Anal. C, calcd., 69.41, found, 69.63; H, calc., 4.16, found 4.45. NMR measurements in deuterochloroform, conc. approx. 1% showed 3 kinds of hydrogens, with chemical shifts at 3.21, 3.22 τ for one H next to carbonyl, one H next to methyl, and 7.86 τ for methyl hydrogens.[12] Ratios of areas under peaks approximately 1:1:3, but the low concentration of the solution made this difficult to determine exactly. Half-reduced form: m.p., 215–215.5°; u.v.$_{max}$ 254, 296, Ext., 609, 527; vis.$_{max}$ 455, Ext. 27.1; Anal. C, calcd. 68.84, found, 68.56; H, calcd., 5.07, found, 4.77. Mol. wt. (camphor) 234; calcd., 242.

Dimer from p-xylo-p-quinone. Oxidized form: m.p., 149–150°; u.v.$_{max}$, 256, Ext. 1,160; vis.$_{max}$, 430, Ext. 10.58; Anal. C, calcd., 71.11, found, 70.73; H, calcd., 5.22, found, 4.98. Half-reduced form: m.p., 163–164°; u.v.$_{max}$, 246, 288, Ext. 580, 396; vis.$_{max}$ 430, Ext. 14.49; Anal. C, calcd., 70.58, found, 70.22; H, calcd., 5.92, found, 6.4.

Acknowledgment. We are pleased to acknowledge that this work was supported by a grant from Research Corporation and a PHS research grant, A-3304, National Institute of Arthritis and Metabolic Diseases, Public Health Service.

STERLING CHEMISTRY LABORATORY
YALE UNIVERSITY
NEW HAVEN, CONN.

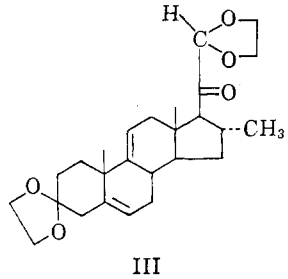
16-Methylated Steroids. III. The Synthesis of 9α - Fluoro -16α,17α- dimethyl -4- androstene-11β,17β-diol-3-one

JOHN H. FRIED, ANTHONY N. NUTILE, GLEN E. ARTH, AND L. H. SARETT

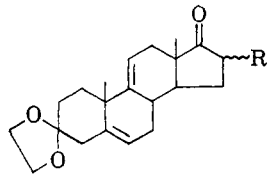
Received August 25, 1961

The greatly enhanced anti-inflammatory potency of 16α-methylcorticoids[1-4] prompted us to prepare some 16α-methyl derivatives in the testosterone series. The 16α-methyl analog of 9α-fluoro-17α-methyl-4-androstene-11β,17β-diol-3-one[5] was of particular interest since the parent compound has been utilized clinically as a potent androgenic and anabolic agent.[6] A 2:1 ratio of anabolic to androgenic activity has been demonstrated in animals.[7]

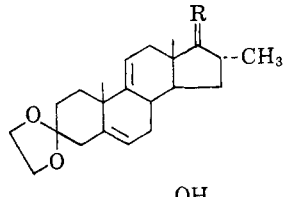
This note describes the partial synthesis of 9α-fluoro-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one IX from 16α-methyl-4,9(11)-pregnadiene-17α,21-diol-3,20-dione 21-acetate[2] (I). Selective ketal formation of I at C-3 afforded 3-ethylenedioxy - 5,9(11) - pregnadiene - 17α,21- diol - 20-one 21-acetate (II). In addition to II a slightly less polar compound III, $C_{26}H_{36}O_5$ was isolated in 3% yield, by chromatography of the reaction mixture. This compound showed only saturated carbonyl, λ_{max} 5.79 μ, in the infrared. The NMR spectrum[8] indicated two distinct dioxolane functions, τ 5.89 and 5.95. One of these can be assigned to a dioxolane of an aldehyde on the basis of the companion proton at τ 4.84 and the positive Schiff aldehyde test obtained with III after acid treatment. The data are best interpreted on the basis of structure III, 16α-methyl-3,21-bisethylenedioxy-5,9(11)-pregnadiene-20-one. Formation of III may proceed *via* hydrolysis at C-21, Fischer rearrangement[9] to the 21-aldehyde 17,20-diol, followed by dehydration and dioxolanation. The C-17 side chain is presumed to be in the thermodynamically more stable β-configuration.[10]

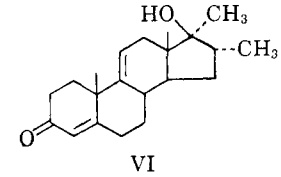


III

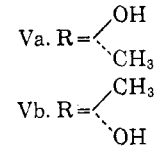


IVa. R = --CH_3
IVb. R = —CH_3

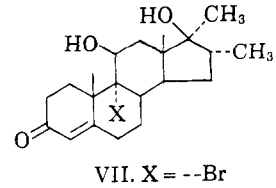




VI



Va. R = $\overset{OH}{\underset{CH_3}{}}$
Vb. R = $\overset{CH_3}{\underset{OH}{}}$



VII. X = --Br
IX. X = --F

Reduction of II with lithium aluminum hydride followed by periodic acid cleavage afforded 16α-methyl-3-ethylenedioxy-5,9(11)-androstadiene-17-one IVa. An idea of the conformational stability of

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

(2) G. E. Arth, J. Fried, D. B. R. Johnston, D. R. Hoff, L. H. Sarett, R. H. Silber, H. C. Stoerk, and C. A. Winter, *J. Am. Chem. Soc.*, **80**, 3161 (1958).

(3) E. P. Oliveto, R. Rausser, A. L. Nussbaum, W. Gebert, E. B. Herschberg, S. Tolksdorf, M. Eisler, P. L. Perlman, and M. M. Pechet, *J. Am. Chem. Soc.*, **80**, 4428 (1958).

(4) E. P. Oliveto, R. Rausser, L. Weber, A. L. Nussbaum, W. Gebert, C. T. Coniglio, E. B. Hershberg, S. Tolksdorf, M. Eisler, P. L. Perlman, and M. M. Pechet, *J. Am. Chem. Soc.*, **80**, 4431 (1958).

(5) M. E. Herr, J. A. Hogg, and R. H. Levin, *J. Am. Chem. Soc.*, **78**, 500 (1956).

(6) T. H. McGaback and W. Seegers, *Am. J. Med. Sc.*, **235**, 125 (1958).

(7) S. C. Lyster, G. H. Lund, and R. O. Stafford, *Endocrinology*, **58**, 781 (1956).

(8) NMR Spectra were run on a Varian 60 mc. spectrometer at a concentration of *ca.* 20 mg. in 0.3 ml. deuterochloroform. $\tau = \nu/60 + 3.5$ where ν is the observed band position in c.p.s. relative to benzene as external standard. *Cf.* G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

(9) H. O. L. Fischer, C. Taube, and E. Baer, *Ber.*, **60**, 479 (1927).

(10) A. Butenandt and G. Fleischer, *Ber.*, **70**, 96 (1937); A. Butenandt and L. Mamoli, *Ber.*, **68**, 1847 (1935).

the 16-methyl group can be gained from equilibration experiments. Treatment of IVa with refluxing methanolic potassium hydroxide yielded a 2:1 mixture of 16β-methyl-3-ethylenedioxy-5,9(11)-androstadiene-17-one IVb and recovered IVa. Earlier experiments in the estrone series[11] established that the 16α-methyl is also the stable configuration in that series.

Reaction of IVa with methylmagnesium iodide afforded the 17-methylated product Va in addition to a small quantity of the C-17-epimeric product Vb. Examination of models indicates formation of the 17α-methyl product, Va, to be favored sterically. Compound Va should also be less polar than Vb since the 17-hydroxyl group is *quasi-axial* compared to the *quasi-equatorial* 17-hydroxyl group present in Vb. Experimentally the major product of the reaction was also the less polar product. The molecular rotation difference between Va and Vb is −7°. For comparison the molecular rotation difference between the epimeric 17α- and 17β-methyltestosterones is −36°.[12]

Hydrolysis of Va afforded 16α,17α-dimethyl-4,9(11)-androstadiene-17β-ol-3-one (VI) which was converted to the bromohydrin VII with *N*-bromosuccimide and perchloric acid in acetone.[2] Conversion of VII to the oxide[13] VIII followed by treatment with hydrogen fluoride[13,14] yielded 9α-fluoro - 16α,17α - dimethyl - 4 - androstene-11β-17β, diol-3-one (IX).

Compounds VI and IX were tested in the Merck Institute for Therapeutic Research.[15,16] Compound IX was about one-seventh as active as 9α-fluoro-17α-methyl-4-androstene-11β,17β-diol-3-one in the levator ani response and did not effect the seminal vesicles at the level tested. Compound VI exhibited about one-fourth the levator ani and one-sixth the seminal vesicle response of 9α-fluoro-17α-methyl-4-androstene-11β,17β-diol-3-one.

EXPERIMENTAL[17]

16α-Methyl-3-ethylenedioxy-5,9-(11)-pregnadiene-17α,21-diol-20-one (II) *and 16α-methyl-3,21-bisethylenedioxy-5,9(11)-pregnadiene-20-one* (III). A mixture containing 25.3 g. of 16α-methyl-4,9(11)-pregnadiene-17α,21-diol-3,20-dione 21-acetate (I) and 1.50 g. of *p*-toluenesulfonic acid in 600 ml. of butanone dioxolane was refluxed 0.5 hours under an atmosphere of nitrogen. At the end of this time *ca.* 325 ml. of solvent was allowed to distill out of the reaction mixture over a period of 5 hr. The solution was cooled and poured into aqueous sodium bicarbonate. The organic phase was separated and the aqueous layer extracted with ether. The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. Direct crystallization from ether afforded 12.3 g. of II, m.p. 209–222°. A benzene solution of the mother liquors was chromatographed on 40 g. acid-washed alumina (Merck). Elution with ether–petroleum ether (7:3) afforded 0.741 g. (3%) of 16α-methyl-3,21-bisethylenedioxy-5,9 (11)-pregnadiene-20-one III, m.p. 212–225°. The sample for analysis was crystallized from ethyl acetate, m.p. 228–229.5°. Infrared: λ_{max}^{Nujol} 5.79, 9.12 μ. N.M.R.: two ethylenedioxy functions, τ 5.89 and 5.95, and a proton on a carbon bearing two oxygen functions and a carbonyl, τ 4.84.

Anal. Calcd. for $C_{26}H_{36}O_5$: C, 72.86; H, 8.47. Found: C, 72.68; H, 8.65.

Acid hydrolysis of III yielded an amorphous solid which exhibited a positive Schiff aldehyde test and showed $\lambda_{max}^{CH_3OH}$ 239 mμ, ε 13,200.

Further elution with ether–petroleum ether (8:2) afforded an additional 3.6 g. of II, m.p. 207–214°. A sample for analysis was crystallized twice from methanol, m.p. 218–225°; α_D^{23} +10°.

16α-Methyl-3-ethylenedioxy-5,9(11)-androstadiene-17-one (IVa). A suspension of 1.5 g. of lithium aluminum hydride and 1.3 g. of II, m.p. 210–225°, in 100 ml. of anhydrous tetrahydrofuran was refluxed overnight. The suspension was cooled and excess lithium aluminum hydride was destroyed by the careful addition of ethyl acetate. Subsequently 17 ml. of water was added with stirring. The organic phase was separated by filtration, and the filter cake was washed with chloroform. The combined organic layer was dried over sodium sulfate and concentrated *in vacuo*.

The crude product above was dissolved in 10 ml. of pyridine and 10 ml. of methanol and maintained at 25°. A solution of 2.53 g. of periodic acid in 10 ml. of water was added with stirring over a period of 15 min. After an additional 45 min., saturated aqueous sodium bicarbonate (25 ml.) was added, and the solution was extracted with chloroform. The organic phase was dried over sodium sulfate and concentrated *in vacuo*. The crude product in benzene was chromatographed on 35 g. of acid-washed alumina (Merck). Elution with ether–petroleum ether (1:9 and 2:8) yielded 0.82 g. (84%) of IVa, m.p. 170–175° (from ether). Recrystallization from ether afforded the analytical sample of 16α-methyl-3-ethylenedioxy-5,9-(11)-androstadiene-17-one, m.p. 174–176°; α_D^{25} +66°.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 77.15; H, 8.83. Found: C, 77.41; H, 8.64.

16β-Methyl-3-ethylenedioxy-5,9-(11)-androstadiene-17-one (IVb). A sample of 16α-methyl-3-ethylenedioxy-5,9(11)-androstadiene-17-one, m.p. 174–176°, dissolved in 20 ml. of 0.9*N* methanolic potassium hydroxide was refluxed overnight under an atmosphere of nitrogen. The cooled solution was poured into water, extracted with chloroform, dried over sodium sulfate, and concentrated *in vacuo*. A benzene solution of the steroid was chromatographed on 25 g. of acid-washed alumina (Merck). Elution with ether–petroleum ether (2:8) yielded 77 mg. of recovered 16α-methyl-3-ethylenedioxy-5,9(11)-androstadiene-17-one, m.p. 167–176°. Further elution with ether–petroleum ether (2:8) yielded 146 mg. of 16β-methyl-3-ethylenedioxy-5,9(11)-androstadiene-17-one, m.p. 179–185°. Crystallization from methanol afforded a sample for analysis, m.p. 181–184°; α_D^{24} +93°.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 77.15; H, 8.83. Found: C, 76.91; H, 8.71.

(11) Unpublished results of G. E. Arth, Phyllis Tormo, and L. H. Sarett.

(12) K. Miescher and W. Klarer, *Helv. Chim. Acta*, **22**, 962 (1939).

(13) Josef Fried and E. F. Sabo, *J. Am. Chem. Soc.*, **76**, 1455 (1954); **79**, 1130 (1957).

(14) R. Hirschmann, R. Miller, J. Wood, and R. E. Jones, *J. Am. Chem. Soc.*, **78**, 4956 (1956).

(15) We are indebted to Dr. R. H. Silber for carrying out this determination.

(16) E. Eisenberg and C. S. Gordan, *J. Pharm. and Exp. Therap.*, **99**, 38 (1950).

(17) Melting points were determined on a Kofler micro hot stage and are corrected. Rotations were determined in chloroform at concn. = 10 mg./ml., unless otherwise noted. We wish to thank R. Boos and his associates for microanalyses, A. Kalowsky for ultraviolet absorption spectra, R. Walker and N. Allen for the infrared spectra, and Dr. N. R. Trenner and B. Arison for the NMR spectra herein reported.

16α,17α-Dimethyl-3-ethylenedioxy-5,9(11)-androstadiene-17β-ol, (Va) *and 16α-17β-dimethyl-3-ethylenedioxy-5,9(11)-androstadiene-17α-ol* (Vb). A solution of 8.8 g. of IVa, m.p. 172–175°, in 200 ml. of benzene was dried by azeotropic distillation. This was added to a solution of methylmagnesium iodide (prepared from 1.50 g. of magnesium, 24 ml. of methyl iodide, and 240 ml. of anhydrous ether) and allowed to stir overnight at 25°. Water was added and the product extracted with chloroform. The chloroform phase was dried over sodium sulfate and concentrated *in vacuo*. Chromatography on 300 g. of acid-washed alumina (Merck) and elution with ether–petroleum ether (1:9) yielded 1.02 g. (12%) of starting material. Elution with ether–petroleum ether (3:7 and 4:6) afforded 5.91 g. (75%) of 16α,17α-dimethyl-3-ethylenedioxy-5,9(11)-androstadiene-17β-ol, m.p. 170–191°. A sample for analysis was obtained from methanol, m.p. 194–198°; α_D^{27} −35° (*c* 1.3, benzene).

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.06; H, 9.35.

Further elution with ether–petroleum ether (5:5) afforded 260 mg. (3%) of 16α,17β-dimethyl-3-ethylenedioxy-5,9(11)-androstadiene-17α-ol, m.p. 152–156°. Recrystallization from methanol afforded the analytical sample, m.p. 155–157°; α_D^{23} −37° (*c* 1.0, benzene).

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.65; H, 9.94.

16α,17α-Dimethyl-4,9(11)-androstadiene-17β-ol-3-one (VI). A solution containing 0.4 g. of Va and 50 mg. of *p*-toluenesulfonic acid in 50 ml. of acetone was allowed to stand overnight. The solution was diluted with aqueous sodium bicarbonate and extracted with chloroform. The chloroform layer was dried over sodium sulfate and concentrated *in vacuo*. The crude product was adsorbed on 25 g. acid-washed alumina (Merck) from benzene. Elution with ether–petroleum ether (6:4, 7:3, and 8:2) afforded 0.25 g. crude VI. Several crystallizations from methanol–ether yielded a sample of 16α,17α-dimethyl-4,9(11)-androstadiene-17β-ol-3-one for analysis, m.p. 187–189°; α_D^{26} +34°; $\lambda_{max}^{CH_3OH}$ 240 mμ, ε 16,600.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.49; H, 9.86.

9α-Bromo-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one (VII). A suspension of 175 mg. of VI and 140 mg. of *N*-bromosuccinimide in 2.34 ml. of acetone was cooled in an ice bath and 0.56 ml. of 0.27*N* perchloric acid (0.46 g. 70% perchloric acid in 16.5 ml. water) was added. The suspension was stirred by means of a magnetic stirrer for 30 min., an additional 1 ml. of acetone was added and stirring continued for 40 min. Excess *N*-bromosuccinimide was destroyed by the addition of 0.24 ml. of allyl alcohol. The reaction mixture was diluted with 30 ml. of water and the product, 180 mg., was separated, and air dried. The crude 9α-bromo-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one, m.p. 183–188° dec., was used in the next step.

16α,17α-Dimethyl-9β,11β-oxido-4-androstene-17β-ol-3-one (VIII). A suspension of 90 mg. of bromohydrin above and 75 mg. of anhydrous potassium acetate in 2 ml. of anhydrous ethanol was refluxed under nitrogen for forty min. The suspension was cooled, diluted with ice water, and extracted with chloroform. The chloroform layer was dried over sodium sulfate and concentrated *in vacuo*. The crude product was adsorbed on 7.0 g. of acid-washed alumina (Merck) from benzene. Elution with ether–chloroform (9:1 to 5:5) afforded 32 mg. of 16α,17α-dimethyl-9β,11β-oxido-4-androstene-17β-ol-3-one. The analytical sample was crystallized from ether, m.p. 178–181°; α_D^{23} −67°; $\lambda_{max}^{CH_3OH}$ 244 mμ, ε 14,800.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 75.66; H, 8.99.

9α-Fluoro-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one (IX). A solution of 200 mg. of VIII in 1.6 ml. of chloroform cooled to −40° was added to 10 ml. of a reagent consisting of 10 ml. of a 2:1 mixture by weight of hydrogen fluoride in tetrahydrofuran, 10.8 ml. of tetrahydrofuran, and 10 ml. of chloroform maintained at −80°. The tetrahydrofuran was reagent grade dried over potassium hydroxide and filtered. The reaction mixture was placed in a Dewar flask and maintained at −30° for 4 hr. After this time the reaction mixture was poured into a stirred mixture consisting of 5 g. of anhydrous potassium carbonate, 15 ml. of ice and water, and 25 ml. of chloroform. The chloroform layer was separated, and the aqueous phase was re-extracted with chloroform. The combined chloroform layer was dried over sodium sulfate and concentrated *in vacuo*. Chromatography on 20 g. acid-washed alumina (Merck) and elution with chloroform–ether (8:2) afforded 94 mg. of crude 9α-fluoro-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one. Crystallization from ethyl acetate afforded 71 mg., m.p. 256–259°; α_D^{23} +85°; $\lambda_{max}^{CH_3OH}$ 239 mμ, ε 17,800.

Anal. Calcd. for $C_{21}H_{31}O_3F$: C, 71.95; H, 8.91; F, 5.42. Found: C, 72.37; H, 8.89; F, 5.21.

MERCK, SHARP AND DOHME
RESEARCH LABORATORIES
RAHWAY, N. J.

Synthesis of *N*-Substituted Derivatives of Carnosine and Homocarnosine

GARY BAILIN AND AARON LUKTON

Received August 28, 1961

The preparation of *N*-substituted derivatives of dipeptides in high yield cannot readily be achieved by direct attack on the free amino group of the dipeptide. The alternative method is to condense the carboxyl group of the *N*-substituted amino acid with the amino group of the second esterified amino acid such as in the *p*-nitrophenyl ester procedure which is used here. It has been previously applied, with considerable success, to the synthesis of large peptides.[1]

These compounds have been prepared for use as substrate analogs for enzyme specificity studies, including carnosine forming and splitting enzymes and the acylases. They will also serve as starting materials for the preparation of substituted lactam derivatives of the dipeptides which will be reported later.

TABLE I

Compound	Yield, %	M.P., Obs. (uncorr.)	M.P., Lit.	Ref.
Acetyl-β-alanine	85	78–80	78–81	[a]
Benzoyl-β-alanine[b]			133–134	[c]
Phthaloyl-β-alanine	90	152–153	152–153	[d]
Phthaloyl-γ-amino-butyric acid	95	117–119	115–117.5	[e]

[a] P. J. Fodor *et al.*, *J. Biol. Chem.*, **178**, 503 (1949). [b] Obtained from Mann Research Labs. Inc., N. Y. [c] C. C. Barker, *J. Chem. Soc.*, 317 (1954). [d] R. A. Turner, *J. Am. Chem. Soc.*, **75**, 2388 (1953). [e] G. Kushinsky *et al.*, *Biochem. Z.*, **327**, 314 (1955); *Chem. Abstr.*, **50**, 7164 (1956).

(1) M. Bodansky and V. duVigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).