Anabolic Agents. 2-Methylene-5α-androstane Derivatives

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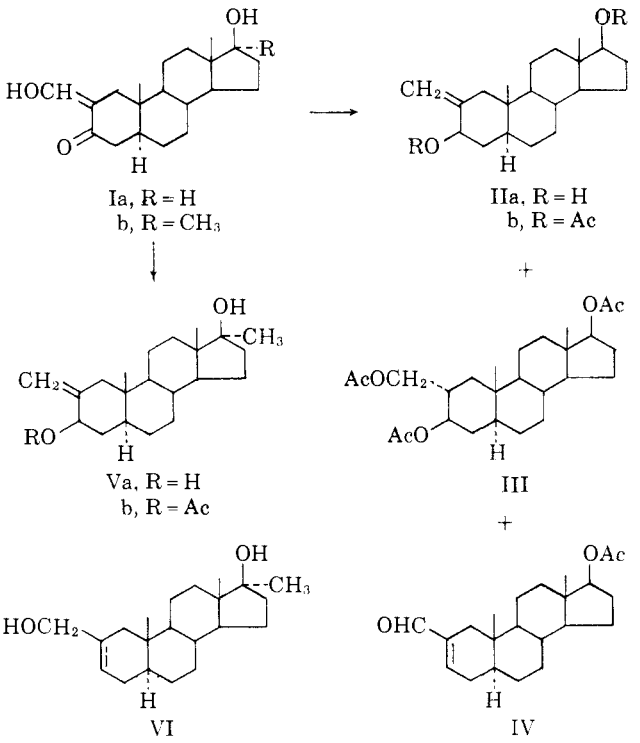
Several derivatives of 2-methylene-5α-androstane were prepared in the hope of obtaining compounds with high anabolic and minimal androgenic activity. The synthesis and biological properties of these compounds are described.

Recent publications from the Syntex laboratories[1,2] concerning 2-methylene-3-oxygenated androstanes prompt us to report some of our efforts in this area. In a previous publication,[3] we had noted that shifting the double bond of testosterone and 17α-methyltestosterone from the 4,5-position to the 1,2-position enhanced anabolic and, to a lesser extent, androgenic activity. Moreover, it was found that methylation of these isomeric products at C-2 had little effect on their relative potencies. As a consequence of these findings it seemed of interest to determine the biological effect of shifting the double bond in the A ring to an exocyclic position. As a result, several 2-methylene-3-oxygenated androstane derivatives were synthesized and evaluated biologically.



In 1953, Dreiding and Hartman[4] reported that reduction of 2-hydroxymethylenecyclohexanone with an excess of lithium aluminum hydride (LAH) in boiling ether gave a mixture of 2-methylenecyclohexanol, 1-hydroxymethylcyclohexene, and 2-hydroxymethylcyclohexanol in a ratio of 5:2:1. More recently, Knox and Velarde[1] treated 2-hydroxymethylene-17α-methyl-5α-androstan-17β-ol-3-one (Ib) with an excess of LAH in ether at room temperature. They obtained 2α-hydroxymethyl-17α-methyl-5α-androstan-17β-ol-3-one in 45% yield along with 2-methylene-17α-methyl-5α-androstane-3β,17β-diol (Va) and 2α-hydroxymethyl-17α-methyl-5α-androstane-3β,17β-diol in 15 and 3.5% yield, respectively. In contrast with the study by Dreiding and Hartman, 2-hydroxymethyl-17α-methyl-5α-androst-2-en-17β-ol (VI) could not be detected in their reduction mixture.

In our studies, the 2-hydroxymethylene derivatives (I) were reduced with an excess of LAH in refluxing dioxane. Treatment of Ia in this manner followed by acetylation of the crude product and column chromatography afforded the 2-methylene diacetate (IIb) and the triol triacetate (III) in yields of 62 and 12%, respectively. To our surprise, these products were accompanied by 2-formyl-5α-androst-2-en-17β-ol acetate (IV) in 3.7% yield and not the expected 2-hydroxymethyl-5α-androst-2-en-17β-ol diacetate. Although 2-formyl-Δ^2-steroids have been prepared by metal hydride reduction of ethers of 2-hydroxymethylene-3-ketosteroids,[5] the starting materials used in our experiments were prepared in such a way as to preclude the presence of any 2-alkoxymethylene impurities. Moreover, the physical constants and spectral properties of Ia and Ib were in agreement with those reported in literature.[6] Thus, to our knowledge, this is the first instance of forming a 2-formyl-Δ^2-steroid by direct metal hydride reduction of a 2-hydroxymethylene-3-ketosteroid.

In contrast with the products obtained by the reduction of Ia, similar treatment of Ib furnished 2-methylene-17α-methyl-5α-androstane-3β,17β-diol 3-acetate (Vb) as the only readily isolable product. Further elution of the chromatographic column, however, gave some noncrystalline material which upon saponification afforded Va along with a trace of lower melting solid. The n.m.r. and infrared spectra of this latter product suggested 2-hydroxymethyl-17α-methyl-5α-androst-2-en-17β-ol (VI) as the probable structure.[7] The assignment of this structure was subsequently confirmed by comparison of the infrared spectrum with that of an authentic sample of VI prepared according to the procedure described by Orr and co-workers.[5c] Thus, in this instance, any of the 2-formyl-Δ^2 product

(1) L. H. Knox and E. Velarde, *J. Org. Chem.*, **27**, 3925 (1962).

(2) J. A. Edwards, M. C. Calzada, and A. Bowers, *J. Med. Chem.*, **6**, 178 (1963).

(3) R. E. Counsell, P. D. Klimstra, and F. B. Colton, *J. Org. Chem.*, **27**, 248 (1962).

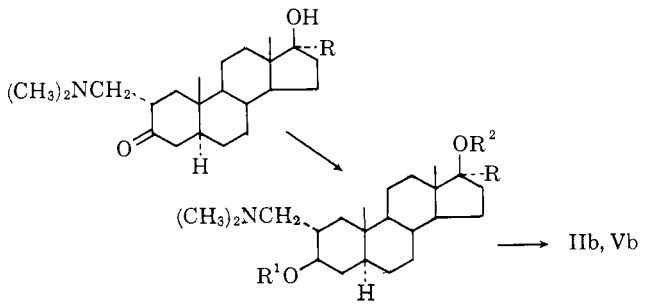
(4) A. S. Dreiding and J. A. Hartman, *J. Am. Chem. Soc.*, **75**, 939 (1953).

(5) (a) B. Fuchs and H. J. E. Loewenthal, *Tetrahedron*, **11**, 199 (1960); (b) J. C. Orr, O. Halpern, and A. Bowers, *J. Med. Pharm. Chem.*, **5**, 409 (1962); (c) J. C. Orr, O. Halpern, P. G. Holton, F. Alvarez, I. Dódan, A. de la Roz, A. M. Ruiz, and A. Bowers, *ibid.*, **6**, 166 (1963).

(6) (a) J. Edwards and H. J. Ringold, *J. Am. Chem. Soc.*, **81**, 5262 (1959); (b) H. J. Ringold, E. Batres, O. Halpern, and E. Necoechea, *ibid.*, **81**, 427 (1959); see also R. O. Clinton, A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, W. B. Dickinson, and C. Carabateas, *ibid.*, **83**, 1478 (1961).

(7) We are grateful to Dr. R. H. Bible of our Laboratories for his comments regarding the n.m.r. data.

which may have been initially formed was reduced further to the 2-hydroxymethyl compound. As noted previously, Knox and Velarde[1] were unable to detect the formation of VI under their experimental conditions. As suggested by these workers, the differences in solubilities of the intermediary enolate salts and aluminum hydride complexes in the solvents employed is probably the best explanation for these observed differences in product formation and yield.



VII a, R = H
b, R = CH_3

VIII a, R = R^1 = R^2 = H
b, R = H; R^1 = R^2 = Ac
IX a, R = CH_3; R^1 = R^2 = H
b, R = CH_3; R^1 = Ac; R^2 = H

During the course of this investigation, the desired 2-methylene derivatives also were prepared by a less ambiguous route. The appropriate 2α-dimethylaminomethyl-3-ketosteroids (VII)[3,8] were reduced with either LAH or lithium tri-*t*-butoxyaluminum hydride to the corresponding 3β-ols (VIIIa, IXa). The assignment of the β-configuration for the C-3 hydroxyl in these compounds was based on the appearance of an unresolved multiplet from 185 to 220 c.p.s. in the n.m.r. spectrum which is characteristic for C-3 axial protons.[9] These aminoalcohols were converted to their acetates (VIIIb, IXb) and oxidized with perbenzoic acid. The resulting N-oxides were not isolated but immediately pyrolyzed in refluxing *t*-butyltoluene. This gave the desired 2-methylene-3β-acetoxy derivatives (IIb, Vb) identical with the products described before. These results confirm the 3β-hydroxyl configuration for II and V which had been assigned previously by Knox and Velarde[1] on the basis of mechanistic considerations and molecular rotation data.

Biological Activity.[10]—Edwards and co-workers[2] previously reported that 2-methylene-17α-methyltestosterone possessed 15% the androgenic and 50% the anabolic activity of 17α-methyltestosterone. This reduction in biological activity produced by introduction of a 2-methylene group was even more dramatic in our cases. Whereas the 3β-hydroxy-5α-androst-1-ene derivatives were extremely potent myotrophic agents (Table I) the corresponding 2-methylene derivatives (IIb and Vb) displayed weak anabolic and androgenic activity. The low order of activity displayed by these 2-methylene derivatives has prompted interest in their evaluation as potential inhibitors of tumor growth. The results of these studies will be reported elsewhere.

18) R. Mauli, H. J. Ringold, and C. Djerassi, *J. Am. Chem. Soc.*, **82**, 5494 (1960).

19) J. N. Shoolery and M. T. Rogers, *ibid.*, **80**, 5121 (1958).

(10) We are grateful to Drs. F. J. Saunders, H. D. Lennon, and E. F. Nutting of our Endocrinology Division for furnishing us with this information.

TABLE I

ANABOLIC AND ANDROGENIC ACTIVITIES[a]

Compound	I.m. admin. Myotrophic	I.m. admin. Androgenic	Oral admin. Myotrophic	Oral admin. Androgenic
Testosterone propionate	100	100		
5α-Androst-1-ene-3β,17β-diol diacetate[3]	40	50		
IIb	<5	1		
17α-Methyltestosterone	26	24	100	100
17α-Methyl-5α-androst-1-ene-3β,17β-diol 3-acetate[3]	200	100	300–400	75–100
Va	<5	<1		
Vb	5	1	<5	<5

[a] The compounds were injected intramuscularly or given orally to castrated male rats. The potencies are given in terms of % activity of testosterone propionate (i.m.) or 17α-methyltestosterone (oral) and were determined from the minimal levels at which significant increases in seminal vesicle or levator ani muscle weights were obtained.

Experimental[11]

Lithium Aluminum Hydride Reduction of 2-Hydroxymethylene-3-ketoandrostanes. (a) Reduction of 2-Hydroxymethylene-17β-hydroxy-5α-androstan-3-one (Ia).—A solution of Ia (10 g.) in dioxane[12] (250 ml.) was added with stirring to a refluxing slurry of lithium aluminum hydride (3.8 g.) in dioxane (250 ml.). The mixture was refluxed for an additional 2 hr. and the excess hydride decomposed by the sucessive addition of water (3.8 ml.) in dioxane (50 ml.), 4 *N* sodium hydroxide solution (3.8 ml.), and water (13.3 ml.). The inorganic salts were separated and washed with isopropyl alcohol. The filtrate was concentrated to dryness and the residue dissolved in pyridine (200 ml.) and acetic anhydride (10 ml.). The solution was allowed to stand at room temperature for 24 hr. and poured into cold water (1 l.). The water was decanted from the gummy precipitate and the latter taken up in ether (400 ml.). The ether solution was washed with water, dilute hydrochloric acid (1:3), and water. The ether layer was dried over anhydrous sodium sulfate and the solvent removed by distillation under reduced pressure. The residual oil was dissolved in benzene (50 ml.) and adsorbed onto silica gel (1 kg.). Elution with benzene–ethyl acetate (49:1) afforded crude 2-methylene-5α-androstane-3β,17β-diol diacetate (IIb, 7.6 g.). Recrystallization from methanol–water gave a pure sample, m.p. 117.5–119°, $[\alpha]^{24}D$ −40°; lit.[3] m.p. 116–118°, $[\alpha]D$ −18°.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 74.26; H, 9.30.

Elution with benzene–ethyl acetate (19:1) gave 2-formyl-5α-androst-2-en-17β-ol acetate (IV, 0.4 g.), m.p. 158.5–162.5°; lit.[5b] m.p. 161–163°; ϵ^{241}_{max} 12,750; ν_{max} 3.41, 5.65, 5.78, 5.94, 6.07, and 7.94 μ; n.m.r. 43, 48 (C-18 and C-19 methyls), 123 (acetoxy), 260–285 (C-17 proton), 395–412 (C-3 proton), and 564 c.p.s. (formyl proton). Further elution with benzene–ethyl acetate (9:1) gave 2α-hydroxymethyl-5α-androstane-3β,17β-diol triacetate (III, 1.7 g.), m.p. 156.5–158°, $[\alpha]^{26}D$ −29.5°; lit.[1] m.p. 162–163°, $[\alpha]D$ −23.5°.

Anal. Calcd. for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 69.56; H, 8.97.

(b) Reduction of 2-Hydroxymethylene-17β-hydroxy-17α-methyl-5α-androstan-3-one (Ib).—Reduction of Ib (10 g.) with

(11) Optical rotations, spectra, and analytical data were furnished by Dr. R. T. Dillon, Mr. E. Zelinski, and Mr. J. Damascus of our analytical department. The optical rotations and infrared spectra were obtained in chloroform and the ultraviolet spectra in methanol unless otherwise specified. The n.m.r. spectra were obtained with a Varian A-60 spectrometer and are reported in c.p.s. downfield from tetramethylsilane which was used as the internal standard. Deuteriochloroform was used as the solvent unless otherwise specified. The melting points were obtained on a Fisher-Johns apparatus and are corrected.

(12) Purified dioxane as obtained from Pierce Chemical Company was used in these experiments.

lithium aluminum hydride and acetylation of the crude product was carried out as described in section a. The acetylated product was dissolved in benzene (125 ml.) and adsorbed onto silica gel (1 kg.). Elution with ethyl acetate–benzene (1:19) gave crude 2-methylene-17α-methyl-5α-androstane-3β,17β-diol 3-acetate (Vb, 8.3 g.) which upon crystallization from acetone–heptane afforded essentially pure material (5.4 g.), m.p. 120.5–121.5°. Recrystallization from methanol–water gave an analytical sample, m.p. 126–127°, $[\alpha]^{25}D$ −43°; lit.[2] m.p. 125–127° $[\alpha]D$, −49°.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 76.37; H, 9.84.

Further elution with the same solvent system gave oils (0.8 g.) which resisted crystallization. Saponification of this material with methanolic sodium hydroxide followed by addition of water gave a solid from which two products were obtained by fractional crystallization from acetone–water. The higher melting product was identical with 2-methylene-17α-methyl-5α-androstane-3β,17β-diol (Va) described later. The lower melting material (50 mg.), m.p. 160–162°, exhibited an n.m.r. spectrum suggestive of 2-hydroxymethyl-17α-methyl-5α-androst-2-en-17β-ol (VI) [*i.e.*, n.m.r. (CD_3COCD_3), 47, 52 (C-18 and C-19 methyls), 70 (C-17 methyl), 234 (2-hydroxymethyl), and 325–345 c.p.s. (C-3 vinyl proton)]. That this was the case was shown by comparison of the infrared spectrum with that of an authentic sample of VI (m.p. 169–171°, $[\alpha]^{23}D$ +31.5°) prepared according to the procedure of Orr and co-workers.[5c]

2α-Dimethylaminomethyl-5α-androstane-3β,17β-diol (VIIIa).—A solution of 2α-dimethylaminomethyl-17β-hydroxy-5α-androstan-3-one[8] (VIIa, 10.5 g.) in tetrahydrofuran (100 ml.) was added dropwise with stirring to a refluxing slurry of lithium aluminum hydride (3.8 g.) in tetrahydrofuran (100 ml.). After refluxing the reaction mixture for 30 min., the excess hydride was decomposed by the successive dropwise addition of water (3.8 ml.) in dioxane (100 ml.), 4 *N* sodium hydroxide solution (3.5 ml.), and water (13.3 ml.). The insoluble salts were removed by filtration of the hot reaction mixture. The salts were washed with dioxane and the filtrate concentrated to dryness *in vacuo*. The residue was recrystallized from methanol–ethyl acetate to give VIIIa (7.5 g.), m.p. 202–208°. One additional recrystallization afforded an analytical sample, m.p. 217–219°, $[\alpha]^{25}D$ +13.5° (MeOH).

Anal. Calcd. for $C_{22}H_{39}NO_2$: C, 75.59; H, 11.25. Found: C, 75.49; H, 11.46.

2α-Dimethylaminomethyl-5α-androstane-3β,17β-diol Diacetate (VIIIb).—A solution of VIIIa (3.5 g.) in pyridine (35 ml.) and acetic anhydride (5 ml.) was allowed to stand at room temperature for 18 hr. The reaction was poured into ice and water (75 ml.) and made basic by the addition of a 4 *N* sodium hydroxide solution. The resulting precipitate was collected by filtration and washed with water to give crude VIIIb (3.9 g.), m.p. 117–121°. Recrystallization from pentane afforded an analytical sample, m.p. 127–128.5°, $[\alpha]^{25}D$ −55.5°.

Anal. Calcd. for $C_{26}H_{43}NO_4$: C, 72.01; H, 10.00. Found: C, 72.19; H, 9.80.

2α-Dimethylaminomethyl-17α-methyl-5α-androstane-3β,17β-diol (IXa).—A solution of lithium-tri-*t*-butoxyaluminum hydride (45 g.) in tetrahydrofuran (300 ml.) was added all at once with stirring to an ice-cold solution of 2α-dimethylaminomethyl-17β-hydroxy-17α-methyl-5α-androstan-3-one[9] (VIIIb) (20.0 g.) in tetrahydrofuran (300 ml.). The reaction mixture was stirred for 20 min. and poured into ice–water (3.5 l.) containing acetic acid (200 ml.). The clear solution was washed repeatedly with ether and made basic with aqueous saturated carbonate solution. The aqueous mixture was extracted with chloroform and the combined extract washed with water. The extract was dried over anhydrous potassium carbonate containing Darco and the solvent removed *in vacuo*. The solid residue was recrystallized from acetone to give IXa (17.3 g., m.p. 255–256.5°, $[\alpha]^{25}D$ +7°, n.m.r. 51, 52 (C-18 and C-19 methyls), 72 (C-17 methyl), 136 (dimethylaminomethyl), and 185–220 c.p.s. (C-3 axial proton)).[13]

Anal. Calcd. for $C_{23}H_{41}NO_2$: C, 75.97; H, 11.37. Found: C, 75.98; H, 11.29.

2α-Dimethylaminomethyl-17α-methyl-5α-androstane-3β,17β-diol 3-Acetate (IXb).—A mixture of IXa (4.9 g.) in triethylamine (75 ml.) and acetic anhydride (20 ml.) was stirred at room temperature for 18 hr. The reaction gradually became homogeneous and was poured into ice–water (125 ml.). The solution was made basic by the addition of 5% aqueous sodium bicarbonate. The crude product was collected, washed with water, and recrystallized from methanol–water to give pure IXb (3.2 g.), m.p. 220–224° dec.; $[\alpha]^{25}D$ −69°.

Anal. Calcd. for $C_{25}H_{43}NO_3$: C, 74.03; H, 10.69. Found: C, 73.68; H, 10.68.

2-Methylene-5α-androstane-3β,17β-diol Diacetate (IIb). To a solution of VIIIb (2.0 g.) in chloroform (50 ml.) was added a solution of perbenzoic acid in benzene (1.54 *N*, 10 ml.). The reaction vessel was surrounded with an ice–water bath during the addition. The solution was allowed to stand at room temperature for 20 min. and then washed successively with three 50-ml. portions of 10% sodium carbonate solution, 10% sodium iodide solution (50 ml.), and two 50-ml. portions of 1% sodium thiosulfate solution. The chloroform layer was dried over anhydrous sodium sulfate and the solvent removed by distillation. The resulting N-oxide was refluxed in *t*-butyltoluene (10 ml.) for 0.5 hr. Ether (50 ml.) was added to the cooled reaction mixture and the solution washed with 2 *N* hydrochloric acid and water. The solution was dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. Crystallization of the resulting product from methanol afforded pure IIb (0.7 g.) identical with that described previously.

2-Methylene-17α-methyl-5α-androstane-3β,17β-diol 3-Acetate (Vb).—Formation and pyrolysis of the N-oxide of IXb (2.0 g.) as described for IIb gave a crude product which crystallized from methanol–water as pure Vb (0.8 g.) identical with the material obtained previously.

2-Methylene-17α-methyl-5α-androstane-3β,17β-diol (Va).—A solution of Vb (2.0 g.) and potassium hydroxide (1.0 g.) in methanol (40 ml.) and water (1 ml.) was stirred at room temperature for 1 hr. Addition of water (50 ml.) gave a quantitative yield of crude Va, m.p. 220.5–223°. Two recrystallizations from ethanol gave needles (1.6 g.), m.p. 229.5–230.5°, $[\alpha]^{25}D$ −41°; lit.[2] m.p. 233–235°, $[\alpha]D$ −36°.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.19; H, 10.76. Found: C, 79.12; H, 10.77.

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(13) 2α-Dimethylaminomethyl-17α-methyl-5α-androstane-3α,17β-diol (m.p. 171.5 (73%, $[\alpha]^{25}D$ −2.5°), the C-3 epimer of IXa, has been isolated in our laboratories along with IXa as a minor by-product in the catalytic hydrogenolysis of VIIIb.[9] The n.m.r. spectrum of this substance displayed peaks at 47, 50 (C-18 and C-19 methyl), 72 (C-17 methyl), 133 (dimethylaminomethyl), and 237–241 c.p.s. (3β-proton).

Anal. Calcd. for $C_{23}H_{41}NO_2$: C, 75.97; H, 11.37. Found: C, 75.58; H, 11.05.