Synthesis of Isomeric 5α-Androstane-3,15,17β-triols

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3α-Acetoxy-16α-bromo-5α-androstan-17-one (1) was converted into 16α-bromo-17,17-ethylenedioxy-5α-androstan-3α-ol (2), which was dehydrobrominated and hydrolysed to give 3α-hydroxy-5α-androst-15-en-17-one (5). Addition of benzyl alcohol to the Δ^{15}-3α-ol (5) followed by hydrogenolysis yielded 3α,15β-dihydroxy-5α-androstan-17-one (7). This was converted into 5α-androstane-3α,15β,17β-triol (8). Similarly 5α-androstane-3β,15β,17β-triol (10) was prepared from 3β-hydroxy-5α-androst-15-en-17-one (3). Hydroboration of 5α-androst-14-ene-3α,17β-diol (12) afforded 5α-androstane-3α,15α-17β-triol (13). The spectral properties of 5α-androstane-3,15,17β-triols are markedly affected by the configuration of the hydroxy-group at C-15.

In a paper [1] dealing with the biliary metabolites of testosterone sulphate in the rat, we reported the production of considerable quantities of disulphates of 5α-androstane-3α,17β-diol and polar hydroxylated steroids in the female. Gustafsson and Ingelman-Sundberg [2] recently described the 15β-hydroxylation of the 3,17-disulphates of 5α-androstane-3α,17β-diol and the corresponding 3β-epimer in female rat liver microsomes. These results strongly suggest the occurrence of 15β-hydroxylated steroids in biliary metabolites. As authentic standards, 5α-androstane-3,15,17β-triols were prepared by incubation of 15α- and 15β-hydroxyandrost-4-ene-3,17-dione with rat liver microsomes, and the respective trimethylsilyl ethers were subjected to g.l.c.–mass spectrometric analysis.[2] Of the four isomeric 3,15,17β-triols, 5α-androstane-3β,15α,17β-triol (15) has been prepared by Nussim and his co-workers.[3] We now describe the synthesis of 5α-androstane-3α,15β,17β-triol (8), -3β,15β,17β-triol (10), and -3α,15α,17β-triol (13).

The 15β-hydroxy-group has been introduced into the steroid nucleus by nucleophilic addition of a benzyl alcohol to a Δ^{15}-17-oxo steroid, followed by catalytic hydrogenolysis,[4,5] and 15α-hydroxy-steroids have been prepared by hydroboration [3] of Δ^{14}-steroids. We have applied these reactions to the synthesis of the 5α-androstane-3,15,17β-triols.

3α-Acetoxy-16α-bromo-5α-androstan-17-one (1) [6] was acetalised with ethylene glycol; [7] dehydrobromination of the bromo-acetal (2) with potassium t-butoxide in refluxing xylene [7] then gave the unexpected 3β-epimerisation product, 3β-hydroxy-5α-androst-15-en-17-one (3) (after hydrolysis of the acetal with toluene-*p*-sulphonic acid). The structure (3) was confirmed by catalytic hydrogenation to give 3β-hydroxy-5α-androstan-17-one (4), identical with an authentic sample.[7] However, mild dehydrobromination of the bromo-acetal (2) with potassium t-butoxide in dimethyl sulphoxide at 37 °C [5] and subsequent deacetalisation provided a route to 3α-hydroxy-5α-androst-15-en-17-one (5), catalytic hydrogenation of the 15,16-double bond affording 3α-hydroxy-5α-androstan-17-one (6). Base-catalysed addition of benzyl alcohol to the Δ^{15}-3α-ol (5) followed by hydrogenolysis over palladium–charcoal [4] gave 3α,15β-dihydroxy-5α-androstan-17-one (7), which was converted into the 3α,15β,17β-triol (8) with sodium borohydride. Similarly, condensation of the Δ^{15}-3β-ol (3) with benzyl alcohol and hydrogenolysis yielded 3β,15β-dihydroxy-5α-androstan-17-one (9), which was reduced with sodium borohydride to the 3β,15β,17β-triol (10).

TABLE 1

Molecular rotation and ^{1}H n.m.r. data for the 3,15,17β-triols

	Molecular rotation		^{1}H N.m.r. (δ)	
Compd.	$[\alpha]_D$ (°)	M_D (°)	18-H_3	19-H_3
(8)	+17	+54	1.01	0.83
(10)	−16	−49	1.01	0.87
(13)	+97	+299	0.76	0.81
(15)	+63	+194	0.76	0.84

3α-Hydroxy-5α-androst-14-en-17-one (11), prepared by acid-catalysed epimerisation [5] of the Δ^{15}-3α-ol (5), was reduced with sodium borohydride to give 5α-androst-14-ene-3α,17β-diol (12). Hydroboration followed by oxidation with alkaline hydrogen peroxide [3] then afforded the 3α,15α,17β-triol (13). The 3β,15α,17β-triol (15) [3] was also prepared from 5α-androst-14-ene-3β,17β-diol (14) [8] by the hydroboration method.

[1] M. Matsui, Y. Kinuyama, and M. Hakozaki, *Steroids*, 1975, **25**, 637.

[2] J. A. Gustafsson and M. Ingelman-Sundberg, *F.E.B.S. Letters*, 1973, **31**, 292; *J. Biol. Chem.*, 1974, **249**, 1940; 1975, **250**, 3451.

[3] M. Nussim, Y. Mazur, and F. Sondheimer, *J. Org. Chem.*, 1964, **29**, 1120.

[4] E. W. Cantrall, R. Littell, and S. Bernstein, *J. Org. Chem.*, 1964, **29**, 64; 214.

[5] R. W. Kelly and P. J. Sykes, *J. Chem. Soc.* (*C*), 1968, 416.

[6] J. Fajkos and F. Sorm, *Coll. Czech. Chem. Comm.*, 1959, **24**, 766.

[7] F. Sondheimer, S. Burstein, and R. Mechoulam, *J. Amer. Chem. Soc.*, 1960, **82**, 3209.

[8] A. F. St. André, H. B. MacPhillamy, J. A. Nelson, C. A. Shabica, and C. R. Scholz, *J. Amer. Chem. Soc.*, 1952, **74**, 5506.

TABLE 2

Relative intensities of principal fragments in the mass spectra of the 3,15,17β-triols (75 eV)

	m/e									
Compound	308 (M^+)	290	275	272	264	246	217	147	121	107
(8)	3	63	30	45	15	35	100	48	48	87
(10)	3	66	22	44	18	30	100	42	52	90
(13)	25	32	21	20	100	13	44	29	28	52
(15)	18	39	23	31	100	17	39	40	37	59

The spectral properties of the isomeric 3,15,17β-triols are markedly affected by the configuration of the C-15 hydroxy-group. Molecular rotation analysis (Table 1) indicated that the 15α-ols [(13) and (15)] were more dextrorotatory than the 15β-epimers [(8) and (10)], in accord with other epimeric 15-hydroxy-steroids.[4,9] The ^{1}H n.m.r. data (Table 1) showed a large downfield shift of the 13-methyl signal of the 15β-ols due to pseudo-1,3-diaxial deshielding[10] by the 15β-hydroxy-group. The mass spectrometric fragmentations (Table 2) provided strong evidence for the configuration of the 15-hydroxy-group. Prominent ions at *m/e* 107 and 264 are characteristic of 15α-ols, whereas the mass spectra of the 15β-ols are dominated by fragments of *m/e* 107, 217, and 290. Generally the 15α-hydroxy-group confers greater polarity than does the 15β-hydroxy-group.[9,11] T.l.c. showed that the 15β-ols were more mobile than the 15α-ols. G.l.c. of the trimethylsilyl ether derivatives on 0.5% CHDMS (or 1.5% SE-30) gave retention times (relative to 5α-cholestane) of 0.34 (0.70), 0.43(0.71), 0.55(0.90), and 0.68(0.99) for the 3α,15β,17β-triol (8), the 3α,15α,17β-triol (13), the 3β,15β,17β-triol (10), and the 3β,15α,17β-triol (15), respectively.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. U.v. spectra were recorded on a Hitachi 124 spectrophotometer. Optical rotations were measured for ethanolic solutions with a JASCO DIP-SL automatic polarimeter. ^{1}H N.m.r. spectra were determined for solutions in deuteriochloroform with tetramethylsilane as internal standard on a JEOL JNM-MH-100 spectrophotometer. Mass spectra were measured with a JEOL JMS-D100 spectrometer with a direct inlet system. G.l.c. was performed on a Shimadzu GC-4BM flame ionisation chromatograph with 0.5% cyclohexane-1,4-dimethanol succinate polyester (CNDMS) (2.0 m × 3 mm) or 1.5% SE-30 (1.5 m × 3 mm) at 200 or 230 °C, respectively. Analytical and preparative t.l.c. were carried out on glass plates (5 × 20 and 20 × 20 cm, respectively) coated with a 0.5 mm thick film of silica gel GF (Merck).

16α-*Bromo*-17,17-*ethylenedioxy*-5α-*androstan*-3α-*ol* (2).—A solution of 3α-acetoxy-16α-bromo-5α-androstan-17-one (1)[6] (m.p. 184—187°; 2.7 g), toluene-*p*-sulphonic acid monohydrate (0.46 g), and ethylene glycol (25 ml) in toluene (120 ml) was distilled slowly for 50 h; then half the solvent was removed. The solution was cooled and washed with aqueous 5% sodium hydrogen carbonate, and water. The organic layer was dried and evaporated *in vacuo*. The residue was crystallised from methanol to give the *bromo-acetal* (2) (2.1 g), m.p. 164—172° (170—177° after recrystal-

[9] G. F. Gibbons and K. Ramananda, *J.C.S. Chem. Comm.*, 1975, 213.

[10] Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, and K. Tsuda, *Chem. and Pharm. Bull. (Japan)*, 1962, **10**, 338.

[11] C. Djerassi, G. von Muzenbecher, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *J. Amer. Chem. Soc.*, 1965, **87**, 817.

lisation from methanol) (Found: C, 61.3; H, 7.9%; M^+, 413. $C_{21}H_{33}BrO_3$ requires C, 61.0; H, 8.0%; M, 413); $[\alpha]_D^{28}$ −21° (*c* 0.1); δ 0.76 (3 H, s, 18-H_3), 0.86 (3 H, s, 19-H_3), 3.90—4.20 (5 H, m, O·CH_2·CH_2·O and 3-H), and 4.36 (1 H, m, 16-H).

3β-*Hydroxy*-5α-*androst*-15-*en*-17-*one* (3).—A solution of potassium (1.4 g) in t-butyl alcohol (70 ml) was distilled to dryness under reduced pressure. Xylene (70 ml) was added to the residue, and removed by distillation, and this procedure was repeated twice more. A solution of the bromo-acetal (2) (2.0 g) in xylene (70 ml) was added, and the mixture was heated under reflux in nitrogen for 18 h. The mixture was cooled, poured into water, and extracted with ether. The organic layer was washed with water, dried, and evaporated. The residue (1.36 g) was dissolved in acetone (90 ml), a solution of toluene-*p*-sulphonic acid monohydrate (60 mg) in water (15 ml) was added, and the resultant solution was kept at 20 °C for 3 h. The solution was then extracted with ether, washed with water, aqueous 5% sodium hydrogen carbonate, and water, and dried. Evaporation left a residue (1.2 g), which was purified by preparative t.l.c. (11 plates) in chloroform–acetone (20 : 1). The strongly u.v.-absorbing zones (R_F 0.3) were scraped off and eluted with methanol–ethyl acetate (1 : 1). Evaporation left a residue (0.77 g), which was crystallised from acetone–petroleum to give the Δ^{15}-3β-ol (3) (0.41 g), m.p. 155—158° (lit.,[7] 162—163°), identical (mixed m.p., u.v., and n.m.r. spectra, and t.l.c.) with an authentic specimen.[7]

3β-*Hydroxy*-5α-*androstan*-17-*one* (4).—A solution of the Δ^{15}-3β-ol (3) (16 mg) in ethanol (5 ml) was hydrogenated over pre-hydrogenated platinum oxide (14 mg) at 20 °C for 20 min at atmospheric pressure. The catalyst was removed and the solution was evaporated. The residue was crystallised from acetone–n-hexane to afford the 3β-hydroxy-17-one (4) (12 mg), m.p. 166—170° (lit.,[12] 174—175°), identical (mixed m.p., n.m.r. spectrum, and t.l.c.) with an authentic sample.

3α-*Hydroxy*-5α-*androst*-15-*en*-17-*one* (5).—A solution of potassium (1.1 g) in t-butyl alcohol (60 ml) was distilled to dryness *in vacuo*. Xylene (60 ml) was added to the residue and removed by distillation, and this procedure was repeated twice. A solution of the bromo-acetal (2) (5.6 g) in dimethyl sulphoxide (90 ml) was added under nitrogen, and the flask was stoppered and kept at 37 °C for 16 h. The mixture was poured into water, and extracted with ether. The organic layer was washed with water, dried, and evaporated. The residue was dissolved in acetone (300 ml), and a solution of toluene-*p*-sulphonic acid monohydrate (300 mg) in water (20 ml) was added. The resultant solution was kept at 20 °C for 7 h. Water (100 ml) was added, and the solution was evaporated to half its volume, then extracted with ethyl acetate. The extract was washed with water, aqueous 5% sodium hydrogen carbonate, and water, and dried. Evaporation left a residue (5.0 g), which was crystallised from acetone–n-hexane to give the Δ^{15}-3α-ol (5) (1.8 g), m.p. 153—160° (158—162° after recrystallisation from acetone–n-hexane) (Found: C, 78.6; H, 9.9%; M^+, 288. $C_{19}H_{28}O_2$ requires C, 79.1; H, 9.8%; M, 288); $[\alpha]_D^{20}$ −48° (*c* 0.1); $\lambda_{max.}$ (EtOH) 232 nm (ε 7 210); δ 0.84 (3 H, s, 19-H_3), 1.05 (3 H, s, 18-H_3), 4.08 (1 H, m, 3-H), 6.04 (1 H, m, 15-H), and 7.57 (1 H, d, *J* 6 Hz, 16-H).

3α-*Hydroxy*-5α-*androstan*-17-*one* (6).—A solution of the Δ^{15}-3α-ol (5) (28 mg) in ethanol (5 ml) was hydrogenated over pre-reduced platinum oxide (25 mg) as described for the 3β-hydroxy-17-one (4); the product was crystallised from acetone–n-hexane to afford the 3α-hydroxy-17-one (6) (23 mg), m.p. 178—182° (lit.,[13] 181—182°), identical (mixed m.p., n.m.r. spectrum, and t.l.c.) with an authentic sample.

3α,15β-*Dihydroxy*-5α-*androstan*-17-*one* (7).—To a solution of the Δ^{15}-3α-ol (5) (200 mg) in benzyl alcohol (10 ml) was added powdered potassium hydroxide (300 mg). The mixture was stirred under nitrogen at 20 °C for 3.5 h, then extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated *in vacuo* to dryness. The residue was dissolved in acetic acid (10 ml) and 5% palladium–charcoal (100 mg) was added. The mixture was hydrogenated at 20 °C for 24 h at atmospheric pressure. The catalyst was removed, and the solution was evaporated. The residue (230 mg) was purified by preparative t.l.c. (4 plates) in chloroform–acetone (5 : 1). The zones at R_F 0.2 were located by spraying with water, scraped off, and eluted with methanol–ethyl acetate (1 : 1). Evaporation left a residue (177 mg) which was crystallised from acetone to afford the 3α,15β-*hydroxy*-17-*one* (7) (144 mg), m.p. 214—219° (Found: C, 74.5; H, 9.8%; M^+, 306. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%; M, 306); $[\alpha]_D^{20}$ +87° (*c* 0.1); δ 0.84 (3 H, s, 19-H_3), 1.17 (3 H, s, 18-H_3), 2.52 (2 H, m, 16-H_2), 4.08 (1 H, m, 3-H), and 4.56 (1 H, m, 15-H).

5α-*Androstane*-3α,15β,17β-*triol* (8).—To a solution of the 3α,15β-hydroxy-17-one (7) (65 mg) in methanol (5 ml) was added sodium borohydride (50 mg) with cooling in ice–water. After 0.5 h, the solution was poured into water, and extracted with chloroform. The organic layer was washed with water, dried, and evaporated to give a crystalline residue (65 mg), which was crystallised from acetone to give the 3α,15β,17β-*triol* (8) (48 mg), m.p. 231—233° (Found: C, 71.7; H, 10.3%; M^+, 308. $C_{19}H_{32}O_3$,0.5H_2O requires C, 71.9; H, 10.5%; M, 308); $[\alpha]_D^{20}$ +17° (*c* 0.1); δ 0.83 (3 H, s, 19-H_3), 1.01 (3 H, s, 18-H_3), 3.60 (1 H, m, 17-H), 4.08 (1 H, m, 3-H), and 4.20 (1 H, m, 15-H).

3β,15β-*Dihydroxy*-5α-*androstan*-17-*one* (9).—To a solution of the Δ^{15}-3β-ol (3) (200 mg) in benzyl alcohol (10 ml) was added powdered potassium hydroxide (300 mg). Work-up as described for the 3α,15β-hydroxy-17-one (7) gave the 3β,15β-*hydroxy*-17-*one* (9) (92 mg), m.p. 210—217° (Found: C, 74.5; H, 9.9%; M^+, 306. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%; M, 306); $[\alpha]_D^{28}$ +69° (*c* 0.1); δ 0.88 (3 H, s, 19-H_3), 1.18 (3 H, s, 18-H_3), 2.55 (2 H, m, 16-H_2), 3.60 (1 H, m, 3-H), and 4.60 (1 H, m, 15-H).

5α-*Androstane*-3β,15β,17β-*triol* (10).—The 3β,15β-hydroxy-17-one (9) (50 mg) was reduced with sodium borohydride as described for the 3α,15β,17β-triol (5) to afford the 3β,15β,17β-*triol* (10) (38 mg), m.p. 237—238° (Found: C, 74.1; H, 10.8%; M^+, 308. $C_{19}H_{32}O_3$ requires C, 74.0; H, 10.5%; M, 308); $[\alpha]_D^{28}$ −16° (*c* 0.1); δ 0.87 (3 H, s, 19-H_3), 1.01 (3 H, s, 18-H_3), 3.60 (2 H, m, 3- and 17-H), and 4.20 (1 H, m, 15-H).

3α-*Hydroxy*-5α-*androst*-14-*en*-17-*one* (11).—The Δ^{15}-3α-ol (5) (500 mg) and toluene-*p*-sulphonic acid monohydrate (50 mg) were dissolved in toluene (60 ml), and heated under reflux for 2 h. The solution was cooled, poured into water, and extracted with ethyl acetate. The organic layer was washed with water, aqueous 5% sodium hydrogen carbonate, and water, and dried. Evaporation left a syrup (490 mg), which was purified by preparative t.l.c. (6 plates) in chloroform–acetone (20 : 1). The products were located by

[12] L. Ruzicka, M. W. Goldberg, J. Meyer, H. Brüngger, and E. Eichenberger, *Helv. Chim. Acta*, 1934, **17**, 1395.

[13] L. Cagliotti, G. Cainelli, G. Maina, and A. Selva, *Tetrahedron*, 1964, **20** 957.

spraying with water, and the zone corresponding to the Δ^{14}-3α-ol (11), which was more mobile than the Δ^{15}-3α-ol (5), was scraped off and eluted with methanol–ethyl acetate (1 : 1). Evaporation left a syrup (282 mg), which was crystallised from acetone–n-hexane to afford the Δ^{14}-3α-ol (11) (251 mg), m.p. 102—105° (Found: C, 78.7; H, 9.8%; M^+, 288. $C_{19}H_{28}O_2$ requires C, 79.1; H, 9.8%; M, 288); $[\alpha]_D^{20}$ +166° (*c* 0.1); δ 0.83 (3 H, s, 19-H_3), 1.09 (3 H, s, 18-H_3), 2.90 (2 H, m, 16-H_2), 4.07 (1 H, m, 3-H), and 5.50 (1 H, m, 15-H).

5α-*Androst*-14-*ene*-3α,17β-*diol* (12).—The Δ^{14}-3α-ol (11) (125 mg) was dissolved in methanol (3 ml) and sodium borohydride (130 mg) was added with cooling in ice–water. After 0.5 h, the mixture was poured into water, and extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated *in vacuo* to give a crystalline residue (125 mg), which was crystallised from acetone to afford the Δ^{14}-diol (12) (94 mg), m.p. 215—222° (Found: C, 78.3; H, 10.5%; M^+, 290. $C_{19}H_{30}O_2$ requires C, 78.6; H, 10.4%; M, 290); $[\alpha]_D^{20}$ +55° (*c* 0.1); δ 0.80 (3 H, s, 19-H_3), 0.97 (3 H, s, 18-H_3), 4.07 (2 H, m, 3- and 17-H), and 5.10 (1 H, m, 15-H).

5α-*Androstane*-3α,15α,17β-*triol* (13).—The Δ^{14}-diol (12) (90 mg) was dissolved in freshly distilled tetrahydrofuran (30 ml) and cooled in ice–water. Boron trifluoride–ether complex (1.5 g) was added, and subsequently a solution of lithium aluminium hydride (200 mg) in dry ether (9 ml) was added dropwise during 1 h under nitrogen with stirring and continuous cooling in ice–water. The mixture was then stirred at 20 °C for 2 h. Water was added dropwise, and the mixture was then extracted with ether. The organic layer was washed with water, aqueous 5% sodium hydrogen carbonate, and water, and dried. Evaporation left a residue, which was dissolved in tetrahydrofuran (9 ml). Aqueous sodium hydroxide (10%; 3 ml) was added, followed dropwise by 30% hydrogen peroxide (2.5 ml) during 10 min with stirring and cooling in ice–water. The mixture was stirred for 1 h in ice–water, poured into water, and extracted with ether. The organic layer was washed with aqueous sodium hydrogen sulphite, and water, and dried. Evaporation yielded a residue (87 mg), which was purified by preparative t.l.c. (2 plates) in benzene–ethanol (7 : 1). The zones at R_F 0.2 were located by spraying with water, scraped off, and eluted with methanol–ethyl acetate (1 : 1). Evaporation left a residue (39 mg), which was crystallised from acetone–n-hexane to afford the 3α,15α,17β-*triol* (13) (30 mg), m.p. 138—146° (softening at 130°) (Found: C, 73.8; H, 10.8%; M^+, 308. $C_{19}H_{32}O_3$ requires C, 74.0; H, 10.5%; M, 308); $[\alpha]_D^{20}$ +97° (*c* 0.1); δ 0.76 (3 H, s, 18-H_3), 0.81 (3 H, s, 19-H_3), and 3.80—4.10 (3 H, m, 3-, 15-, and 17-H).

5α-*Androstane*-3β,15α,17β-*triol* (15).—5α-Androst-14-ene-3β,17β-diol (14) [8] (m.p. 135—136°; 40 mg) was treated as described for the 3α,15α,17β-triol (13) to afford the 3β,15α,-17β-triol (15) (15 mg), m.p. 259—262°, M^+ 308; $[\alpha]_D^{28}$ +63° (*c* 0.1); δ † 0.76 (3 H, s, 18-H_3) and 0.84 (3 H, s, 19-H_3) {lit.,[3] m.p. 263—265°; $[\alpha]_D$ +49° (pyridine)}.

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† Chemical shifts of other protons are not given, because the triol was only slightly soluble in deuteriochloroform.