SHORT PAPER

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The microbiological hydroxylation of 3α,17β- and 3β,l7α-dihydroxy-5α-androstanes by *Cephalosporium aphidicola*†

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Both 3α,l7β- and 3β,l7α-dihydroxy-5α-androstanes are hydroxylated at C-6β by the fungus, *Cephalosporium aphidicola* but the 3α,l7β-diol is also hydroxylated at C-7α whereas the 3β,17α-diol is hydroxylated at C-11β.

Keywords: microbiological hydroxylation, steroids, *Cephalosporium aphidicola*

Models for the microbiological hydroxylation of steroids envisage three active centres on the hydroxylase, two of which bind the substrate and one delivers the oxygen.[l,2] It has been suggested that[3] if a steroid was bound to the microbial hydroxylase at C-3 and C-17, rotation about the C-3:C-17 axis could bring different sites into juxtaposition with the hydroxylating centre of the enzyme. As part of our studies[4] on the application of the fungus, *Cephalosporium aphidicola* to microbiological hydroxylation, we have compared the hydroxylation of 3α,17β-dihydroxy-5α-androstane **l**[5] with 3β, 17α-dihydroxy-5α-androstane **4**[6] in which the opposite relative configurations of the 3- and 17-alcohols might favour different orientations about the C-3:C-17 axis.

3α, l7β-Dihydroxy-5α-androstane **1** was obtained by reduction of 17β-acetoxy-5α-androstan-3-one to the 3β-alcohol and inversion of the configuration at C-3 using a Mitsunobu reaction with chloroacetic acid as the nucleophile. 3β, l7α-Dihydroxy-5α-androstane was one of the products of hydroboration of 3β-hydroxy-5α-androst-16-ene.[7]

Incubation of the 3α, l7β-dihydroxy-5α-androstane **1** with *C.aphidicola* gave two metabolites, **2** and **3** which were separated by chromatography. The ^{13}C NMR spectrum (see Table 1) of the major metabolite **2** contained a new CH(OH) signal at δ_C 71.7. The signals assigned to C-5 and C-7 had moved downfield whilst there were γ-carbon upfield shifts for C-4 and C-8. Hence the new hydroxyl group was at C-6. The new CH**(**OH) ^{l}H NMR signal (δ_H 3.70) was a broad singlet and the 19-H signal showed a significant downfield shift consistent with a 6β-stereochemistry for the alcohol. The ^{13}C NMR spectrum of the second metabolite also revealed a new CH(OH) resonance (δ_C 67.5 ppm). There were downfield shifts for C-6 and C-8 and γ-gauche upfield shifts for C-5,C-9 and C-l4 consistent with the location of the new hydroxyl group at C-7. The ^{l}H NMR signal (δ_H 3.83) was a broad singlet typical of a C-7α (axial) alcohol. The metabolites were therefore assigned the structures of 3α,6β,17β- and 3α,7α,l7β-trihydroxy-5α-androstanes, **2** and **3**.[8] Incubation of 3β,17α-dihydroxy-5α-androstane **4** gave two metabolites **5** and **6**. In the ^{13}C NMR spectrum of 5 the high-field resonance associated with C-l1 had been replaced by a CH(OH) signal (δ_C 68.2) whilst there were downfield shifts for the signals assigned to C-9 and C-l2 and a γ-gauche shift for C-8 and C-13. The new CH(OH) resonance (δ_H 4.08) was a broad singlet whilst both the 18-H and 19-H resonances showed significant downfield shifts consistent with interactions with an 11β-hydroxyl group. The major metabolite was the 3β,6β,17β-trio1 **6**. There was a new CH(OH) resonance at δ_C 72.8. The signals assigned to C-5 and C-7 showed downfield shifts whilst C-4 and C-8 showed a characteristic γ-gauche upfield shift. The multiplicity of the new CH(OH) resonance was obscured by the 3-H and 17-H signals but the 19-H signal showed a downfield shift consistent with a diaxial interaction with a 6β-hydroxyl group.

Table 1 ^{13}C NMR data for the metabolites

Carbon atom	Compound 1	2[a]	3	4[b]	5[b]	6[b]
1	33.0	33.9	32.0	3a.9	37.4	40.9
2	29.9	29.6	2a.9	33.a	33.2	34.0
3	65.6	66.2	66.4	71.9	70.8	70.6
4	36.9	32.8	35.5	40.6	39.2	34.7
5	39.6	41.7	31.6	46.4	46.4	50.0
6	29.1	71.7	36.3	30.5	29.1	72.8
7	32.2	39.0	67.5	34.2	32.7	43.1
8	35.9	30.3	39.6	37.2	32.3	33.0
9	55.0	54.3	46.1	55.0	58.9	56.5
10	36-6	36.0	36.3	35.1	36.5	37.5
11	20.9	20.1	20.2	22.5	68.2	22.8
12	37.6	36.5	36.3	34.3	41.8	38.8
13	43.6	42.9	42.9	48.0	45.4	47.2
14	51.5	50.7	45.3	50.4	51.1	50.1
15	23.8	23.2	22.8	26.4	25.4	26.9
16	31.0	29.9	30.4	33.6	33.7	34.3
17	81.4	81.5	81.8	80.5	79.5	79.5
18	11.6	11.0	10.1	13.8	15.7	18.9
19	12.0	14.6	10.9	18.8	20.2	19.2

[a]In $CDCl_3$:CD_3OD; [b]In C_5D_5N.

The hydroxylation of **1** at C-11β and of 4 at C-7α are interesting because these two centres are related by rotation about the C-3:C-17 axis. This suggests that a model involving a common hydroxylase with different substrate binding modes may be used to rationalise the hydroxylations by *Cephalosporium aphidicola.*

Experimental

Silica for chromatography was Merck 9385. ^{1}H and ^{13}C NMR spectra were determined in deuteriochloroform, unless otherwise stated, at 360 and 90 MHz respectively. IR spectra were determined as nujol mulls. Light petroleum refers to the fraction b.p. 60–80°C. Extracts were dried over sodium sulfate.

Preparation of 3α,l7β-dihydroxy-5α-androstane. Diethyl azodicarboxylate (3 cm^3) was added to a solution of 17β-acetoxy-5α-androstan-3β-ol[9] (3 g), triphenylphosphine (4.5 g) and dry chloroacetic acid (2.1 g) in dry toluene (90 cm^3). The resulting orange solution was stirred at room temperature overnight. The solvent was evaporated and the residue was chromatographed on silica. Elution with 3% ethyl acetate:light petroleum gave 17β-acetoxy-3α-chloroacetoxy-5α-androstane (2.83 g) which crystallised from ethyl acetate as needles, m.p. 120°C, (Found: C, 67.3: H,8.6. $C_{23}H_{35}ClO_4$

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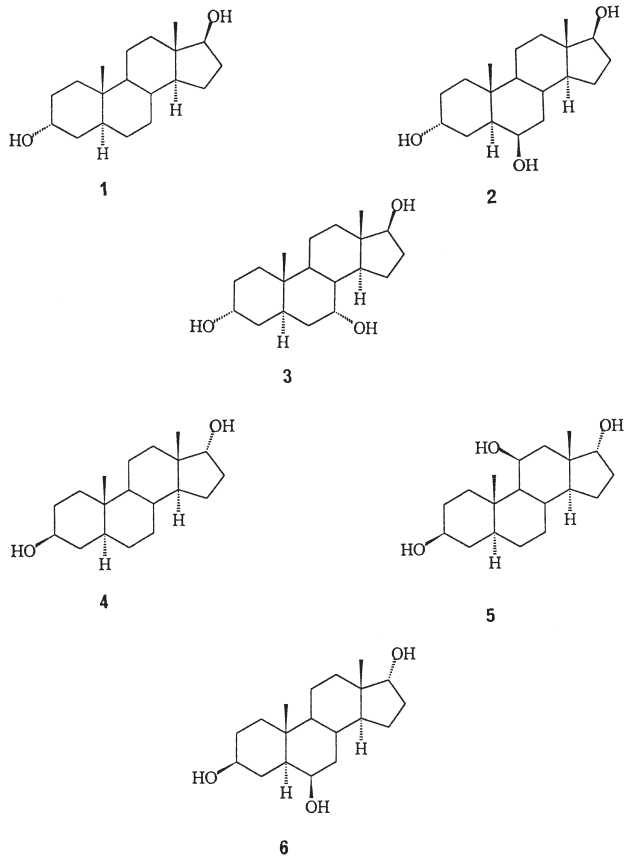
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*



requires C, 67.2: H,8.6%), ν_{max}/cm^{-1} 1751,1726; δ_H 0.78(3H,s, 18-H), 0.81(3H,s, 19-H), 0.70-1.80 (22H,overlapping multiplets), 2.03(3H,s, OAc), 4.07 (2H,s,OCCH$_2$Cl), 4.58(lH,t,J 8.5 Hz,l7-H), 5.11(lH,br.s.3-H).

The chloroacetate (2.7 g) in methanol (120 cm^3) was treated with a solution of potassium carbonate (8 g) in water (40 cm^3) at room temperature for 24 h. Acetic acid (15 cm^3) was added and the mixture was stirred for 5 min. The methanol was evaporated and the residue was extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, water, brine and dried. The solvent was evaporated to give 3α,l7β-dihydroxy-5α-androstane 1 (1.8 g) which crystallised from ethyl acetate as needles, m.p. 223–224°C, (lit., [5] 222–224°C),ν_{max}/cm^{-1} 3418,3386; δ_H 0.73(3H,s,18-H), 0.79(3H,s,19-H), 0.80–1.95(22H, overlapping multiplets), 3,64(1H,t,*J* 8.7 Hz,l7-H), 4.05(1H,t,*J* 2.8 Hz,3-H).

Incubation of substrates: The fungus, *Cephalosporium aphidicola* was grown on shake culture in 250 cm^3 conical flasks on a medium (100 cm^3) comprising (per litre), glucose (50 g), potassium dihydrogen phosphate (5g),magnesium sulfate (2 g), glycine (2 g), potassium chloride (1 g) and a trace elements solution (2 cm^3). The trace elements solution contained (per litre), zinc sulfate (1.6 g), ferrous sulfate (1 g), cobalt nitrate (1 g) ammonium molybdate (1 g), copper sulfate (0.1 g) and manganese sulfate (0.1 g), The fungus was grown for 2 days before the substrate (1.5 g) in ethanol (50 cm^3) was distributed over 50 flasks. The fermentation was then continued for a further 7 days. The broth was filtered and the mycelium was washed with ethyl acetate. The broth was acidified with dil. hydrochloric acid and extracted with ethyl acetate. The extracts were washed with water and dried. The solvent was evaporated and the residue was chromatographed on silica and eluted with an increasing gradient of ethyl acetate in light petroleum.

3α,l7β-Dihydroxy-5α-androstane 1 gave 3α,6β,l7β-trihydroxy-5α-androstane 2 (101 mg) which crystallised as needles, m.p. 219°C, (Found: C,72.1; H,10.2. $C_{19}H_{32}O_3.0.5H_2O$ requires C,C,71.8: H,10.5%), ν_{max}/cm^{-1} 3353 (br), δ_H 0.77(3H,s,l8-H), l.02(3H,s,19-H),0.80-1.90(20H, overlapping multiplets), 3.65(lH,t,*J* 9 Hz, 17-H), 3.70(1H,br.s,6-H), 4.18(lH,m.3-H). Further elution gave 3α,7α,l7β-trihydroxy-5α-androstane 3 (57 mg) which crystallised as needles, m.p. 194–196°C, (lit.,[8] 195–197°C), ν_{max}/cm^{-1} 3450; δ_H 0.74(3H,s,18-H), 0.79(3H,s,l9-H), 0.80-1.90(20H, overlapping multiplets), 3.69(1H,t,*J* 9 Hz,l7-H), 3.83(1H,br.s.7-H), 4.18(1H,m,3-H).

3β,17α–Dihydroxy-5α-androstane **4** gave 3β,11β,17α-trihydroxy-5α-androstane 5 (118 mg) which crystallised from acetone as needles, m.p.221–223°C, (Found: C, 73.7; H,10.5. $C_{19}H_{32}O_3$ requires C,74.0; H,10.5%),ν_{max}/cm^{-1} 3495, 3412, 3343; δ_H(pyridine-d_5) 1.22(3H,s,l8-H), 1.39(3H,s,19-H),0.90–2.20(20H, overlapping multiplets), 3.97 (2H,m, 3-H and 17-H), 4.08(lH,br.s,11-H). Further elution gave 3β,6β,17α-trihydroxy-5α-androstane 6 (203 mg) which crystallised from ethyl acetate as needles, m.p. 204–205°C, (Found: C,73.7; H,10.3. $C_{19}H_{32}O_3$ requires C,74.0; H,10.5%), ν_{max}/cm^{-1} 3370; 32 δ_H(pyridine), 0.71(3H,s,18-H), 1.39(3H,s,19-H), 90–2.20(20H, overlapping multiplets), 4.0(3H,br.m. 3-,6- and 17-H).

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