SHORT PAPER

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The biotransformation of 5β-methylestr-9-enes by *Cephalosporium aphidicola*†

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The products of the microbiological hydroxylation of 5β-methylestr-9-enes by the fungus *Cephalosporium aphidicola* have been shown to include the 3-keto-1,9-diene, the 3-keto-10β-hydroxy-9(11)-alkene and the 3-keto-11β-hydroxy-9(10)-alkene.

Keywords: microbiological hydroxylation, steroids, *Cephalosporium aphidicola*

Microbiological hydroxylation is a useful transformation of steroids which provides access to sites that are often chemically relatively inaccessible.[1,2] We have been studying the microbiological hydroxylation of a range of steroids[3-7] using the fungus, *Cephalosporium aphidicola*, in order to map the factors which determine the regiochemistry of the transformation by this organism. Incubation of 5α-androstane-3,17-dione with *C.aphidicola* resulted[8] in hydroxylation at the C-1lα and C-14α positions. A 5β-methylestrane has a significantly different shape from a 5α-steroid. Hence there are different possible orientations of the steroid within the microbial hydroxylases. In this paper, we report the hydroxylation of the 5β-methylestr-9-enes **3** and **4** by *C.aphidicola*.

The substrates were prepared as follows. The Westphalen rearrangement[9] of 3β-acetoxy-5α-hydroxy-6β-chloroandrostan-17-one gave 3β-acetoxy-6β-chloro-5β-methylestr-9-en-l7-one 1.[10] The 6β-chlorine atom was removed by hydrogenolysis with tri-*n*-butyltin hydride. Hydrolysis of the 3β-acetate **2** gave the alcohol **3** which was oxidised with chromium trioxide to the 3-ketone **4**.[10]

The steroids **3** and **4** were incubated with *C.aphidicola* for 7 days in shake culture. 3β-Hydroxy-5β-methylestr-9-en-17-one **3** gave four metabolites which were separated by chromatography. The first metabolite to be eluted from the column was the 3-ketone **4** which was identified by comparison with an authentic sample. The second metabolite had two additional alkene resonances in its NMR spectra (δ_H 7.39 and 5.86, each doublets *J* 10.2 Hz, and δ_C 141.5 and 125.7 ppm). There was intense UV absorption at λ_{max} 305 nm (ε 18,000) consistent with a dienone (calculated 309 nm) in the structure **5**. The third metabolite was a tertiary alcohol (δ_C 73.83) containing a trisubstituted double bond (δ_H 5.88; δ_C 140.1 and 120.7). Since there were several alternative possible structures which could accommodate these data, the structure and stereochemistry **6** were established by X-ray crystallography (Fig. 1). The final metabolite possessed a secondary alcohol (δ_H 5.04: δ_C 66.3) and a tetra-substituted alkene (δ_C 135.5 and 138.7). The structure and stereochemistry **7** for this metabolite were also established by X-ray crystallography (Fig. 2).

Incubation of the 3,17-dione **4** with *C.aphidicola* gave the dienone **5** and the 10β-alcohol **6** both of which were identified by comparison with authentic samples.

The results may be rationalised in terms of the initial oxidation of the C-3 alcohol to a C-3 ketone. Microbial oxidation of the allylic C-1 or C-11 positions may then lead to elimination to form the dienone or to axial hydroxylation. These results differ from those observed with 5α-androstanes.

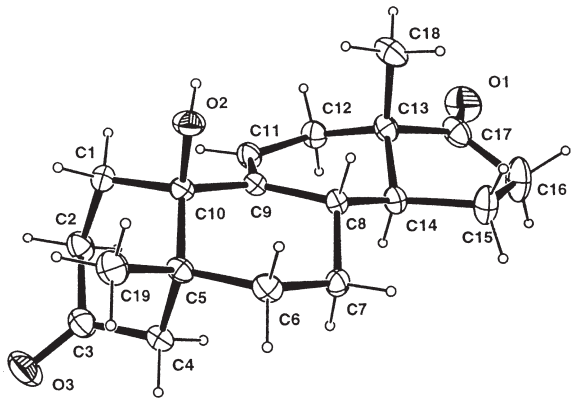

Fig. 1 The X-ray crystal structure of compound **6**.

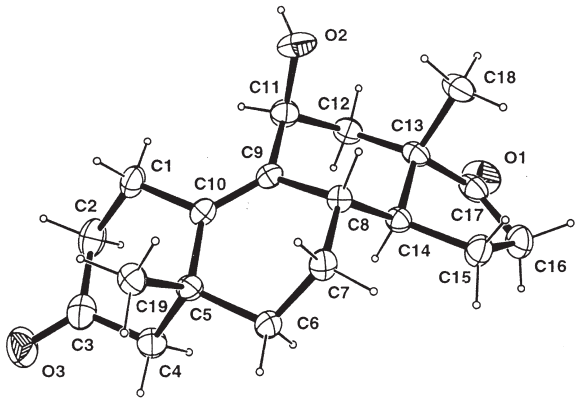

Fig. 2 The X-ray crystal structure of compound **7**.

No metabolites were detected arising from hydroxylation at the C-14α position.

Experimental

General experimental details: Silica for chromatography was Merck 9385. Light petroleum refers to the fraction b.p. 60–80°C. Extracts were dried over sodium sulfate. IR spectra were determined as nujol mulls. ^{1}H and ^{13}C NMR spectra were determined at 300 and 75 MHz respectively for solutions in deuteriochloroform. 3β-Hydroxy-5β-methylestr-9-en-l7-one 3, m.p. 163–165°C (lit.,[10] 167–168°C) and 5β-methylestr-9-ene-3,17-dione **4**, m.p. 126–128°C (lit.,[10] 130–132°C) were prepared by literature methods.[10]

General fermentation details: Cephalosporium aphidicola was grown in shake culture (100 cm^3 medium per flask) on a medium comprising (per litre):– glucose (50 g), potassium dihydrogenphosphate (5 g), magnesium sulfate (2 g), glycine (2 g), potassium chloride (1 g) and a trace elements solution (2 cm^3) which 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

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

Table 1 ^{13}C NMR data for compounds 3–7 determined in $CDCl_3$ at 75 MHz

Carbon no.	Compound 3	4	5	6	7
1	35.5	36.8	141.5	39.1	36.7
2	37.3	38.9	125.7	36.1	41.9
3	67.6	211.9	199.7	211.5	210.9
4	47.4	54.8	52.6	48.8	54.8
5	35.3	38.7	36.7	41.8	38.3
6	20.4	23.7	19.3	22.6	23.4
7	21.9	24.7	24.5	26.4	24.1
8	37.2	37.8	37.3	35.4	34.6
9	136.0	132.8	147.3	140.1	138.7
10	130.8	132.9	131.8	73.8	135.5
11	27.7	24.7	24.5	120.7	66.3
12	32.8	32.0	33.1	33.2	38.4
13	48.1	47.5	48.3	45.7	46.7
14	50.9	51.3	50.1	48.2	50.6
15	22.0	22.2	21.7	22.5	22.1
16	35.8	35.8	35.8	33.7	35.4
17	221.0	220.6	219.0	221.3	219.6
18	13.4	131.1	13.7	14.0	15.3
5β-Me	28.4	27.7	25.8	22.7	27.7

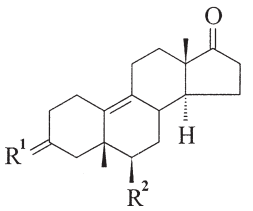
sulfate (0.1 g). The fungus was grown for 2 days. The substrate in ethanol (25 cm^3) was then evenly distributed between 25 flasks. After 7 days incubation with the substrate, the culture was filtered and the mycelium was washed with ethyl acetate. The broth was adjusted to pH 2 and extracted (× 3) with ethyl acetate. The extracts were washed with water and dried. The solvent was evaporated and the residue was chromatographed on silica. The metabolites were eluted with increasing concentrations of ethyl acetate in light petroleum.

3β-Hydroxy-5β-methylestr-9-en-l7-one 3 (750 mg) gave 5β-methylestra-3,17-dione **4** (5 mg) which was eluted with 5% ethyl acetate in light petroleum and identified by its NMR spectrum. Elution with 10% ethyl acetate:light petroleum gave 5β-methylestra-1,9-diene-3,17-dione 5 (7 mg) as a gum, (Found: M^+ 284.175, $C_{19}H_{24}O_2$ requires 284.177); ν_{max}/cm^{-1} 1742, 1712, 1654, 1630; λ_{max} 305 nm (ε 18,000): δ_H 1.06 (3H,s,l8-H), 1.12 (3H,s,5β-Me), 1.10–2.55(15H, overlapping multiplets), 2.86(1H, d, *J* 13.8 and 3.0 Hz,H-4), 5.86 (1H, d, *J* 10.2 Hz,2-H), 7.39 (1H,d, *J* 10.2 Hz, 1-H). Elution with 15% ethyl acetate:light petroleum gave 10β-hydroxy-5β-methylestr-11-ene-3,17-dione **6** (26 mg) which was crystallised from ethyl acetate:light petroleum as needles, m.p. 159–161°C, (Found: M^+ 302.189, $C_{19}H_{26}O_3$ requires 302.188); ν_{max}/cm^{-1} 3456,1738, 1714, 1633; δ_H 0.84(3H,s,l8-H), 1.00(3H,s,5β–Me), 1.05–2.50(18H, overlapping multiplets), 5.88 (1H,br.s.,11-H). Further elution with 15% ethyl acetate:light petroleum gave 11β-hydroxy-5β-methylestr-9-en-3,17-dione **7** (7 mg) which was crystallised from ethyl acetate:light petroleum as needles, m.p. 153–155°C, (Found: M^+ 302.185 $C_{16}H_{26}O_3$ requires 302.188);ν_{max}/cm^{-1} 3420, 1730, 1710, and 1640; δ_H 1.10 (3H,s, 18-H), 1.20(3H,s, 5β-Me), 1.10–2.50(17H, overlapping multiplets), 1.97(1H,m,4-H), 5.04 (1H,br.d. *J* 2.7 Hz, 11-H).

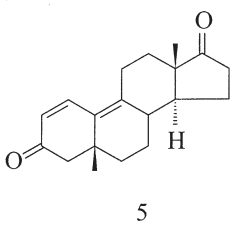
Under similar conditions 5β-methylestr-9-ene-3,17-dione **4** (500 mg) gave as metabolites, compounds 5 (8 mg) and **6** (18 mg) which were identified by their NMR spectra.

X-ray crystallographic data and structure determination: (a) Compound **6**, $C_{l9}H_{26}O_3$, M_r = 302.40, monoclinic, space group $P2_1$ (no.4), *a* = 7.3846(3),*b* = 11.3251(5), *c* = 10.1927(3)A: α = γ = 90°, β = 108.677(3)°, *v* = 807.54(5)$Å^3$, *Z* = 2, D_C =1.24 g/cm^3, F(000) = 328, μ = 0.08 mm^{-l}, crystal size 0.3 × 0.3 × 0.3 mm. A total of 6182 reflections were collected for 4.59 < θ × 27.91° and –9<=*h*<=9, –14<=*h*<=ll, –10<=*l*<=13 on a Nonius Kappa CCD diffractometer. There were 3024 independent reflections and 2927 reflections with $I > 2\sigma(I)$ were used in the refinement. No absorption correction was applied. The structure was solved by direct methods and refined using SHELXL-97. The final R indices were [$I > 2\sigma(I)$] R_1 = 0.035, ωR_2 = 0.095 and all data R_l = 0.036, ωR_2 = 0.096. The goodness-of-fit on F^2 was 0.858 and the largest difference peak and hole was 0.23 and -0.17e$Å^{-3}$.

(b) Compound **7**, $C_{19}H_{26}O_3$, M_r = 302.40, monoclinic, space group $P2_1$ (no.4), *a* = 5.8288(2),*b* = 10.7436(4), *c* = 12.6699(5)A; α = γ = 90°, β = 90.607(2)°, V = 793.37(5)A^3, *Z* = 2, D_C = 1.27 g/cm^3, F(000) = 328, μ = 0.08 mm^{-l}, crystal size 0.3 × 0.3 × 0.3 mm. A total of 10538 reflections were collected for 3.79 < θ < 27.86^0 and –7<=*h*<=7, –14<=*h*<=l4, -16 =*1*<=16 on a Nonius Kappa CCD diffractometer, There were 3740 independent reflections and 3541 reflections with $I > 2\sigma(I)$ were used in the refinement. No absorption correction was applied. The structure was solved by direct methods and refined using SHELXL-97. The final R indices were [$I > 2\sigma(I)$] R_1 = 0.037, ωR_2 = 0.103 and all data R_l = 0.040, ωR_2 = 0.1095. The goodness-of-fit on F^2 was 0.407 and the largest difference, peak and hole was 0.23 and –0.l6e$Å^{-3}$. The data have been deposited at the Cambridge Crystallographic Data Centre.



1 R^1= α -H,β - OAc ; R^2= Cl
2 R^1= α -H,β - OAc ; R^2= H
3 R^1= α -H,β - OH; R^2=H
4 R^1= O; R^2=H



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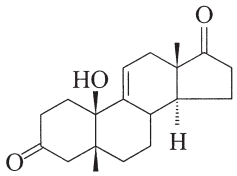

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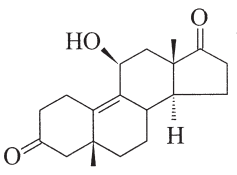

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