogesterone (VII). A solution containing 150 mg. of 5α-hydroxy-6β,16α-dimethylpregnan-3,20-dione (VI) and 0.5 ml. of 5% sodium hydroxide in 10 ml. of methanol was heated to reflux for 1 hr. under nitrogen. The mixture was acidified with a few drops of acetic acid and concentrated to a colorless viscous oil *in vacuo* on a steam bath. The crude product was dissolved in *ca.* 30 ml. of ether, and ether solution was washed with 5% sodium hydroxide, water, and saturated sodium chloride solution. The extract was concentrated to a viscous oil, and the latter was crystallized from aqueous methanol to give 123 mg. (87%) of fine colorless needles, m.p. 109.5–113.5° (Kofler). A sample for analysis melted at 113–116.5° (Kofler); $[\alpha]_D$ +145°; λ_{max} 242 mμ (ε = 16,100); ν_{max}^{KBr} 1705, 1678 and 1610 cm.$^{-1}$

Anal. Calcd. for $C_{23}H_{34}O_2$ (342.50): C, 80.65; H, 10.01. Found: C, 80.41; H, 10.32.

3β-Acetoxy-20-ethylenedioxy-16α-methyl-5-pregnene (VIII). To a solution containing 1.88 g. of 16α-methylpregnenolone acetate (I) and 49 mg. of *p*-toluenesulfonic acid (monohydrate) in 63 ml. of benzene was added 1.90 ml. of ethylene glycol. The resulting mixture was heated to reflux for 16 hr., and the water formed during the reaction was collected in a Dean-Stark apparatus. Pyridine (0.3 ml.) was added, and the solution was washed successively with ice-cold 5% sodium hydroxide, water, and saturated sodium chloride solutions. On evaporation *in vacuo* of the benzene solution, a semicrystalline solid was obtained which crystallized from methanol (containing a few drops of pyridine) to give 1.33 g. (63%) of fine, colorless needles, m.p. 134.5–140.5°. A sample for analysis melted at 144.5–146°; $[\alpha]_D$ −62.5°; ν_{max}^{KBr} 1740, 1255, 1238, and 1037 cm.$^{-1}$

Anal. Calcd. for $C_{26}H_{40}O_4$ (416.58): C, 74.96; H, 9.68. Found: C, 75.02; H, 9.83.

3β-Acetoxy-5α,6α-epoxy-20-ethylenedioxy-16α-methylpregnane (IX). To a solution containing 913 mg. of 3β-acetoxy-20-ethylenedioxy-16α-methyl-5-pregnene (VIII) in 20 ml. of benzene–methylene chloride (1:1) cooled to −10° was added 7.45 ml. of a 0.37*M* solution of perbenzoic acid in benzene dropwise with stirring over 17 min. The mixture was let stand at −5° for 23 hr. and washed successively with ice cold 5% sodium hydroxide, water, and saturated sodium chloride solutions. On evaporation *in vacuo* a semicrystalline solid was obtained. The latter on crystallization from methylene chloride–petroleum ether afforded 533 mg. of colorless needles, m.p. 169–171.5°. A sample for analysis melted at 171.5–172.5°; $[\alpha]_D$ −61°; ν_{max}^{KBr} 1730, 1243, 1218, 1100, 1047, and 1033 cm.$^{-1}$

Anal. Calcd. for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 72.09; H, 9.56.

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ORGANIC CHEMICAL RESEARCH SECTION
LEDERLE LABORATORIES
A DIVISION OF AMERICAN CYANAMID COMPANY
PEARL RIVER, N. Y.

The Triterpenes of *Befaria racemosa* (Vent.)[1]

K. UEDA, WERNER HERZ,[2] AND IRWIN J. PACHTER[3]

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Befaria racemosa (Vent.), a striking shrub found in the pinelands of the Coastal Plain of Florida and Georgia, gave a negative test for andromedotoxin in a recent survey of *Ericaceae*,[4] but seems not to have been investigated in other respects. This note describes the isolation and identification of ursolic acid, taraxerol, β-amyrin, lupeol, and β-sitosterol. In addition there was isolated what appears to be crude α-amyrin and a poor yield (0.07%) of an alkaloid mixture which was reserved for future investigation.

The major triterpene constituents were ursolic acid (0.5%), of common occurrence in *Ericaceae* species,[5,6] and taraxerol (0.2%), which has recently been found in *Pieris japonica* (D. Don.)[7] The former was identified through preparation of acetylursolic acid, methyl ursolate, methyl ursolate acetate, and methyl ursolate benzoate. The latter was identified through the acetate and benzoate and by conversion to taraxerone. The other triterpenes were isolated in small amounts only and were identified by comparison with authentic samples of β-sitosterol, β-amyrin acetate, lupeol benzoate, and α-amyrin acetate.

EXPERIMENTAL

Dried *Befaria racemosa* (Vent.) leaves, collected near Tallahassee in summer 1959, 4.1 kg., were extracted continuously with 20 l. of 95% ethanol for 48 hr. The solution was concentrated to a small volume and filtered from crystalline material (precipitate A). The filtrate was concentrated almost to dryness, stirred with several portions of 3% phosphoric acid, the acid extract made basic with concd. ammonia, and extracted with chloroform until exhausted of alkaloids. The chloroform solution was concentrated to dryness at reduced pressure, the residue taken up in 50 ml. of fresh chloroform and diluted with ether to the point where an insoluble precipitate began to form. The solution was extracted repeatedly with 3% phosphoric acid, the acid extracts were made basic with ammonia and the alkaloids extracted with chloroform. Removal of chloroform left 3 g. of gummy residue (positive to Mayer's reagent) which paper chromatography showed to be a mixture of alkaloids.

Precipitate A was taken up in hot chloroform-ethanol, filtered and allowed to stand. There precipitated crude taraxerol which was recrystallized five times from chloroform-methanol, yield 2.5 g., m.p. 268–272° (Hershberg), 280–281° (Kofler), $(\alpha)_D^{25}$ +2.0 (*c*, 1.5, chloroform).[8]

The acetate, prepared by refluxing with acetic anhydride, crystallized from methanol as colorless scales, m.p. 302°

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(2) To whom correspondence should be addressed, Department of Chemistry, The Florida State University, Tallahassee, Fla.

(3) Smith, Kline and French Laboratories, Philadelphia 1, Pa.

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