A SYNTHESIS OF 7α-HYDROXYANDROST-4-ENE-3,17-DIONE

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ABSTRACT

The first convenient chemical synthesis of 7α-hydroxy-androst-4-ene-3,17-dione is reported. Androsta-4,6-diene-3,17-dione was converted into its 6α,7α-epoxy-derivative; reduction of the epoxide with aluminium amalgam gave 7α-hydroxyandrost-4-ene-3,17-dione. This reducing agent is more efficient than chromous acetate for the purpose.

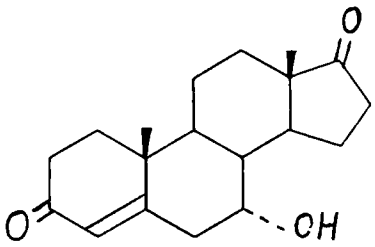
7α-Hydroxyandrost-4-ene-3,17-dione (I) has been isolated from the testicular microsomal fraction of adult rats, where it is formed from androst-4-ene-3,17-dione by the action of a 7α-hydroxylase (1). The 7α-hydroxy compound is suggested as a regulator of testosterone biosynthesis, since it inhibits enzyme reactions involved in the transformation of pregnenolone into testosterone (1).

The only reported chemical preparation of 7α-hydroxy-androst-4-ene-3,17-dione was by side-chain degradation (sodium bismuthate) of 7α,17α,21-trihydroxypregn-4-ene-3,20-dione (2) produced by 7α-hydroxylation of 'Reichstein's Compound S' by micro-organisms. Direct 7α-hydroxylation of androst-4-ene-3,17-dione by Neurospora has also been reported (3). We now describe the first wholly chemical synthesis of 7α-hydroxy-androst-4-ene-3,17-dione, starting from the readily available

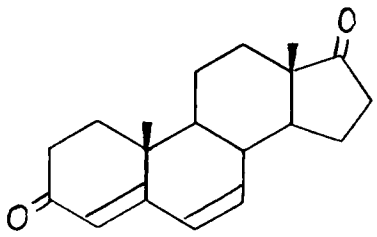
androst-4-ene-3,17-dione.

Androst-4-ene-3,17-dione was transformed into androsta-4,6-diene-3,17-dione (II) by dehydrogenation of the derived $\Delta^{3,5}$-dien-3-yl ethyl ether with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (4). Epoxidation (5,6) with 3-chloroperbenzoic acid then afforded the 6α,7α-epoxide (III), accompanied by traces of by-products from which the epoxide was separated by preparative t.l.c. We recently (7) converted the analogous 6α,7α-epoxy-17β-hydroxyandrost-4-en-3-one (IV) into 7α-hydroxytestosterone by the two-step procedure (reduction by $LiAlH_4$, and selective re-oxidation at C-3 by DDQ) used (8) in an earlier preparation of 7α-hydroxy-4-methyltestosterone. The 17-oxo group in the androstenedione derivative, however, is incompatible with this sequence. Another possible two-step route, through a 6β-bromo-7α-hydroxy derivative (5), seemed unlikely to proceed with the desired efficiency. A single-step selective reduction of a 6α,7α-epoxy-Δ^4-3-oxo steroid with chromous acetate was recently reported (9) to give the corresponding 7α-hydroxy-4-en-3-one, but even the use of a buffered medium could not prevent the simultaneous formation of an equal proportion of the 4,6-dien-3-one. Applied to our present synthesis, the chromous acetate procedure gave 7α-hydroxyandrost-4-ene-3,17-dione, but in such impure condition and poor yield (10%) that we sought an alternative reducing agent.

Aluminium amalgam, a very familiar reagent to chemists of an earlier generation, has recently reappeared as a highly efficient reducing agent for the preparation of



I



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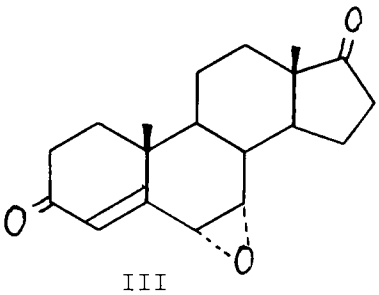

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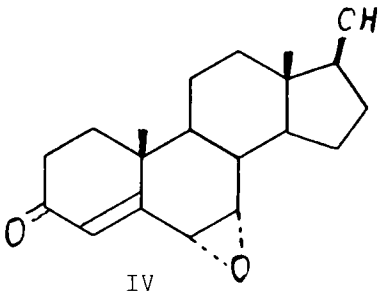

IV

β-hydroxyketones from α,β-epoxyketones (10). As a selective reductant for 6α,7α-epoxyandrost-4-ene-3,17-dione it proved to be much more satisfactory than chromous acetate, giving 7α-hydroxyandrost-4-ene-3,17-dione (I) in 62% yield after purification by preparative t.l.c. The 4,6-dienone (II) and an unidentified by-product were formed only in traces by this reagent.

EXPERIMENTAL

Preparative t.l.c. was carried out on Merck silica gel HF 254. Chromous acetate was from Lancaster Synthesis Ltd., or was freshly prepared (11). I.r. spectra were determined for KBr discs. N.m.r. spectra were determined at 100 MHz for solutions in $CDCl_3$, with Me_4Si as internal standard, by Dr. R.E. Morgan.

Androsta-4,6-diene-3,17-dione (II) was prepared from testosterone, by oxidation with Jones' reagent to give androst-4-ene-3,17-dione, which was converted to 3-ethoxy-androsta-3,5-dien-17-one and dehydrogenated with DDQ as described (4). The diene-dione had m.p. 170-172° [lit. (4) m.p. 169-170°]. Yield: 71% over-all.

6α,7α-Epoxyandrost-4-ene-3,17-dione (III). Androsta-4,6-diene-3,17-dione (756 mg) in dichloromethane (15 ml) was treated with 3-chloroperbenzoic acid (690 mg), added in small portions with stirring until a clear solution was obtained. Anhydrous sodium sulphate (250 mg) was then added (6), and the mixture was stirred at room temperature for 24 hr. Solids were then collected on a filter and washed with a little dichloromethane, and the filtrates, diluted with dichloromethane (50 ml), were washed with sodium hydrogen carbonate solution (10%) and water, dried ($MgSO_4$), and taken to dryness under reduced pressure. The resulting crude epoxide was purified by preparative t.l.c., with benzene-ether (1:1) as the developing solvent. The principal u.v.-absorbing band was collected and eluted with ethyl acetate to give the 6α,7α-epoxide (III), (286 mg) m.p. 216-222° (from ethyl acetate), ν_{max} 1740 (17-CO), 1670, 1615 (Δ^4-3-CO), 1269, 1230, and 875 cm^{-1}; $[\alpha]_D$ +121° (c 1.1, dioxan); n.m.r. τ 9.07 (s, 18-H_3), 8.89 (s, 19-H_3), 4.51, 4.46 (dd, J=4Hz, 6,7-H_2), 3.86 (s, 4-H). (Found: C, 76.1; H, 7.8. $C_{19}H_{24}O_3$ requires C, 76.0; H, 8.0%).

A more mobile band on the t.l.c. plate afforded unreacted androsta-4,6-diene-3,17-dione (153 mg). Attempts to improve the conversion to epoxide by using more per-acid or longer reaction time led to the appearance of more-polar by-products.

Reduction of the 6α,7α-epoxide (III): Preparation of 7α-hydroxyandrost-4-ene-3,17-dione (I).-

(a) with Chromous acetate. A solution of the 6α,7α-epoxide (1.8 g) in acetone (350 ml) was stirred magnetically during the addition of a solution of sodium acetate trihydrate (20 g) in water (50 ml) and glacial acetic acid (10 ml), then solid chromous acetate (9 g) was added all at once and the mixture was stirred vigorously for 45 min. Most of the solvent was then removed under reduced pressure (rotary evaporator, bath temperature 25°), and the residue was triturated with water and extracted several times with ethyl acetate. The combined extracts were washed with water until neutral, and dried (Na_2SO_4). The solvent was removed under reduced pressure at 25°, and the product was purified by preparative t.l.c., using benzene-acetone (4:1) as the developing solvent. The u.v.

absorbing band corresponding in polarity to 7α-hydroxy-androst-4-ene-3,17-dione was collected and eluted with ethyl acetate. Crystallisation from acetone-hexane gave the 7α-hydroxy compound (178 mg; 10%), m.p. 230-234°, essentially identical with the sample prepared by procedure (b), although of slightly lower m.p. The t.l.c. plate contained several additional bands, one of which indicated the presence of a considerable proportion of androsta-4,6-dione-3,17-dione. The other products were not identified.

(b) with aluminium amalgam (recommended procedure).- The amalgam was prepared by adding aluminium (5 g; B.D.H. Analar grade, cut into small pieces) to a solution of mercuric chloride (1.2 g) in water (20 ml) containing concentrated hydrochloric acid (0.5 ml), with shaking for 5 min. The liquid was then decanted, and the amalgam was washed with distilled water until the washings were neutral, then used immediately. The 6α,7α-epoxide (150 mg) in 5% aqueous ethanol (5 ml) was treated with 10% sodium hydrogen carbonate solution (0.5 ml) and stirred in an ice-salt bath below -5°C. Freshly prepared aluminium amalgam (1.5 g) was added, and stirring was continued at the same temperature for 6-7 hr, when t.l.c. showed complete disappearance of the epoxide. Chloroform was then added, and the mixture was filtered. Evaporation of solvents afforded the crude 7α-hydroxy compound which was purified by preparative t.l.c. as described under (a). This time the 7α-hydroxy compound appeared as the major u.v.-absorbing band. Crystallisation from acetone gave 93 mg (61.6%), m.p. 240-245° [lit.:(2b) 220-222.5°; (2a) 249°; or (3) 255-256.5°]; ν_{max} 3422 (OH), 1740 (17-CO), 1660, 1615 (Δ^4-3-CO), 1276, 1040, 1016 (C-O), and 870 [C(4)-H] cm^{-1}; $[\alpha]_D$ +155° (c, 0.5, dioxan) [lit.: 178° (2b); 155 or 164° ($CHCl_3$)(3)]; τ 9.09 (s, 18-H_3), 8.79 (s, 19-H_3), 5.90 (m, W = 10Hz, 7β-H), 4.18 (s, 4-H). (Found: C, 75.35; H, 8.5. Calcd. for $C_{19}H_{26}O_3$, C, 75.5; H, 8.6%).

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