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The hydroxylation of the sesquiterpenoid valerianol by *Mucor plumbeus*

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Abstract

The bio-transformation of the sesquiterpenoid valerianol and its 1α-hydroxy and 1-oxo derivatives by *Mucor plumbeus* has been shown to take place at C-1 and C-10. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Mucor plumbeus*; Microbiological transformation; Sesquiterpenoids; Valerianol

1. Introduction

The sesquiterpenoid valerianol **2** is a constituent of valerian oil (Jommi, Krepinsky, Herout & Sorm, 1969) and it may be prepared by the oxy-mercuration of commercially available valencene **1** (Fontes Arantes, Hanson & Hitchcock, 1999). Valerianol is identical to kusenol which was obtained (Hikino, Suzuki & Takemoto, 1968) from the higher boiling fraction of camphor blue oil. More highly hydroxylated sesquiterpenoids possessing the eremophilane carbon skeleton are of interest (Fraga, 1998 and previous reports) because of their biological activity as phytoalexins (Katsui, Yagihashi & Murai, 1982) and as toxic fungal metabolites (Moreau et al., 1980). Although there is a report (Paknikar & Dhavlikar, 1975) of the bacterial conversion of valencene to dihydro-α-agarofuran, there have not been any reports (Lamare & Furstoss, 1990) on the microbiological hydroxylation of valerianol. We have therefore examined the biotransformation of valerianol and that of some derivatives by the fungus, *Mucor plumbeus*.

2. Results and discussion

Valerianol **2** was obtained by the oxy-mercuration of valencene **1**. Hydroboration of valerianol gave an epimeric mixture of C-1 alcohols which were oxidized with chromium trioxide in acetone to give a single ketone **5** (Jommi et al., 1969). Epimerization at C-10 of the minor *cis* isomer to afford the more stable *trans* ring junction probably took place during the work-up of the oxidation. Reduction of the C-1 ketone with sodium borohydride gave the C-1α alcohol **6**. The multiplicity of the CHOH signal (δ_H 3.77) and the magnitude of the coupling constants (quartet, J 3.0 Hz) indicated that the alcohol **6** possessed the 1α-axial configuration. This alcohol had been described previously (Hikino et al., 1968) but our melting point was significantly different. The X-ray crystal structure of its metabolite **7**, vide infra, confirmed its structure. The alkene **2**, ketone **5** and alcohol **6** were then incubated with *Mucor plumbeus*.

Valerianol **2** gave three metabolites which were separated by chromatography. The first metabolite was identified as 10β,11-oxy-4α,5α,7β-eremophil-1-ene **3**. The ^{13}C NMR spectrum (see Table 1) contained quaternary carbon resonances at δ_C 73.3 and 74.1 characteristic of the ether bridge and alkene resonances at δ_C 129.4 and 130.7. The multiplicity of the corresponding alkene ^1H NMR signals at δ_H 5.33 [doublet (J 9.7 Hz)

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Table 1
^{13}C NMR data for compounds **2**–**8**

	2	3	4	5	6	7[a]	8
1	119.7	130.7	112.4	213.1	71.9	75.8	213.9
2	25.7	129.4	125.7	41.2	33.9	32.7	36.8
3	27.1	21.5	27.0	31.3	25.7	26.8	30.6
4	40.9	32.2	38.7	42.7	43.4	35.3	33.6
5	37.5	37.0	35.9	41.7	36.3	39.8	43.1
6	40.4	32.2	35.6	39.2	41.0	34.8	31.9
7	44.3	35.1	40.5	43.0	43.6	43.9	42.3
8	28.8	27.7	32.0	26.0	27.7	22.4	20.8
9	32.5	35.8	128.3	20.5	25.9	30.4	27.4
10	143.0	74.1	141.9	57.7	49.1	73.6	78.2
11	72.6	73.3	72.5	72.6	72.6	71.9	72.9
12	26.9	28.7	26.1	26.8	27.0	27.5	27.2
13	26.9	28.9	27.4	26.9	27.3	27.9	27.8
14	18.4	18.8	17.0	12.0	15.7	15.7	13.0
15	15.6	14.6	14.6	14.5	14.7	16.2	14.3

[a] Determined in pyridine-d_5.

of doublets (J 1.7 Hz)] and 5.76 [doublet (J 9.7 Hz) of doublets (J 4.6 Hz) of doublets (J 2.2 Hz)] served to locate the double bond at the 1(2) position. The second minor metabolite was a diene which showed multiplet alkene resonances at δ_H 5.42, 5.60 and 5.95. Selective decoupling of the latter showed that it was coupled to the signal at δ_H 5.60. The diene was tentatively assigned the structure **4** although the 1(10); 8(9) isomer could not be ruled out. The third metabolite was the triol **7** which was also obtained from the biotransformation of the 1-ketone **5** and the 1α alcohol **6**. The triol possessed a secondary alcohol ^{13}C NMR signal at δ_C 75.8 and two tertiary alcohol signals at δ_C 71.9 and 73.6. The ^{1}H NMR signal assigned to the secondary alcohol was a broad singlet at δ_H 4.05. The full structure and stereochemistry of the triol was established by X-ray crystallography (see Fig. 1). Since the triol **7** was also obtained as a hydroxylation product of the 1α-alcohol **6** this also served to confirm the stereochemistry of the latter.

Incubation of the 1-ketone **5** with *M. plumbeus* gave three metabolites, which were separated by chromatography on silica. The first metabolite was identical to the alcohol **6**. The second product was the α-ketol **8** (ν_{max}/cm^{-1} 3471, 3334, 1704). The ^{13}C NMR spectrum contained a carbonyl resonance at δ_C 213.9 and a quaternary carbon signal at δ_C 78.2 in addition to the resonance assigned to C-11 (δ_C 72.9). The downfield shift of the resonance assigned to C-9 and the loss of the methine resonance assigned to C-10 when compared to the starting material, indicated that the new hydroxyl group was located at C-10. The third metabolite was the lα,10β,11-triol **7** described above. Incubation of the 1α-alcohol **6** with *M. plumbeus* gave the 1-ketone **5**, the α-ketol **8** and the triol **7**.

The biotransformation of the 1-ketone and the 1α-alcohol at C-10 involves hydroxylation on the β-face of the molecule. The biological epoxidation of an alkene may follow the same stereochemistry as the hydroxylation of the corresponding saturated centre.(Bloom & Shull, 1955) This would lead to the 1β:10β-epoxide in the case of valerianol. Diaxial cleavage of this epoxide would afford the 1α,10β-glycol. Elimination of the 1α-axial hydroxyl group would afford the 1-ene whilst elimination of the 10β-axial hydroxyl group would afford the 9(10)-alkene. Ether formation could take place between the 10β- and 11-

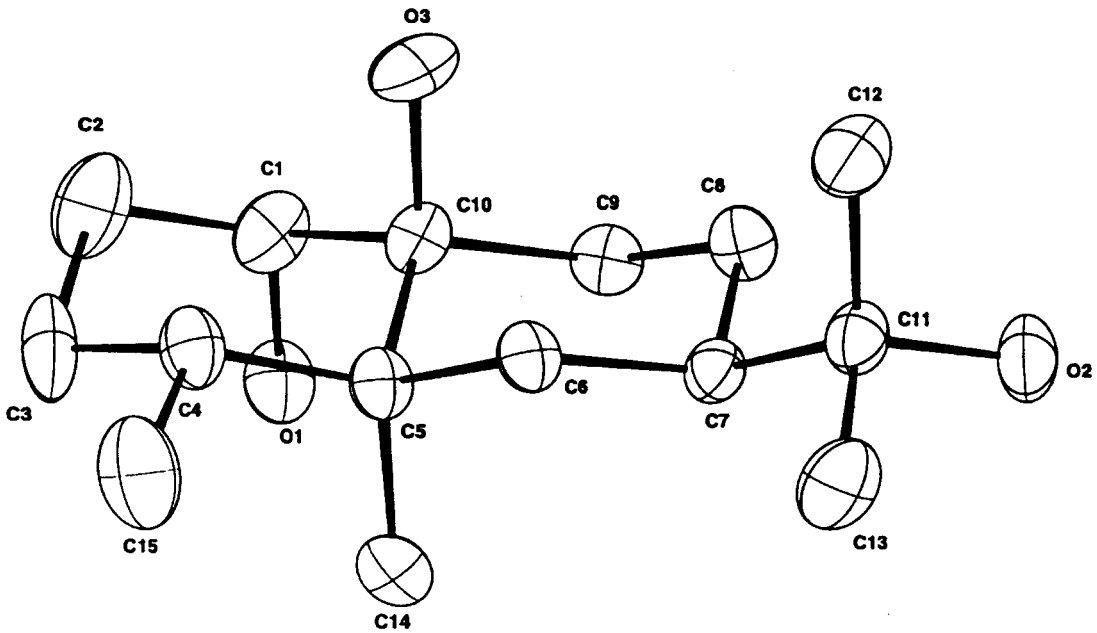
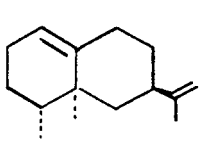
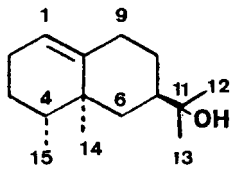


Fig. 1. Crystal structure of compound **7**.

hydroxyl groups to afford the 10β:11-ether in **3**. Although there may not be a sequential relationship interlinking the microbiological hydroxylation products of the alkene, the 1-ketone and the 1α-alcohol, the possible formation of the 1(10)-epoxide from the alkene may lead to a convergence in the products of these transformations.



1



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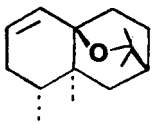

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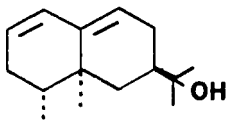
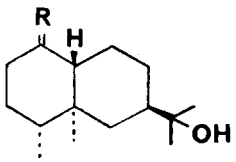
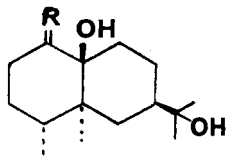


4



5 R = =O.

6 R = α-OH,β-H.



7 R = α-OH,β-H.

8 R = =O.

3. Experimental

3.1. General experimental details

^{1}H NMR spectra were determined in deuteriochloroform at 300 and 500 MHz; ^{13}C NMR spectra were determined at 75 MHz. IR spectra were determined as thin films or as nujol mulls on a Perkin Elmer 1710 spectrometer. Silica gel for chromatography was Merck 9385. Light petroleum refers to the fraction b.p. 60–80°C. Extracts were dried over anhydrous sodium sulfate. Valerianol was obtained from valencene as described previously (Fontes Arantes et al., 1999).

3.2. Hydroboration and oxidation of valerianol

Valerianol **2** (600 mg) in dry tetrahydrofuran (10 cm^3) was treated with a solution of borane (1 M) in tetrahydrofuran (11 cm^3) at 0°C under nitrogen for 6 h. Water (5 cm^3) followed by 10% aqueous sodium hydroxide (10 cm^3) and 30% aqueous hydrogen peroxide (15 cm^3) were added and the mixture was stirred for 15 h. Sodium sulfite (1 g), acetic acid (1 cm^3), water (50 cm^3) and ethyl acetate (150 cm^3) were then added and the solution was stirred for a further 25 min. The organic phase was separated, washed with water, brine and dried. The solvent was evaporated to give a gum. The ^{1}H NMR spectrum showed that this was an epimeric mixture of alcohols. The gum (540 mg) in acetone (5 cm^3) was treated with the Jones chromium trioxide reagent at 0°C until the orange colour persisted. The mixture was left at room temperature for 30 min. and then quenched with methanol. The solution was concentrated in vacuo, diluted with water and the product recovered in ether. The extract was washed with aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated to give 11-hydroxy-1-oxo-4α,5α,7β,10β-eremophilane **5** (520 mg), which crystallized from ethyl acetate: light petroleum as needles, m.p. 82–84°C (lit., Jommi, 1968, 88–89°C). (Found: C, 75.4; H, 11.0. Calc. for $C_{15}H_{26}O_2$: C, 75.6; H, 11.0%),.ν_{max}/cm^{-1} 3436, 1702; δ_H 0.63 (3H, s, H-14), 0.88 (3H, d, *J* 6.7 Hz, H-15), 1.15 and 1.17 (each 3 H, s, H-12, H-13), 2.09 (1H, ddd, *J* 12.2, 3.3 and 1.0 Hz, H-10β), 2.27 (1H, ddd, *J* 13.9, 5.4, and 1.9 Hz, H-2α), 2.33 (1H, ddd, *J* 13.9, 8.4 and 1.0 Hz, H-2β).

3.3. Reduction of 11-hydroxy-1-oxo-4α,5α,7β,10β-eremophilane

The above ketone (1 g) in dry methanol (20 cm^3) was treated with sodium borohydride (250 mg) at 0° for 30 min. Acetic acid (2 cm^3) and water (2 cm^3) were added, the solution was concentrated in vacuo, diluted with water and the organic product was recovered in ether. The extract was washed with aqueous sodium hydrogen carbonate, water, brine and dried. The solvent was evaporated and the residue chromatographed on silica. Elution with 20% ethyl acetate: light petroleum gave 1α,11-dihydroxy-4α,5α,7β,10β-eremophilane **6** (510 mg), which crystallized from ethyl acetate: light petroleum as prisms, m.p. 127°C (lit., Hikino et al., 1968, 187°C) (Found: C, 75.1; H, 12.0. Calc. for $C_{15}H_{28}O_2$: C, 74.9; H, 12.0%), ν_{max}/cm^{-1} 3608, 3583; δ_H 0.81 (3H, d, *J* 6.8 Hz, H-15), 0.90 (3H, s, H-14), 1.13 and 1.14 (each 3H, s, H-12 and H-13), 3.77 (1H, quartet, *J* 3.0 Hz, H-1β).

3.4. Incubations with Mucor plumbeus

Mucor plumbeus CMI 116688 was grown in shake flasks (100 cm^3 medium per 250 cm^3 conical flask) on a medium comprising (per dm^3), glucose (50 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), ferrous ammonium sulfate (0.2 g) and a trace elements solution (2 cm^3). The substrate was added in ethanol 1 day after inoculation and the fermentation was continued for a further 5 days before the mycelium was filtered and the broth extracted with ethyl acetate. The extracts were dried over sodium sulfate and the solvent evaporated to give a residue, which was chromatographed on silica.

(a) Valerianol (1.39 g) in ethanol (46 cm^3) was evenly distributed between 46 flasks of *M. plumbeus*. The metabolites were separated by chromatography on silica. Elution with 5% ethyl acetate: light petroleum gave 10β,11-oxido-4α,5α-eremophil-1-ene **3** (20 mg) as an oil, (Found: M^+ 220.183. $C_{15}H_{24}O$ requires 220.181), ν_{max}/cm^{-1} 1643; δ_H 0.81 (3H, s, H-14), 0.88 (3H, d, *J* 6.7 Hz, H-15), 1.23 and 1.29 (each 3H, s, H-12 and H-13), 5.33 (1H, dd, *J* 9.7 and 1.7 Hz, H-1), 5.76 (1H, ddd, *J* 9.7, 4.6 and 2.2 Hz, H-2). Elution with 10% ethyl acetate: light petroleum gave the starting material (500 mg) followed by 11-hydroxy-4α,5α,7β-eremophila-1(10),8-diene (30 mg) as an oil,(Found: M^+ 202.172. $C_{15}H_{24}$ (M-H_2O) requires 202.172), ν_{max}/cm^{-1} 3609, 3393, 1655; δ_H 0.89 (3H, s, H-14), 0.90 (3H, d, *J* 6.8 Hz, H-15), 1.23 and 1.25 (each 3H, s, H-12 and H-13), 5.42 (1H, m, H-1), 5.60 (1H, m, H-8), 5.95 (1H, m, H-9). Elution with 15% ethyl acetate: light petroleum gave 1α,10β,11-trihydroxy-4α,5α,7β-eremophilane (30 mg) as needles, m.p. 126–127° (Found: C, 69.7; H, 11.0. $C_{15}H_{28}O_3$ requires C, 70.3; H, 11.0%), ν_{max}/cm^{-1} 3608, 3583, 3377; δ_H (pyridine-d_5) 0.92 (3H, d, *J* 6.9 Hz, H-15), 1.43 (6H, s, H-12 and H-13), 1.48 (3H, s, H-14), 4.05 (1H, br.s. H-1).

(b) Under similar conditions incubation of 11-hydroxy-1-oxo-4α,5α,7β,10β-eremophilane **5** (1.0 g) in 48 flasks for 5 days gave, after chromatography on silica and elution with 15% ethyl acetate: light petroleum, the starting material (350 mg) and 1α,11-dihydroxy-4α,5α,7β,10β-eremophilane **6** identified by their ^1H and ^{13}C NMR spectra. Further elution with 30% ethyl acetate: light petroleum gave 10β,11-dihydroxy-1-oxo-4α,5α,7β-eremophilane **8** (46 mg) which crystallized from ethyl acetate: light petroleum as needles, m.p. 182–183° (Found: C, 70.7; H, 10.3. $C_{15}H_{26}O_3$ requires C, 70.8; H, 10.3%), ν_{max}/cm^{-1} 3471, 3334, 1704; δ_H 0.81 (3H, d, *J* 6.7 Hz, H-15), 0.71 (3H, s, H-14), 1.21 (6H, s, H-12 and H-13). Elution with 70% ethyl acetate: light petroleum gave 1α,10β,11-trihydroxy-4α,5α,7β-eremophilane **7** (30 mg) identified by its ^1H and ^{13}C NMR spectra.

(c) Under similar conditions incubation of 1α,11-dihydroxy-4α,5α,7β,10β-eremophilane **6** (600 mg) in 30 flasks for 5 days, gave after chromatography on silica and elution with 20% ethyl acetate: light petroleum, 11-hydroxy-1-oxo-4α,5α,7β,10β-eremophilane **5** (200 mg). Elution with 25% ethyl acetate: light petroleum gave the starting material (60 mg) followed by 10β,11-dihydroxy-1-oxo-4α,5α,7β-eremophilane **8** (40 mg). Elution with 70% ethyl acetate: light petroleum gave 1α,10β,11-trihydroxy-4α,5α,7β-eremophilane **7** (50 mg). The known metabolites were identified by their NMR spectra.

3.5. Crystal data and structure determination of compound 7

$C_{15}H_{28}O_3$, M_r 256.4, monoclinic, space group C2 (No. 5), a = 27.368(4), b = 15.037(2), 13.001(2)Å, $\alpha=\gamma=90°$, $\beta=117.58(1)°$, V = 4742.3(12) Å^3, Z = 12, $D_{calc.}=1.08$ g cm^{-3}, $F(000)=1704$, $\lambda=1.5418$ Å, $\mu=0.58$ mm^{-1}. Data were collected using a crystal of size 0.2 × 0.1 × 0.1 mm, on an Enraf–Nonius CAD4 diffractometer. A total of 4286 reflections were collected for $2 < \theta < 66°$ and $0 < h < 32$, $0 < k < 17$ and $-15 < l < 13$. There were 4213 independent reflections and 2694 reflections with $I > 2\sigma(I)$ were used in the refinement. The structure was solved by direct methods using SHELXS-86 (Sheldrick, 1986) and SHELXL-93 (Sheldrick, 1993) for the refinement. The nonhydrogen atoms were refined anisotropically by full matrix least squares on F^2. Hydrogen atoms were included in the riding mode with $U_{iso}=1.2U_{eq}(C)$ or $1.5U_{eq}(C)$ for methyl groups. Hydroxyl hydrogen atoms were riding at an idealised staggered geometry chosen to give the best hydrogen bonding interactions and with $U_{iso}(H)=1.5U_{eq}(O)$. Despite repeated attempts only poor quality inter-grown crystals could be produced and a piece cut from one such clump was used in the data collection. Although the presence of three independent molecules in space group C2 suggests the possibility of a higher symmetry rhombohedral space group it was not possible to find such a transformation. The final R indices were $R_1=0.097$, $wR_2=0.263$ and R indices (all data) $R_1=0.140$, $wR_2=0.305$. The goodness-of-fit on F^2 was 1.116 and the maximum shift to e.s.d. was 0.009. Tables of atomic coordinates, bond lengths and angles, anisotropic displacement factors and hydrogen atom coordi-

nates have been deposited at the Cambridge Crystallographic Data Centre.

Acknowledgements

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