The hydroxylation of the enantiomeric hexahydro-10-methylnaphthal-4-en-3-ones† by *Cephalosporium aphidicola*

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The enantiomeric hexahydro-10-methylnaphthal-4-en-3-ones are hydroxylated by the fungus, *Cephalosporium aphidicola* at C-6 and at C-9 (steroid-like enantiomer) or at C-1 (steriod enantiomer).

Keywords: microbiological hydroxylation, enantiomers, hexahydronaphthalenones, *Cephalosporium aphidicola*

We have shown that the fungus *Cephalosporium aphidicola* is a useful organism for the microbiological hydroxylation of steroids.[1] It hydroxylates the steroidal unsaturated ketones progesterone and testosterone at C-6β and C-11α.[2,3] Microorganisms have the ability to distinguish between enantiomers in biotransformations.[4] It was, therefore, of interest to examine the hydroxylation of the commercially available hexahydro-10-methylnaphthal-4-en-3-ones, 1 and 5,[5] by *Cephalosporium aphidicola*. These hexahydronaphthalenones might be considered as models for rings A and B of the steroids. The hydroxylation of the racemate by *Rhizopus arrhizus* has been shown to occur at C-6[6] whilst the transformation at C-6 and C-8 has been reported[7] of the individual enantiomers by a number of common organisms.

The (+)-enantiomer **1** which has the same absolute stereochemistry as the steroids, was incubated with *C. aphidicola* on shake culture for 6 days. Two metabolites, **2** and **3**, were separated by chromatography. The location of the hydroxyl groups followed from changes in the ^{13}C NMR spectrum (see Table 1). In the 6β-hydroxy compound **2**, there was a γ-gauche shielding of C-8 which has been observed in the steroid series.[2,3] The ^{1}H NMR signal for the C-10β methyl group showed significant downfield shift (Δδ 0.23 ppm) whilst the CH(OH) resonance was a typical poorly resolved narrow triplet. The location of the hydroxyl group in **3** followed from a downfield shift for the C-10 ^{13}C NMR signal (Δδ 5.8 ppm) and a γ-gauche shielding of the C-10β methyl group (Δδ 6.5 ppm). The ^{1}H NMR signal for the CH(OH) was a double-doublet (J=11.6 and 4.3 Hz) consistent with an equatorial alcohol. This alcohol **3** was identical to the reduction product of the Wieland–Miescher ketone 4[8] obtained using sodium borohydride in ethanol at 0° for a short time.

Incubation of the enantiomer **5** gave a poor yield of two metabolities. The C-6α (axial) alcohol **6** was identified by its ^{1}H NMR spectrum. The second product **7**, was identified as the C-1α alcohol from the changes in the position of the C-2 and C-10 ^{13}C NMR signals (see Table 1). The stereochemistry was assigned on the basis of the γ-gauche shielding (Δδ 5.8 ppm) and on the mulitiplicity of the CH(OH) signal (dd, *J*=4,6 and 9.3 Hz) in the ^{1}H NMR spectrum.

These results show that the stereochemistry of hydroxylation at the allylic C-6 position is determined by axial attack, possibly on the enolate of the unsaturated ketone,[6] irrespective of the absolute stereochemistry. On the other hand C-1α and C-9β are related by rotation around the C-5:C-10 bond. The site and stereochemistry of hydroxylation of the two enantiomers [C-9β in **1** and C-1α in **5**] may be determined by placing the C-10 methyl group in the same hydrophobic pocket of the hydroxylase.

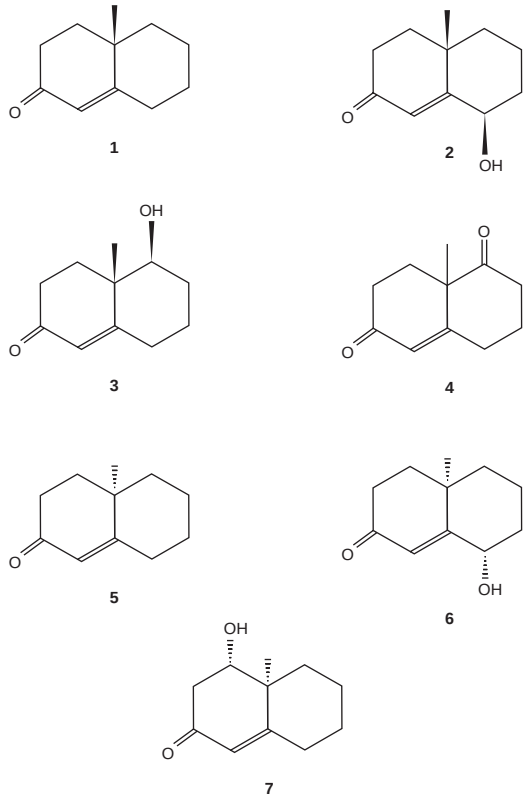

Table 1 ^{13}C NMR data (determined in $CDCl_3$ at 90.5 MHz)

	Compound			
Carbon no.	**1**	**2**	**3**	**4**
1	37.9	39.4	34.2	75.1
2	33.9	33.2	33.0	37.4
3	200.0	200.8	199.7	199.8
4	124.0	126.4	125.4	124.7
5	170.4	167.9	168.6	169.8
6	32.7	72.5	32.0	32.5
7	27.1	34.3	25.1	26.4
8	22.0	16.2	30.2	21.5
9	41.5	41.1	78.2	42.7
10	35.8	35.3	41.6	41.3
10-Me	21.7	24.0	15.2	15.9

Experimental

^{1}H and ^{13}C NMR spectra were determined at 360 and 90.5 MHz respectively for solutions in deuteriochloroform. IR spectra were determined as nujol mulls. Mass spectra were determined on a Fisons Autospec mass spectrometer. Silica for chromatography was a Merck 9385. Petrol refers to the fraction, b.p 60–80°C. Extracts were dried over sodium sulfate.

General fermentation details: *Cephalosporium aphidicola* (IMI 68689) was grown on shake culture at 25°C in conical flasks (250 cm^3) containing sterile medium (100 cm^3) containing (per litre) glucose (80 g), ammonium nitrate (0.48 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(II) 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 2 days growth and the fermentation was continued for a further 6

† Steroid numbering is used for comparison purposes; systematic name, 4,4a,5,6,7,8-hexahydro-4a-methyl-2(3H)-naphthalenone.

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days. The mycelium was filtered and the broth was extracted with dichloromethane. The extract was dried and the solvent was evaporated to give the fermentation products which were separated by chromatography.

Incubation of (+)-hexahydro-10β-methylnaphthal-4-en-3-one: The unsaturated ketone **1** (0.7 g) in ethanol (25 cm^3) was evenly distributed between 30 flasks of *C. aphidicola* 2 days after inoculation. After a further 6 days the fermentation products were a recovered and separated by chromatography on silica. Elution with 20% ethyl acetate:light petroleum gave 6β -hydroxyhexahydro-10β-methylnaphthal-4-en-3-one 2 (50 mg) as an oil, (Found: M^+ 180.115 $C_{11}H_{16}O_2$ requires M^+ 180.115), $[\alpha]_D$ +84° (c 0.08, $CHCl_3$).(lit.,[7] –95° for enantiomer), υ_{max}/cm^{-1} 3404, 1675; δ_H 1.43 (3H, s, 10β-Me), 1.0–2.2 (10H, unresolved multiplets), 4.33 (1H, t, *J*=2.0 Hz, 6-H), 5.79 (1H, s, 4-H). Further elution with 50% ethyl acetate: light petroleum gave 9β-hydroxy-10β-methylhexahydronaphthal-4-en-3-one (100 mg) as an oil, (Found: 180.115, $C_{11}H_{16}O_2$ requires M^+ 180.115), $[\alpha]_D$ + 174° (c 0.07, $CHCl_3$), (lit.,[7] +159°) υ_{max}/cm^{-1} 3410, 1650; δH 1.19 (3H, s, 10β-ME), 1.0– 2.4 (10H overlapping multiplets, 3,43 (1H, dd, *J*=11.6 and 4.3 Hz, 9-H), 5.69 (1H, s, 4-H).

Incubation of (–)-hexahydro-10β-methylnaphthal-4-en-3-one: The unsatureated ketone **5** (0.7 g) was incubated with *C. aphidicola* as above and the metabolites were separated by chromotography. Elution with 20% ethyl acetate:light petroleum gave the starting material (400 mg). Further elution with 35% ethyl acetate light petroleum gave 6α-hydroxyhexahydro-10α-methylnaphthal-4-en-3-one **6** (15 mg) identified by its ^1H NMR spectrum. Elution with 40% ethyl acetate: light petroleum gave 1α-hydroxyhexahydro-10α-methylnaphthal-4-en-3-one **7** (15 mg) as an oil, (Found: M^+ 180.115, $C_{11}H_{16}O_2$ requires M^+ 180.115), $[\alpha]_D$ – 74° (c 0.02, $CHCl_3$), υ_{max}/cm^{-1} 3500, 1660; δ_H 1.20 (3H, s, 10α-Me), 1.0 – 2.3 (10H overlapping multiplets), 3,88 (1H, dd, *J*=9.2 and 4.6 Hz, 1-H), 5.76 (1H, s, 4-H).

Reduction of the Wieland–Miescher ketone: The ketone 4 (500 mg) in ethanol (10 cm^3) was treated with sodium borohydride (150 mg) and stirred at 0° for 5 min. Acetic acid (0.5 cm^3) was added and the solution was stirred for 5 min. The solvent was evaporated and the residue was partitioned between dichloromethane and aqueous sodium chloride. The dichloromethane extract was separated, dried and the solvent evaporated to give a residue which was chromatographed on silica. Elution with 50% ethyl acetate:light petroleum gave 9β-hydroxy-10β-methyl-hexahydronaphthal-4-en-3-one (350 mg) identical (^1H NMR) to the material described above.

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