SHORT PAPER

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The biotransformation of 4-oxa- and 6-oxa-5a-androstan-17-one by *Mucor plumbeus*

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Although the title steroids are metabolised by the *fungus, Mucor plumbeus,* they are not hydroxylated suggesting that the cyclic ether does not facilitate binding to a steroid hydroxylase in this organism.

Keywords: 4-oxa- and 6-oxa-5α-androstan-17-one, *Mucor plumbeus*

The regiochemistry of the microbiological hydroxylation of steroids is determined by a combination of stereo chemical and electronic factors within the substrate molecule.[1-3] The sites of these microbiological hydioxylations have been rationalised in terms of the positions of existing hydroxyl or carbonyl groups on the steroid skeleton which act as directing groups.[1,2] These groups facilitate the binding of the steroid to the hydroxylase possibly by hydrogen bonding and bring the centre which is to be hydroxylated within the ambit of the oxidative co-enzyme. Two such groups, often one on rings A/B and the other on ring D, are usually required for an efficient microbiological hydroxylation. Both the hydroxyl and carbonyl groups can participate in hydrogen bonding and thus serve as locants. Although a number of lactones have been examined, the effect of replacing a methylene in the carbon skeleton of a steroid by an ether oxygen, has not been studied.[3] An ether oxygen may act as a hydrogen bond acceptor and it may also facilitate a radical abstraction reaction such as in the first step in a hydroxylation at an adjacent centre.

The biotransformations of 4-oxa-5a-androstan-17-one **5** and 6-oxa-5α-androstan-17-one 10 by the fungus *Mucor plumbeus* have been examined in the context of the potential role of the 4-oxa and 6-oxa groups in microbiological hydroxylation. The ring A ethers 4-oxa-5α- and 5β-androstan-17-one were prepared from testosterone **1** by the literature method.[4] Cleavage of ring A gave 17β-hydroxy-5-oxo-3, 5-seco-4-norandrostane-3-oic acid **2**. Reduction of the methyl ester with lithium aluminium hydride gave a mixture of epimers of 3, 5, 17-trihydroxy-3, 5-seco-4-norandrostane **3** which were cyclised by treatment with toluene-*p*-sulfonyl chloride. Oxidation of the 17-alcohol **4** with chromium trioxide gave 4-oxa-5α-androstan-17-one **5** and its 5β-epimer which were separated by chromatography.

The 6-oxa-5α-androstan-17-one 10 was prepared by a similar route from androst-5-ene-7, 17-dione **6** involving the cleavage of ring B with potassium permanganate: sodium periodate and reduction of the methyl ester of the acid **7**[5] with lithium aluminium hydride to give the triol **8**. Cyclisation with toluene-*p*-sulfonyl chloride gave the ether, 17β-hydroxy-6-oxa-5α-androstane **9**, which was oxidised with chromium trioxide to give the C-17 ketone **10**. The stereochemistry at C-5 followed from the multiplicity of the H-5α ^{1}H NMR signal (δ_H 3.12, double: doublet, *J* 6.5 and 12 Hz) corresponding to an axial:equatorial and a diaxial coupling respectively.

Incubation of both cyclic ethers with *Mucor plumbeus* for 7 days gave the corresponding 17β-alcohols **4** and **9** in rather poor yield. In the case of the 4-oxa compound some of the alcohol was isolated as its 17β-acetate. The lack of hydroxylation is typical of a monosubstituted steroid possessing only one binding site. Low yields of hydroxylation products have been observed in these circumstances with other organisms.[6] We therefore conclude that with this organism, the combination of a 4- or a 6-oxa moiety and a C-17 ketone do not provide suitable binding groups for a steroid hydroxylase.

Experimental

Silica for chromatography was Merck 9385. Light petroleum refers to the fraction, b.p. 60–80°. ^{1}H and ^{13}C NMR spectra were determined at 300 and 75 MHz respectively for solutions in deuteriochloroform. IR spectra were determined as nujol mulls. Extracts were dried over anhydrous sodium sulfate.

5β, 7, 17β-Trihydroxy-6-nor-5, 7-secoandrostane **8**: Methyl 5-oxo-6-nor-5, 7-secoandrostan-17-one 7-carboxylate 7[5] (2 g) in tetrahydrofuran (250 cm^3) was treated with lithium aluminium hydride (2 g), under reflux in an atmosphere of nitrogen for 2 h. The excess lithium aluminium hydride was destroyed with moist ethyl acetate. The solution was then concentrated and diluted with hydrochloric acid. The steroids were extracted with ethyl acetate. The extract was washed with water, dried and the solvent evaporated. 5β, 7, 17β-Trihydroxy-6-nor-5, 7-secoandrostane **8** (1.2 g) was obtained as a white powder, m.p. 290–293° (Found: C,72.6; H, 10.9. $C_{18}H_{32}O_3$ requires C,72.9; H, 10.9%), ν_{max}/cm^{-1} 3370 (br), δ_H 0.65(3H, s, 18-H), 0.75 (3H,s,19-H), 3.50 (4H,m, 5-H, 7-H and 17-H).

6-Oxa-5α-androstan-17-one **10**: The above. triol 8 (1 g) in dry pyridine (20 cm^3) was treated at 0° with freshly recrystallised toluene-*p*-sulfonyl chloride overnight. The mixture was poured into dilute hydrochloric acid and the steroid was recovered in ethyl acetate. The extract was washed with water, dilute hydrochloric acid, aqueous sodium hydrogen carbonate and dried. The solvent was evaporated to give a residue (600 mg) which was dissolved in acetone (50 cm^3) and treated with Jones' reagent (2 cm^3) dropwise over a period of 10 min. The mixture was left to stand for 1 h at room temperature and treated with methanol. The solution was concentrated, water was added and the products were recovered in ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, water and dried. The solvent was evaporated to give a residue which was chromatographed on silica. Elution with 3% ethyl acetate: light petroleum gave 6-oxa-5α-androstan-17-one **10** (400 mg) as an oil, (Found: M$^+$ 276.227 $C_{18}H_{28}O_2$ requires M$^+$ 276.229), ν_{max}/cm^{-1} 1745; δ_H 0.82 (3H, s, 18-H), 0.94 (3H, s, 19-H), 3.12 (1H, dd, *J* 6.5 and 12 Hz, 5α-H), 3.43 (1H,m,) and 3.98 (1H, d, *J* 15 Hz) (each 7-H).

Incubation of 4-oxa-5α-androstan-17-one **5** with *Mucor plumbeus*: The fungus was grown in shake culture in 250 cm^3 conical flasks (100 cm^3 medium per flask) on a medium comprising (per litre): glucose (15 g), potassium dihydrogen phosphate (1 g), magnesium sulfate (1 g), ammonium tartrate (1 g), yeast extract (0.5 g), calcium chloride (0.25 g), sodium chloride (0.5 g), ferrous ammonium sulfate (0.1 g) and a trace elements solution (1 cm^3). The trace elements solution comprised (per litre), zinc sulfate (1.6 g), ferrous sulfate (1.0 g), cobalt nitrate (1.0 g), ammonium molybdate (1.0 g), copper sulfate (0.1 g) and manganese sulfate (0.1 g). 4-Oxa-5α-androstan-17-one 5[4] (200 mg) in ethanol (10 cm^3) was evenly distributed between 25 flasks after 2 days growth. After a further 7 days the mycelium was

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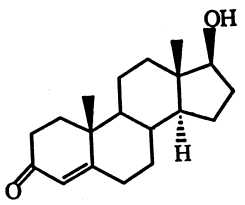
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*



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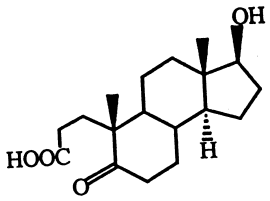

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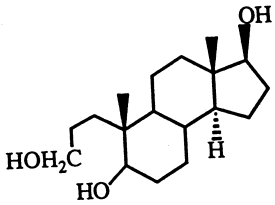

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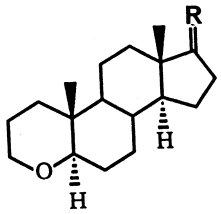
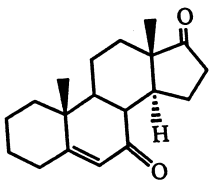


4 R = OH
5 R = O



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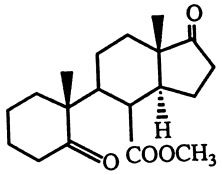

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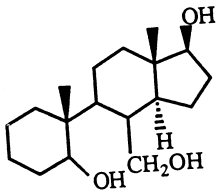

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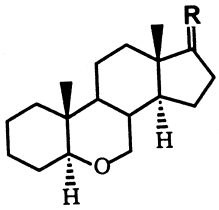

9 R = OH
10 R = O

filtered and the broth was extracted with ethyl acetate. The extract was dried and the solvent was evaporated to give a residue which was chromatographed on silica. Elution with 1% ethyl acetate:light petroleum gave 17β-acetoxy-4-oxa-5α-androstane (25 mg) as an oil (Found: M^+ 320.231 $C_{20}H_{32}0_3$ requires M^+ 320.228), ν_{max}/cm^{-1} 1730; δ_H 0.82 (3H, s, 18-H), 0.94 (3H, s, 19-H), 2.07 (3H, s, OAc), 2.89 (1H, dd, *J* 5.8 and 12 Hz, 5α-H), 3.34 (lH, td, *J* 11.2 and 3 Hz, 3α-H), 3.92 (1H, dd, *J* 11 and 5 Hz, 3β-H), 4.52 (1H, t, *J* 8.3 Hz, 17α-H). Elution with 3% ethyl acetate:light petroleum gave the starting material (40 mg). Elution with 5% ethyl acetate:light petroleum gave 17β-hydroxy-4-oxa-5α-androstane 4 (40 mg) as an oil, (Found M^+ 278.225 $C_{18}H_{30}O_2$ requires M^+ 278.225), ν_{max}/cm^{-1} 3350 (br); δ_H 0.82 (3H, s, 18-H), 0.96 (3H, s, 19-H), 2.93 (1H, dd, *J* 5.8 and 12 Hz, 5α-H), 3.30 (1H, td, *J* 11.2 and 3 Hz, 3α-H), 3.57 (1H, t, *J* 8.4 Hz, 17α-H), 3.89 (1H, dd, *J* 5 and 11 Hz, 3β-H).

Incubation of 6-oxa-5α-andrastan-17-one 10 with Mucor plumbeus: The fungus was grown on shake culture for 2 days as described previously. 6-Oxa-5α-androstan-17-one (300 mg) in ethanol (15 cm^3) was evenly distributed between 15 flasks. After a further 7 days the mycelium was filtered and the broth was extracted with ethyl acetate. The extract was dried and the solvent was evaporated to give a residue which was chromatographed on silica. Elution with 2% ethyl acetate:light petroleum gave the starting material (112 mg). Further elution with 5% ethyl acetate:light petroleum gave 6-oxa-5α-androstan-17-β-ol **9** (34 mg) as an oil, (Found: M^+ 278.225, $C_{18}H_{30}O_2$ requires M^+ 278.225), ν_{maz}/cm^{-1} 3350; δ_H 0.82 (3H, s, 18-H), 0.94 (3H, s, 19-H), 3.12 (1H, dd, *J* 6.5 and 12 Hz, 5α-H), 3.43 and 3.98 (each 1H, m 7-H), 3.57 (lH, t, *J* 8.4 Hz, 17α-H).

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