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The hydroxylation of the sesquiterpenoid guaioxide by *Mucor plumbeus*

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Abstract

Microbiological hydroxylation of guaioxide by *Mucor plumbeus* gave the 7-, 8α- and 9β- and 9α-hydroxy derivatives.

Keywords: *Mucor plumbeus*; Microbiological hydroxylation; Sesquiterpenoid; Guaioxide

1. Introduction

Guaiol **1** is a readily available sesquiterpenoid from guaiac wood oil (Plattner & Lemay, 1940; Plattner & Magyar, 1942). A large number of hydroxylated guaianes are known. Compounds with this azulenoid carbon skeleton have been obtained from both plants and fungi and a number have interesting biological activity (Fraga, 1998). Their preparation by the microbiological hydroxylation of the more abundant members of this family is an attractive target (Lamare & Furstoss, 1990). The hydroxylation of guaioxide **2** by *Mucor parasiticus* gave (Ishii et al., 1970) the 4α- and 8α-monohydroxy- and 4α,8α-dihydroxy derivatives whilst *Streptomyces purpurescens* afforded (Ishii et al., 1971) ring A hydroxylation products and 9α-hydroxyguaioxide.

2. Results and discussion

Despite numerous attempts, it was not possible to hydroxylate guaiol **1** itself using *Mucor plumbeus*. However, the ether bridge of guaioxide **2**, which was readily prepared from guaiol by treatment with perchloric acid in acetic acid (Bates & Slagel, 1962), alters the geometry of the guaiane ring system sufficiently to make it a substrate for *M. plumbeus*.

Incubation of guaioxide **2** with *M. plumbeus* for five days gave four metabolites, which were separated by column chromatography. The first metabolite to be isolated was 7-hydroxyguaioxide **3**. The location of the hydroxyl group at C-7 followed from the appearance of a new tertiary alcohol signal in the ^{13}C NMR spectrum at δ_C 79.8 (see Table 1). The methylene signals assigned to C-6 and C-8 showed significant downfield shifts (δ_C 47.0; $\Delta\delta$ 6.2, C-6; δ_C 39.5, $\Delta\delta$ 9.1, C-8) whilst the C-14 and C-15 methyl groups showed γ-gauche shieldings of 3.6 and 1.7 ppm compared to the starting material.

The 8α- and 8β-hydroxyguaioxides, **4** (Ishii et al., 1970) and **5**, were isolated as an inseparable mixture (1:1) of secondary alcohols (**4**, H-8, δ_H 3.88, multiplet; **5**, H-8, δ_H 3.62, doublet of triplets, J 11.9 and 4.1 Hz). The mixture was oxidized with chromium trioxide to a single ketone (δ_C 208.15; ν_{max} 1695 cm^{-1}) and hence it was a mixture of epimeric alcohols. In order to confirm the structure, the proton resonances on the seven-membered carbocyclic ring of the ketone were assigned as follows. Irradiation of the geminal methyl group signal at δ_H 1.15 (H-14) gave a nuclear Overhauser effect (nOe) enhancement to a signal at δ_H 1.90 (double-doublet, J 13.7 and 6.4 Hz, H-6β) and δ_H 2.57

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Table 1
^{13}C NMR data determined in deuteriochloroform at 75 MHz

	Compound			
Carbon	2	3	6	7
1	54.8	53.2	55.6	53.2
2	28.5	28.2	29.0	29.2
3	31.1	30.7	31.6	31.2
4	46.0	46.4	52.0	46.4
5	90.9	87.0	91.4	90.9
6	40.8	47.0	36.4	40.5
7	41.1	79.8	63.0	44.2
8	30.4	39.5	208.1	40.9
9	33.8	32.2	45.9	72.4
10	37.5	37.5	32.9	44.2
11	80.9	81.0	81.0	81.2
12	23.3	23.7	23.1	17.6
13	16.3	17.1	16.5	16.8
14	30.4	26.8	29.8	30.3
15	23.0	21.3	24.3	23.9

(multiplet, H-7). The ^{1}H:^{13}C correlation spectrum showed that the signal at δ_H 1.90 was linked to a methylene carbon (δ_C 36.4) and to a second proton (δ_H 2.10, doublet of doublets, J 13.7 and 1.7 Hz). There was also a correlation between a methine resonance at δ_C 63.0 and the signal (δ_H 2.57) assigned to H-7. Irradiation of H-15 (δ_H 1.22) produced an nOe enhancement of δ_H 2.57 (H-7, 1.7%) and at δ_H 2.61 (1H, m, 3.8%). The ^{1}H:^{13}C correlation spectrum showed a correlation between the signal at δ_H 2.61, a methylene carbon at δ_C 52.0 and a further proton at δ_H 2.30 (doublet, J 14.5 Hz, of triplets, J 1.5 Hz). Irradiation of this signal showed that it was coupled to the signal at δ_H 2.61 and to a signal at δ_H 1.56 which was in turn coupled to the methyl group doublet (δ_H 1.00, J 7.0 Hz). This led to the assignment of the signals at δ_H 1.56 as H-10, δ_H 1.90 as H-6β, δ_H 2.10 as H-6α, δ_H 2.30 as H-9α, δ_H 2.57 as H-7 and δ_H 2.61 as H-9β and to the structure of the ketone as 8-oxo-guiaoxide **6**. Hence the original metabolites were the 8α- and 8β-alcohols, **4** and **5**.

The site of hydroxylation (C-9) of the fourth metabolite **7** followed from changes in the ^{13}C NMR spectrum to the position of the signals assigned to C-8 (δ_C 40.9; $\Delta\delta$ 10.5 ppm) and C-10 (δ_C 44.2, $\Delta\delta$ 6.7 ppm). The ^{1}H NMR spectrum of 9α-hydroxyguaioxide **7** contained a CH(OH) resonance at δ_H 3.75 which appeared as a doublet (J 10.8 Hz) of doublets (J 9.8 Hz) of doublets (J 4.0 Hz) (Ishii et al., 1971, δ_H 3.83, multiplet). This multiplicity corresponded to a secondary alcohol possessing two diaxial and one axial:equatorial coupling. Irradiation of the H-15 (methyl group) signal (δ_H 1.28) gave a nOe enhancement (4.4%) of the CH(OH) signal at δ_H 3.75 whilst irradiation of the methyl group doublet at δ_H 0.98 (H-12) also produced nOe enhancements of the H-1 (δ_H 1.54, 3.2%) and H-9 (δ_H 3.75, 1.1%) signals. Hence the metabolite was assigned the stereochemistry of 9α-hydroxyguaioxide **7**.

Guaioxide as a cyclic ether, is chemically relatively unreactive thus restricting its use as a starting material for partial synthesis. Free radical oxidation with *m*-chloroperbenzoic acid has been shown (Tori et al., 1990) to occur at the tertiary centres C-1, C-4 and C-10 in low yield. However the microbiological hydroxylation by *M. plumbeus* takes place at methylene carbons that are chemically difficult to functionalize. The distribution of metabolites obtained with *M. plumbeus* differed from that found with other organisms.

3. Experimental

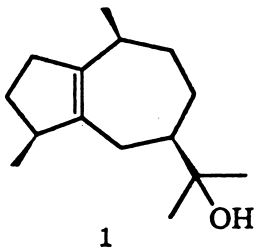
^{1}H NMR spectra were recorded in $CDCl_3$ at 300 or 500 MHz. ^{13}C NMR spectra were determined at 75 MHz. IR spectra were recorded as nujol mulls. Chromatography was carried out on silica, Merck 9385. Light petroleum refers to the fraction, b.p. 60–80°. Extracts were dried over anhydrous sodium sulfate. Jones reagent refers to a solution of chromium trioxide (26.72 g) in concentrated sulfuric acid (23 cm^3) diluted to 100 cm^3 with water.

3.1. Fermentation conditions

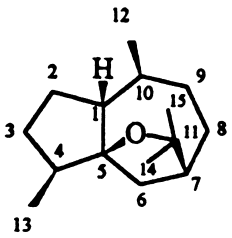
M. plumbeus (IMI 116688) was grown on shake culture on a medium comprising (per litre): glucose (30 g), potassium dihydrogen phosphate (2 g), magnesium sulfate (2 g), ammonium tartrate (2 g), yeast extract (1 g), calcium chloride (0.1 g), sodium chloride (1 g), iron(II) ammonium sulfate (0.2 g) and a trace elements solution (2 cm^3). The latter contained (per litre): zinc sulfate (1 g), iron(II) sulfate (1 g), cobalt nitrate (1 g), ammonium molybdate (1 g), copper sulfate (0.1 g) and manganese sulfate (0.1 g). The culture was grown in shake culture in 250 cm^3 conical flasks each containing 100 cm^3 medium for 36 h prior to the addition of the substrate.

3.2. Incubation of guaioxide with M. plumbeus

Guaioxide (710 mg) dissolved in ethanol (30 cm^3), was evenly distributed between 29 flasks of *M. plumbeus* and the fementation continued for a further five days. The mycelium was filtered and the broth was extracted with diethyl ether. The extract was dried and the solvent evaporated to give a gum which was chromatographed on silica. Elution with 5% diethyl ether:-light petroleum gave 7α-hydroxyguaioxide **3** (23 mg) which crystallized from light petroleum as needles, m.p. 118–120° (found: HREIMS 238.192 $C_{15}H_{26}O_2$



1



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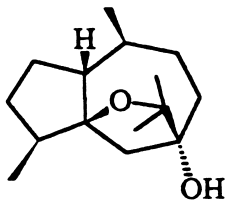

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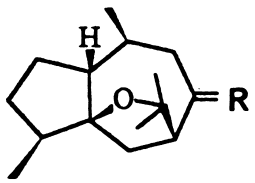
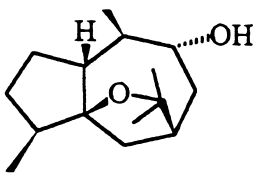


4 R = α-OH, β-H

5 R = α-H, β-OH

6 R = =O



7

requires 238.193), ν_{max} 3388, 1086, 1064 cm^{-1}; δ_H 0.90 (3H, *d, J* 6.3 Hz, H-12), 0.95 (3H, *d, J* 7.0 Hz, H-13), 0.99 (1H, *m*, H-2α), 1.12 (3H, *s*, H-14), 1.16 (3H, *s*, H-15), 1.57 (1H, *td, J* 13.8, 4.1 Hz, H-8β), 1.91 (1H, *m*, H-2β), 1.96 (1H, *dd, J* 11.7, 2.0 Hz, H-6β), 2.12 (1H, *d, J* 11.7 Hz, H-6α). Elution with 7% diethyl ether: light petroleum gave a mixture of alcohols **4** and **5** (25 mg) which were oxidized to 8-oxoguaioxide (vide infra). Further elution with 10% diethyl ether:light petroleum gave 9α-hydroxyguaioxide **7** (60 mg) which crystallized from light petroleum as needles, m.p. 117–119° (found: HREIMS 238.193 $C_{15}H_{26}O_2$ requires 238.193). ν_{max} 3380, 1107 cm^{-1}, δ_H 0.95 (3H, *d, J* 7.1 Hz, H-13), 0.98 (3H, *d, J* 6.5 Hz, H-12), 1.12 (3H, *s*, H-14), 1.28 (3H, *s*, H-15), 1.31 (1H, *m*, H-8α), 1.54 (1H, *dt, J* 10.1, 8.5 Hz, H-1), 2.08 (1H, *m*, H-8β), 3.75 (1H, *ddd, J* 10.8, 9.8, 4.0 Hz, H-9β).

3.3. Oxidation of the alcohols *4* and *5*

The mixture of alcohols **4** and **5** (25 mg) from the above was dissolved in acetone (5 cm^3) and titrated with the Jones reagent until a strong persistent orange colour was obtained. Methanol was then added, the solvent was evaporated and the products recovered in diethyl ether. The extract was washed with aqueous sodium hydrogen carbonate, water, brine and dried. The solvent was evaporated to give 8-oxoguaioxide **6** (24 mg) as an oil, ν_{max} 1695, 1164, 1045 cm^{-1}, δ_H 0.91 (1H, *m*, H-2), 0.93 (3H, *d, J* 6.4 Hz, H-13), 1.00 (3H, *d, J* 7.0 Hz, H-12), 1.15 (3H, *s*, H-14), 1.22 (3H, *s*, H-15), 1.25 (1H, *m*, H-3), 1.56 (1H, *m*, H-10), 1.63 (1H, *dd, J* 10.3, 8.0 Hz, H-1), 1.69 (1H, *m*, H-3), 1.76 (1H, *m*, H-4), 1.90 (1H, *dd, J* 13.7, 6.4 Hz, H-6β), 1.93 (1H, *m*, H-2), 2.10 (1H, *dd, J* 13.7, 1.7 Hz, H-6α), 2.30 (1H,

dt, J 14.5, 1.5 Hz, H-9α), 2.57 (1H, *m*, H-7), 2.61 (1H, *m*, H-9β).

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