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The microbiological hydroxylation of the sesquiterpenoid patchoulol by *Mucor plumbeus*

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Abstract

The microbiological hydroxylation of patchoulol by *Mucor plumbeus* gave the 5α- and 9α-hydroxy derivatives. The 5α-hydroxy compound was also obtained using *Cephalosporium aphidicola* and its structure was established by X-ray crystallography.

Keywords: *Mucor plumbeus*; Microbiological hydroxylation; Sesquiterpenoids; Patchoulol

1. Introduction

The bridged ring system of the readily available sesquiterpenoid, patchoulol **1** (patchouli alcohol) (Dobler et al., 1963; Teisseire, Maupetit & Corbier, 1974) makes it a suitable substrate for the study of the effect of structure on the regiochemistry of microbiological hydroxylation. In view of the importance of norpatchoulenol as the odiferous component of patchouli oil, there have been a number of studies (Bang, Ourisson & Teisseire, 1975; Furstoss & Lamare, 1990; Teisseire, 1980) on the biotransformation of patchoulol directed at the hydroxylation of the secondary methyl group (C-12) in order to facilitate its removal and the synthesis of norpatchoulenol. Recently some hydroxylated patchoulol derivatives have been obtained (Nishiya et al., 1995) from the Chinese medicinal plant, *Valeriana fauriei*.

2. Results and discussion

Incubation of patchoulol **1** with *Mucor plumbeus* for 5 days gave two hydroxylation products which were separated by chromatography. The ^{13}C NMR spectrum (see Table 1) of the first metabolite **2** to be eluted from the column, contained an additional tertiary alcohol resonance at δ_C 76.6 ppm. The downfield shift of the resonances assigned to C-4 ($\Delta\delta$ 6.4 ppm) and C-6 ($\Delta\delta$ 10.3 ppm) compared to the starting material (Nesmelyi & Lukacs, 1981), suggested that the hydroxyl group was located at C-5. This diol **2** had been described previously as a biotransformation product formed by *Aspergillus niger* (Furstoss & Lamare, 1990) and *Gliocladium roseum* (Becher et al., 1978). However, in view of the difference in melting point, the structure was confirmed by X-ray crystallography (see Fig. 1). Unlike the analytical sample,the X-ray sample was prepared by crystallization from methanol and it is a methanol solvate. The formation of solvates may account for the difference in melting point.

The second metabolite was identified as 9α-hydroxypatchoulol **3** (Teisseire, 1980). The location of the site of hydroxylation at C-9 followed from the appearance of a methine ^{13}C NMR signal at δ_C 69.1 (see Table 1) and downfield shifts in the position of the resonances assigned to C-8 ($\Delta\delta$ 8.8 ppm) and to C-10 ($\Delta\delta$ 3.3 ppm) when compared to the starting material. The stereochemistry of the alcohol followed from an analysis of the ^1H NMR spectrum. The C**H**(OH) resonance at δ_H 4.14 was a double-doublet (J 6.2 and 9.9.Hz). A

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Table 1
^{13}C NMR data for patchoulol and its metabolites (determined at 75 MHz in deuteriochloroform)

Carbon	Compound		
	1	**2**	**3**
1	75.7	75.4	75.3
2	32.7	31.8	35.9
3	28.6	29.7	28.1
4	28.1	34.5	27.5
5	43.7	76.6	39.2
6	24.3	34.6	24.4
7	39.1	39.1	35.3
8	24.6	22.9	33.4
9	28.9	23.6	69.1
10	37.7	43.4	40.0
11	40.1	39.4	43.4
12	18.6	14.0	15.9
13	20.6	14.8	18.6
14	26.8	27.0	27.2
15	24.3	24.3	24.3

selective population transfer NMR experiment based on the methyl doublet (H-12) at δ_H 0.82 (J 6.6 Hz) led to the identification of the H-4 signal at δ_H 1.82. Irradiation of the methyl group signal at δ_H 1.02 (H-13) gave a nuclear Overhauser effect enhancement of 7.3% to the signal at δ_H 1.82 (H-4) and 3.0% to the C**H**(OH) signal at δ_H 4.14. Irradiation of the H-15 signal (δ_H 1.04) also gave an enhancement of 4.4% to the C**H**(OH) signal. Hence the secondary alcohol has the axial 9α-configuration.

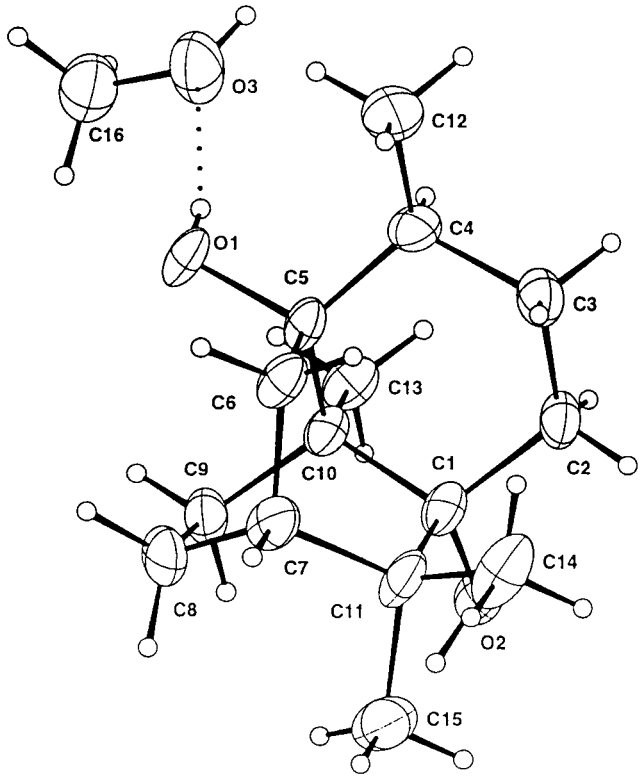
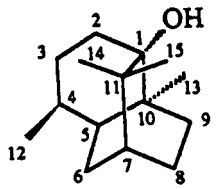
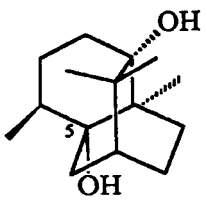


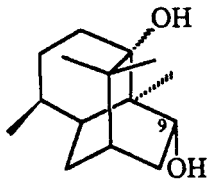
Fig. 1. Crystal structure of 5α-hydroxypatchoulol **2**.



1



2



3

Incubation of patchoulol **1** with the fungus *Cephalosporium aphidicola* for 10 days only gave 5α-hydroxypatchoulol **2** which was identical to the sample obtained from *M. plumbeus*.

These results show the value of microbiological methods in hydroxylating chemically inaccessible centres in polycyclic molecules. Neither C-5 nor C-9 are easily functionalized by conventional chemical means. There is an interesting symmetry in the hydroxylation pattern. The hydroxylation has taken place in a hydrophobic portion of the molecule. The carbon atoms C-5 and C-9 are related by rotation around the C-1:C-10 bond. The calculated distances beween the original C-1 oxygen atom and the oxygen atom that has been introduced are 4.76 and 4.14 Å in **2** and **3**, respectively.

3. Experimental

3.1. General experimental methods

^{1}H NMR spectra were recorded in deuteriochloroform at 300 or 500 MHz. ^{13}C NMR spectra were-

determined at 75 MHz. IR spectra were recorded as nujol mulls. Chromatography was carried out on silica, Merck 9385. Light petroleum refers to the fraction, b.p. 60–80°C. Extracts were dried over anhydrous sodium sulfate. Patchoulol was a gift from Quest International, Ashford, Kent.

3.2. Fermentation conditions

M. plumbeus (IMI 116688) was grown on shake culture on a medium comprising (per litre): glucose (30 g), potassium dihydrogen phosphate (2 g), magnesium sulfate (2 g), ammonium tartrate (2 g), yeast extract (1 g), calcium chloride (0.1 g), sodium chloride (1 g), iron(II) ammonium sulfate (0.1 g) and a trace elements solution (2 cm^3). The latter contained (per litre): zinc sulfate (1 g), iron(II) sulfate (1 g), cobalt nitrate (0.1 g), ammonium molybdate (1 g), copper sulfate (0.1 g) and manganese sulfate (0.1 g). The culture was grown in 250 cm^3 conical flasks each containing 100 cm^3 medium for 36 h at 25°C prior to the addition of the substrate. *C. aphidicola* (IMI 68689) was grown on shake culture on a medium comprising (per litre): glucose (50 g), potassium dihydrogen phosphate (5 g), magnesium sulfate (2 g), glycine (2 g), potassium chloride (1 g) and the above trace elements solution (2 cm^3). The cultures were grown in 250 cm^3 conical flasks each containing 100 cm^3 medium for 48 h at 25°C prior to the addition of the substrate.

3.3. Incubation of patchoulol with M. plumbeus

Patchoulol **1** (2 g) in ethanol (25 cm^3) was evenly distributed between 50 flasks of *M. plumbeus* and the fermentation was continued for a further 5 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 10% ethyl acetate:light petroleum gave the starting material (1.11 g), identified by its ^{1}H NMR spectrum. Further elution with 20% ethyl acetate:light petroleum gave 5α-hydroxypatchoulol **2** (100 mg) which crystallized from ethyl acetate:light petroleum as plates, m.p. 133–1340°C (found: C, 75.1; H, 11.1; $C_{15}H_{26}O_2$ requires C, 75.6; H,11.0%), ν_{max} 3513 (br) cm^{-1}, δ_H 0.85 (3H, s, H-13), 0.87 (3H, d, *J* 6.6 Hz, H-12), 1.06 (3H, s) and 1.10 (3H, s) (H-14 and H-15). The sample for X-ray crystallography was prepared by recrystallization from methanol. Further elution with 25% ethyl acetate:light petroleum gave 9α-hydroxypatchoulol **3** (178 mg) which crystallized from ethyl acetate:light petroleum as cubes, m.p. 196°C (lit. (Teisseire, 1980) 195–196°C), ν_{max} 3387 (br), 1054 cm^{-1}, δ_H 0.82 (3H, d, *J* 6.6 Hz), 1.02 (3H, s, H-13), 1.04 (3H, s, H-14), 1.09 (3H, s, H-15), 1.82 (1H, m, H-4), 4.14 (1H, dd, *J* 6.2 and 9.9 Hz, H-9).

3.4. Incubation of patchoulol with C. aphidicola

Patchoulol **1** (2 g) in ethanol (25 cm^3) was evenly distributed between 50 flasks of *C. aphidicola* and the fermentation was continued for a further 10 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 gum which was chromatographed on silica. Elution with 20% ethyl acetate:light petroleum gave 5α-hydroxypatchoulol (50 mg) identical (^{1}H NMR) to the material described above.

3.5. Crystallographic data and structure determination for 5α-hydroxypatchoulol 2

$C_{15}H_{26}O_2{\cdot}CH_3OH$, M_r 270.4, monoclinic, space group $P2_1$ (No. 4), a = 7.184(2), b = 9.2530(10), c = 11.781(2) Å, $\alpha=\gamma=90°$, $\beta=96.48(2)°$, V = 778.1(3) $Å^3$, Z = 2, $D_{calc}=1.15$ g cm^{-3}, $F(000)=300$, $\lambda=0.71073$ Å, $\mu=0.08$ mm^{-1}. Data were collected using a crystal of size ca. 0.40 × 0.40 × 0.05 mm on an Enraf-Nonius CAD4 diffractometer. A total of 1559 reflections were collected for $2 < \theta < 25°$ and $0 < h < 8$, $0 < k < 10$, $-13 < l < 13$. There were 1454 independent reflections and 939 reflections with $I > 2\sigma(I)$ were used in the refinement. There was no crystal decay and no absorption corrections were applied. The structure was solved by direct methods using SHELXS-86 (Sheldrick, 1986) and refined using SHELXL-93 (Sheldrick, 1993). The non-hydrogen atoms were refined anisotropically by full matrix least squares on F^2. Hydrogen atoms were included in the riding mode with $U_{iso}(H)=1.2$ $U_{eq}(C)$ or 1.5 $U_{eq}(C)$ for methyl groups. Hydroxyl groups were fixed at an idealised geometry but with the torsion angle defining the hydrogen atom position refined and $U_{iso}(H)=1.5$ $U_{eq}(O)$. The final R indices were $R_1=0.068$, $wR_2=0.181$ and R indices (all data) $R_1=0.111$, $wR^2=0.232$. The goodness-of-fit on F^2 was was 0.864 and the maximum shift/e.s.d. was 0.12 (the C16 methyl torsion angle). Tables of atomic co-ordinates, bond lengths and angles, anisotropic displacement parameters and hydrogen atom co-ordinates are deposited with the Cambridge Crystallographic Data Centre.

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