SHORT PAPER

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The microbiological hydroxylation of 4,4-dimethylandrost-5-enes by *Cephalosporium aphidicola*†

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The microbiological hydroxylation of 4,4-dimethylandrost-5-en-3-ones by the fungus, *Cephalosporium aphidicola,* takes place at C-7 rather than in a biosynthetically patterned sense on the C-4 dimethyl groups.

Keywords: microbiological hydroxylation, 4,4-dimethylandrost-5-enes, *Cephalosporium aphidicola*

Aphidicolin **1** is a diterpenoid metabolite of the fungus, *Cephalosporium aphidicola.*[1] Its biosynthesis involves the hydroxylation of the equatorial methyl of the C-4 geminal dimethyl groups of an aphidicolane precursor. We have studied the stereochemistry of this biosynthetic step.[2] Aphidicolin belongs to the same enantiomeric series as the steroids. Consequently it was of interest to see if the fungus might hydroxylate one of the two diastereotopic methyl groups of a 4,4-dimethyl steroid.

The substrates **3** and **6** were prepared from testosterone **2** by alkylation with methyl iodide in the presence of potassium *t*-butoxide.[3] This gave 4,4-dimethyl-17β-hydroxy-androst-5-en-3-one 3 which was oxidised with chromium trioxide to give the corresponding 3,17-dione **6**.[4]

Both substrates were incubated with *C.aphidicola* for 8 days. The metabolites were separated by chromatography and their structures established by ^{13}C and ^{l}H NMR spectroscopy. 4,4-Dimethyl-17β-hydroxyandrost-5-en-3-one 3 gave the 7 β-alcohols **4** and **7** together with the 7α-alcohol **5**. 4,4-Dimethylandrost-5-ene-3,17-dione **6** gave the 3,7,17-trione **9** together with the 7-alcohols **7** and **8**. The ^{13}C NMR signals have been assigned in the 4,4-dimethyl steroids.[5] The presence of the oxygen functions at C-7 was established by the change in the position of the ^{13}C NMR resonances for C-6 and C-8 (see Table 1). The compounds **4** and **5** were interrelated by oxidation with chromium trioxide to afford the unsaturated ketone **9**. The stereochemistry of the alcohols followed from the magnitude of the H-7:H-8 coupling constants [H-7α:H-8β, *J* 8 Hz, (7β-OH);H-7β:H-8β, *J* 4 Hz (7α-OH)].

These hydroxylations parallel those observed for the androst-5-enes.[7] There was no evidence for a biosynthetically patterned hydroxylation and in particular we did not detect any metabolites arising from hydroxylation of the methyl groups. The non-stereospecific allylic hydroxylation at C-7 may arise because a C-7 radical intermediate is stabilised by resonance involving the adjacent double bond and may then be attacked by oxygen from either face.

Table 1 ^{13}C NMR data

Carbon atom	Compound 3	4	5	6	7	8	9
1	31.8	31.8	31.7	32.0	31.6	31.0	31.0
2	33.5	33.5	33.4	33.6	33.8	33.3	33.1
3	218.1	215.6	215.4	216.4	215.7	215.2	212.4
4	48.2	48.4	48.5	48.7	48.2	48.5	47.9
5	149.4	152.9	155.7	150.1	160.0	155.8	174.9
6	119.7	123.6	121.8	119.2	124.6	121.6	124.4
7	31.1	73.5	64.7	30.6	73.3	64.2	200.7
8	31.1	40.4	36.9	31.8	40.4	36.5	45.9
9	49.2	47.6	41.5	49.0	47.9	41.6	49.2
10	36.9	37.2	38.1	37.2	37.7	38.1	38.9
11	20.7	20.9	20.6	20.6	21.1	20.3	21.0
12	36.3	36.3	36.1	31.4	32.1	31.6	30.8
13	42.5	43.2	42.5	47.6	48.8	47.1	49.2
14	51.1	50.6	44.3	51.7	51.6	45.0	43.8
15	23.1	25.6	23.4	21.8	24.5	21.8	24.1
16	30.0	30.7	30.5	35.8	36.4	35.8	35.6
17	81.1	81.4	81.6	220.9	221.2	221.0	219.9
18	10.7	11.0	10.8	13.6	14.1	13.3	13.8
19	19.1	19.0	17.7	19.3	19.4	17.7	16.6
Me	26.9	26.9	27.0	27.2	27.3	27.0	26.1
Me	30.0	30.1	30.3	30.2	30.6	30.3	29.0

Experimental

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

General fermentation details: The fungus, *Cephalosporium aphidicola* was grown on shake culture in conical flasks (250 cm^3) containing medium (100 cm^3) comprising (per litre), glucose (80 g), ammonium nitrate (4.8 g), potassium dihydrogen phosphate (5 g), magnesium sulfate (1 g) and a trace elements solution (2 cm^3). The latter contained (per 100 cm^3), cobalt nitrate (0.01 g), iron(I1) sulfate (0.1 g), copper sulfate (0.015 g), zinc sulfate (0.161 g), manganese sulfate (0.01 g) and ammonium molybdate (0.01 g). The substrates were added after 3 days growth and the fermentation was continued for a further 8 days. The mycelium was filtered and the broth was acidified and extracted with ethyl acetate. The extract was dried and the solvent evaporated to give a residue which was chromatographed on silica.

Incubation of 4,4-dimethyl-17β-hydroxyandrost-5-ene-3-one The substrate **3**[3] (1.2 g) gave in the fraction eluted with 20% ethyl acetate:light petroleum the starting material (0.5 g). Elution with 30% ethyl acetate:light petroleum gave 4,4-dimethyl-7β-hydroxyandrost-5-ene-3,17-dione 7 (50 mg) which crystallised from ethyl acetate:light petroleum as needles, m.p. 202–208°C, (Found: C, 76.3; H, 9.4. $C_{21}H_{3O}O_3$ requires C, 76.3; H, 9.15%), ν_{max}/cm^{-1} 3441, 1737, 1704; δ_H 0.92 and 0.94 (each 3H,s, 18- and 19-H), 1.28 and 1.30(each 3H,s, 4-Me), l.00-2.40(15H, overlapping multiplets), 4.06 (lH,dd, *J* 2 and 8 Hz, 7α-H), 5.53 (lH,d, *J* 2 Hz, 6-H). Elution with 40% ethyl acetate:light petroleum gave 7β,17β-dihydroxy-4,4-dimethylandrost-5-en-3-one **4** (50 mg), which crystallised from ethyl acetate:light petroleum as needles, m.p.189–190°C (Found: M^+ 332.236. $C_{21}H_{32}O_3$ requires M^+ 332.235), ν_{max}/cm^{-l} 3608, 1713; δ_H 0.78(3H,s,18-H), 0.92 (3H,s,19-H), 1.25 and 1.28 (each 3H,s, 4-Me), l.00–2.30(15H, overlapping multiplets), 3.66(1H,t, *J* 8.6 Hz, 17-H), 3.93 (lH,dd, *J* 2 and 8 Hz, 17αH), 5.49(1H,d, *J* 2 Hz, 6-H). Further elution gave 7α,17β-dihydroxy-4,4-dimethylandrost-5-en-3-one 5 (50 mg), which crystallised from ethyl acetate:light petroleum as needles, m.p. 160–165°C, (Found: M^+ 332.236. $C_{21}H_{32}O_3$ requires M^+ 332.235),

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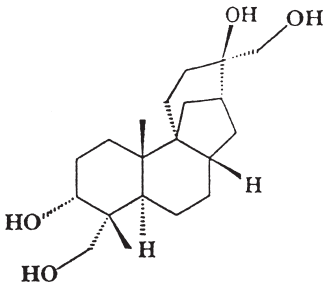
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*



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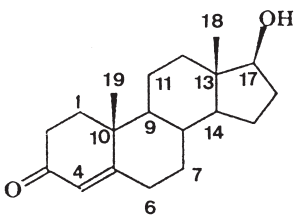

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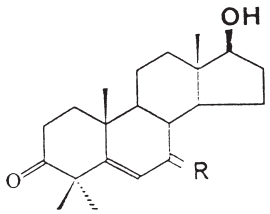

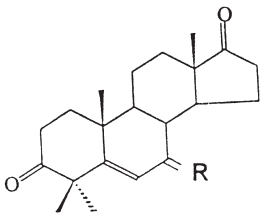

3 R = H_2

4 R = α-H,β-OH

5 R = α-OH,β-H

6 R = H_2

7 R = α-H,β-OH

8 R = α-OH,β-H

9 R = =O

ν_{max}/cm^{-1} 3600, 1715; δ_H 0.78(3H,s,18-H), 0.85 (3H,s,19-H), 1.28(6H, s,4,4-Me), l.00-2.30(15H, overlapping multiplets), 3.73(1H,t, *J* 8.6 Hz, 17-H), 3.97 (lH,t, *J* 4.6Hz, 7β-H), 5.69(1H,d, *J* 4.6 Hz,6-H).

Incubation of 4,4-dimethylandrost-5-ene-3,17-dione: The substrate 6[4] (1.5 g) gave in the fraction eluted with 10% ethyl acetate:light petroleum, the starting material (611 mg). Elution with 20% ethyl acetate:light petroleum gave 4,4-dimethylandrost-5-ene-3,7,17-trione 9 (51 mg) which crystallised from ethyl acetate as prisms, m.p. 162-164°C, (Found: C, 76.7; H,8.4. $C_{21}H_{28}O_3$ requires C,76.8; H,8.6%), ν_{max}/cm^{-1} 1742, 1716, 1652; δ_H 0.92(3H,s, 18-H), l.l0(3H,s, 19-H), 1.35 (6H,s,4,4-Me), 1.00–2.50 (15H, overlapping multiplets), 5.97 (lH,s,6-H). Further elution with 25% ethyl acetate:light petroleum gave 4,4-dimethyl-7β-hydroxyandrost-5-ene-3,17-dione **7** (188 mg) identical to the material described above. Elution with 30% ethyl acetate: light petroleum gave 4,4-dimethyl-7α-hydroxyandrost-5-ene-3,17-dione **8** (280 mg) which crystallised from ethyl acetate:light petroleum as prisms, m.p. 189–192°C,(Found: C, 76.4; H,9.2. $C_{21}H_{30}O_3$ requires C,76.3; H,9.15%), ν_{max}/cm^{-1} 3469,1736, 1695; δ_H 0.86(3H,s, 18-H), 0.90(3H,s, 19-H), 1.29 (6H,s,4,4-Me), l.00-2.50(15H, overlapping multiplets), 4.10 (lH,t, *J* 4 Hz,7β-H), 5.53 (lH,d, *J* 4 Hz, 6-H).

Oxidation of the metabolites: 7α,17-Dihydroxy-4,4-dimethylandrost-5-en-3-one (20 mg) in acetone (2 cm^3) was treated with a few drops of the chromium trioxide (Jones') reagent for 15 min. Methanol was added and the solution was concentrated. The concentrate was diluted with water and extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, water and dried. The solvent was evaporated to give a residue which was crystallised from ethyl acetate:light petroleum to give 4,4-dimethylandrost-5-ene-3,7,17-trione **9** (12 mg), m.p. 155–160°C, identified by its ^{1}H NMR spectrum. Under similar conditions 7α,17β-dihydroxy-4,4-dimethylandrost-5-en-3-one 5 (20 mg) gave the trione **9** (15 mg) identified by its ^{1}H NMR spectrum.

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