Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 67 (2006) 58–61
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Microbial transformation of the sesquiterpenoid (−)-maalioxide by *Mucor plumbeus*

Yanmei Wang [a], Teck-Koon Tan [b], Geok Kheng Tan [a], Joseph D. Connolly [c], Leslie J. Harrison [a,*]

[a] *Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Republic of Singapore*
[b] *Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Republic of Singapore*
[c] *Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, UK*

Received 27 September 2005; accepted 27 September 2005
Available online 15 November 2005

Abstract

Microbial transformation of the sesquiterpenoid (−)-maalioxide by the fungus *Mucor plumbeus* gave three metabolites, 9β-hydroxymaalioxide, 1β-hydroxymaalioxide and 7β-hydroxymaalioxide. 9β-Hydroxymaalioxide and its structure was established on the basis of its spectroscopic properties and chemical reactions.

Keywords: Biotransformation, (−)-Maalioxide; 9β-Hydroxymaalioxide; Hydroxylation; *Mucor plumbeus*

1. Introduction

The conversion of readily available natural products into more useful substances by incubation with biological systems has attracted much attention as this approach allows the ready functionalisation of unactivated carbon atoms. For instance, microbial hydroxylation of progesterone is an important step in the industrial production of the steroid hormone cortisol (Peterson et al., 1952). A number of transformations of terpenoids have been directed at the production of compounds with enhanced biological activity, e.g., formation of insecticidal derivatives of cadinanes by incubation with the deuteromycete *Beauveria bassiana* (Buchanan et al., 2000) or the microbial hydroxylation of patchoulol as a route to fragrant compounds (Suhara et al., 1981). (−)-Maalioxide (**1**) is a natural product which was first isolated from the liverwort *Plagiochila acanthophylla* subsp. *japonica* (Matsuo et al., 1974) and which possesses a pleasant odour (Hashimoto et al., 2004). In an effort to improve its odour, **1** has been hydroxylated by both chemical and biological methods. Refluxing with *m*-chloroperbenzoic acid in chloroform afforded 2α-hydroxymaalioxide, 7β-hydroxymaalioxide (**2**) and 8α-hydroxymaalioxide (Tori et al., 1990). Hydroxylation with *Aspergillus cellulosae* gave 1β-hydroxymaalioxide (**3**) while *Aspergillus niger* produced **2** as well as the 1β,9β- and 1β,12-dihydroxy derivatives (Hashimoto et al., 2004). We now report the results of our study of the incubation of **1** with the fungus *Mucor plumbeus*.

2. Results and discussion

(−)-Maalioxide (**1**) was available from a previous study of the chemistry of the liverwort *Lophozia ventricosa* (Huneck et al., 1984) and its structure was confirmed by X-ray crystallographic analysis. Following incubation with *M. plumbeus* for 5 days, the organic-soluble material was chromatographed to afford the known compounds 1β-hydroxymaalioxide (**3**) and 7β-hydroxymaalioxide (**2**) which were identified by comparison of their physical properties with the literature data (Hashimoto et al., 2004; Tori

* Corresponding author. Tel.: +65 6874 2687; fax: +65 6779 1691.
E-mail addresses: chmhl@nus.edu.sg, leslie@nus.edu.sg (L.J. Harrison).

0031-9422/$ - see front matter © 2005 Esevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.09.030

et al., 1990). The major product, however, was the new compound 9β-hydroxymaalioxide (**4**), $C_{15}H_{26}O_2$ (*m/z* 238.1930). The ^1H and ^{13}C NMR spectra of **4** (see Table 1) showed the presence of one oxygenated methine [δ_H 3.40 (1H, *dd*, $J = 4.0$ and 10.1 Hz); δ_C 81.0 (*d*)], two fully substituted oxygenated carbons [δ_C 78.2 (*s*) and 82.9 (*s*)], and four tertiary methyl groups [δ_H 1.31 (3H, *s*), 1.16 (3H, *s*), 1.03 (3H, *s*), 0.90 (3H, *s*), δ_C 30.8 (*q*), 25.8 (*q*), 22.9 (*q*) and 13.0 (*q*)] as well as one fully substituted carbon [δ_C 38.4 (*s*)], two methines [δ_C 56.5 (*d*) and 42.7 (*d*)] and five methylenes [δ_C 40.3 (*t*), 39.0 (*t*), 30.2 (*t*), 27.0 (*t*), and 22.0 (*t*)]. The compound was thus bicarbocyclic with an additional ether ring. Collins' oxidation of **4** gave a ketone (**5**) [δ_C 214.3 (*s*)] which suggested that **4** was a hydroxy derivative of maalioxide as did comparison of the ^1H and ^{13}C NMR spectra of **4** with those of **1**. A complete structure elucidation was carried out with the help of 2D NMR techniques and the important HMBC correlations are shown in Fig. 1. These established that the compound was 9β-hydroxymaalioxide (**4**). The secondary hydroxyl group was obviously equatorial (β) from the coupling constants of H-9 and this was supported by the results of a NOESY spectrum (Fig. 2) which also aided in the complete assignment of the ^1H and ^{13}C NMR spectra.

Table 1
^1H (500 MHz), HMBC and ^{13}C (125 MHz) NMR data for (−)-9β-hydroxymaalioxide (**4**) in $CDCl_3$ (*J* in Hz in parentheses)

Position	δ_H	HMBC		δ_C
		^2J	^3J	
1α	0.98 *dt* (3.3, 13.4)	C-2, 10	C-5, 9, 14	40.3
1β	1.78 *m*	C-2, 10	C-5, 9, 14	
2α	1.38 *dt* (13.4, 3.2)	C-1, 3	C-10	22.0
2β	1.66 *br d* (13.8)	C-1, 3	C-10	
3α	1.06 *m*	C-2, 4	C-1, 5, 15	27.0
3β	1.73 *m*	C-2, 4	C-5	
4	–			78.2
5	1.14 d (13.4)	C-6	C-1, 9, 11, 14, 15	56.5
6	1.83 *m*	C-5, 7	C-12, 13	42.7
7α	1.54 *m*	C-8	C-9	30.2
7β	1.77 *m*	C-8	C-5, 9, 11	
8α	1.46 *m*	C-9		39.0
8β	1.79 *m*	C-9	C-10	
9	3.40 dd (10.1, 4.0)	C-10	C-1, 5, 7, 14	81.0
10	–			38.4
11	–			82.9
12	1.31 *s*	C-11	C-6, 13	30.8
13	1.03 *s*	C-11	C-6, 12	25.8
14	0.90 *s*	C-10	C-1, 5, 9	13.0
15	1.16 *s*	C-4	C-3, 5	22.9

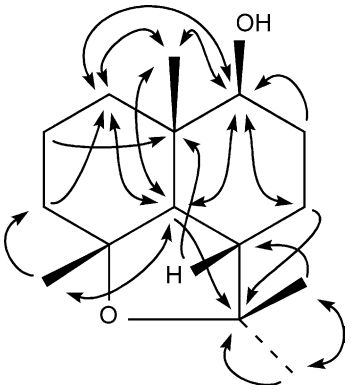


Fig. 1. Selected HMBC correlations of **4**.

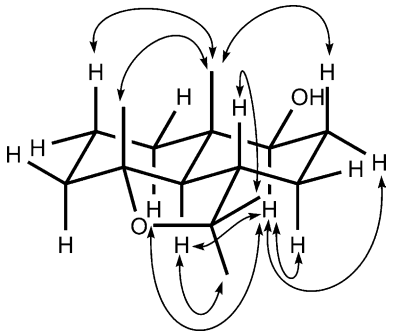
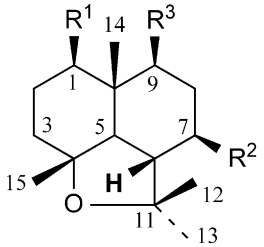
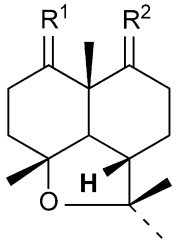


Fig. 2. Selected NOESY correlations of **4**.





1 $R^1=R^2=R^3=H$
2 $R^2=OH$, $R^1=R^3=H$
3 $R^1=OH$, $R^2=R^3=H$
4 $R^3=OH$, $R^1=R^2=H$

5 $R^1=H_2$, $R^2=O$
6 $R^1=O$, $R^2=H_2$

Oxidation of **3** gave the previously unreported 1-oxomaalioxide (**6**). None of the maalioxide derivatives produced in this study had odours which were noticeably different from that of maalioxide itself.

The absolute configuration of synthetic maalioxide was deduced by Büchi et al. (1959) but this compound was only later shown to be (+)-maalioxide (Narayanan et al., 1964). The CD spectra of **5** and **6** showed negative and positive Cotton effects, respectively, at 304 nm, as expected for the (4*R*,5*R*,6*S*,10*S*)-ketones. This also established the absolute configurations of the original alcohols **3** and **4** and confirmed the absolute configuration of (−)-maalioxide (**1**).

3. Experimental

3.1. General experimental details

Mp uncorr.; ^1H and ^{13}C NMR: Bruker DPX300, Bruker AMX500 or Bruker DRX500 in $CDCl_3$; MS: Finnigan TSQ-7000 LC/triple quadrupole MS; IR: Bio-Rad Excalibur Series FTS 3000; Optical Rotation: Perkin–Elmer 241 Polarimeter. X-ray diffraction: Bruker AXS SMART

APEX CCD X-ray Diffractometer; CD: UV/Visible Spectropolarimeter Jasco J-810; CC was carried out on normal phase silica gel 60 (40–63 μm) and HPLC on a Lichrosorb 10 DIOL column (250 × 4.60 mm) with RI detection.

3.2. Fermentation conditions

M. plumbeus (IMI 116688) was obtained from the United Kingdom National Culture Collection (UKNCC). The spores were inoculated on PDA and grown in shake culture in a medium comprising (l^{-1}) (Arantes et al., 1999a,b): 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), ferrous ammonium sulfate (0.1 g) and a trace elements solution (2 cm^3). The latter contained (l^{-1}): zinc sulfate (1 g), ferrous 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 250 cm^3 conical flasks each containing 100 cm^3 of medium for 24 h at 25 °C prior to the addition of the substrate.

3.3. Incubation of (−)-maalioxide with M. plumbeus

(−)-Maalioxide (**1**) (458 mg) was dissolved in ethanol (24 cm^3) and evenly distributed over 30 flasks containing *M. plumbeus* spores. Fermentation was continued for another 5 days, after which the mycelium was filtered off and the broth was extracted with EtOAc. The solvent was evaporated under reduced pressure to give a residue. The mycelium was extracted with MeOH and the residue obtained after solvent removal was defatted by CC on Sephadex LH-20 using MeOH–CH_2Cl_2 (1:1) as eluant. The combined broth and mycelium extracts (in total 703 mg) were chromatographed on silica gel (gradient elution, 0–100% EtOAc–hexane) to afford unreacted starting material (167 mg) and two other fractions (Frs. 2 and 3) which were further purified by HPLC. Fr. 2 (DIOL, 20% acetone–hexane) gave 1β-hydroxymaalioxide (**3**) (20.5 mg) and 7β-hydroxymaalioxide (**2**) (4.6 mg) whilst Fr. 3 afforded 9β-hydroxymaalioxide (**4**) (DIOL, 25% acetone–hexane) (25.0 mg).

3.3.1. (−)-Maalioxide (*1*)

The starting material was isolated from the liverwort *L. ventricosa* (Huneck et al., 1984). Colourless crystals, mp 64–65 °C {lit. mp 66 °C (Matsuo et al., 1974)}; $[\alpha]_D^{20}$ − 30.9 (EtOH; *c* 7.1) {lit. $[\alpha]_D^{20}$ − 34.5 (Matsuo et al., 1974)}.

3.3.2. 9β-Hydroxymaalioxide (*4*)

Colorless gum; $[\alpha]_D^{20}$ − 0.9 (EtOH; *c* 2.5); FT-IR $\nu_{max}^{CHCl_3}$ 3414.0 (–OH), 2976.8, 2937.8, 2866.1, 1382.8, 1214.6 (C–O–C) cm^{-1}; ^1H and ^{13}C NMR shifts are listed in Table 1; HREI-MS 238.1930 ($C_{15}H_{26}O_2$ calcd. as 238.1926); EI-MS *m/z* (rel. int.) 238.3 $[M]^+$ (6), 223.2 (100), 205.2 (50), 195.2 (29), 187.2 (33), 179.2 (98), 162.2 (59), 147.2 (73), 136.2 (42), 123.1 (52), 107.2 (42).

3.3.3. Oxidation of 9β-hydroxymaalioxide (*4*)

9β-Hydroxymaalioxide (**4**) (8.0 mg) was oxidized with Collins reagent (Ratcliffe, 1976) to give (−)-9-oxomaalioxide (**5**) (7.9 mg) as colourless crystals; mp 113.5–115.8 °C (from hexane); $[\alpha]_D^{20}$ − 16.7 (EtOH; *c* 0.79); CD (Δε): -21.5×10^{-3} (304 nm, *c* 1.69, CH_2Cl_2); FT-IR $\nu_{max}^{CCl_4}$ 2974.2, 2938.9, 2868.2, 1711.2 (>C=O), 1380.2, 1263.7, 1141.1, 958.7 cm^{-1}; ^1H NMR (500 MHz) δ 2.58 (1H, *ddd*, *J* = 16.5, 13.5 and 6.5 Hz, H-8β), 2.34 (1H, *ddd*, *J* = 16.3, 5.6 and 1.9 Hz, H-8α), 2.08 (1H, *ddd*, *J* = 11.6, 6.0 and 1.4 Hz, H-7α), 1.96 (1H, *ddd*, *J* = 15.7, 11.6 and 4.2 Hz, H-1α), 1.82 (1H, *dd*, *J* = 13.0 and 5.5 Hz, H-6), 1.74 (3H, *m*, H-7β, 2β, 3α), 1.62 (1H, *d*, *J* = 13.5 Hz, H-5), 1.40 (1H, *m*, H-2α), 1.38 (3H, *s*, H_3-12), 1.35 (3H, *s*, H_3-15), 1.34 (1H, *dt*, *J* = 3.3 and 16.2 Hz, H-3β), 1.13 (3H, *s*, H_3-13), 1.11 (1H, *m*, H-1β), 1.05 (3H, *s*, H_3-14);^{13}C NMR (125 MHz) δ 214.3 (C-9), 82.7 (C-11), 77.2 (C-4), 58.1 (C-5), 47.1 (C-10), 43.2 (C-6), 40.3 (C-1), 36.1 (C-8), 34.7 (C-7), 30.8 (C-12), 26.7 (C-3), 25.8 (C-13), 22.4 (C-15), 21.7 (C-2), 17.1 (C-14); HREI-MS 236.1766 ($C_{15}H_{24}O_2$ calcd. as 236.1770); EI-MS *m/z* (rel. int.) 236.1 $[M]^+$ (7), 221.2 (69), 203.1 (16), 193.0 (23), 179.2 (100), 161.2 (62), 136.1 (67), 121.1 (51), 107.1 (39).

3.3.4. Oxidation of 1β-hydroxymaalioxide (*3*)

1β-Hydroxymaalioxide (**3**) (12.3 mg) was oxidized as above to give (−)-1-oxomaalioxide (**6**) (10.1 mg) as a colourless gum; $[\alpha]_D^{20}$ − 35.7 (EtOH; *c* 0.94); CD (Δε): $+4.86 \times 10^{-3}$ (304 nm, *c* 0.76, CH_2Cl_2); FT-IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2974.3, 2940.0, 2868.9, 1714.2 (>C=O), 1459.3, 1379.3, 1265.2, 1179.8, 942.2; ^1H NMR (500 MHz) δ 2.61 (1H, *dt*, *J* = 6.0 and 13.9 Hz, H-2β), 2.30 (1H, *ddd*, *J* = 16.2, 11.6 and 4.2 Hz, H-3α), 2.25 (1H, *ddd*, *J* = 14.3, 4.6 and 2.8 Hz, H-2α), 1.98 (1H, *dddd*, *J* = 12.9, 6.0, 4.6 and 2.3 Hz, H-7β), 1.84 (1H, *dt*, *J* = 8.8 and 3.2 Hz, H-6), 1.77 (2H, *m*, H-8α, 9β), 1.63 (2H, *m*, H-8β, 3β), 1.57 (1H, *d*, *J* = 13.9 Hz, H-5), 1.49 (1H, *m*, H-7α), 1.39 (3H, *s*, H_3-12), 1.36 (1H, *m*, H-9α), 1.25 (3H, *s*, H_3-15), 1.12 (3H, *s*, H_3-13), 1.04 (3H, *s*, H_3-14); ^{13}C NMR (75 MHz) δ 215.0 (C-1), 80.7 (C-11), 78.1 (C-4), 59.3 (C-5), 48.1 (C-10), 42.3 (C-6), 39.8 (C-3), 37.3 (C-9), 32.3 (C-2), 30.9 (C-12), 27.0 (C-7), 26.0 (C-13), 23.4 (C-15), 20.6 (C-8), 17.1 (C-14); HREI-MS 236.1772 ($C_{15}H_{24}O_2$ calcd. as 236.1770); EI-MS *m/z* (rel. int.) 236.1 $[M]^+$ (54), 221.2 (100), 208.2 (50), 203.1 (41), 193.2 (86), 180.2 (83), 165.1 (95), 136.1 (84), 121.1 (67), 107.1 (69).

3.4. Crystallographic data for (−)-maalioxide (*1*)

$C_{15}H_{26}O$, $M_r = 222.36$, orthorhombic, space group $P2_12_12_1$, $a = 9.4934(6)$ Å, $b = 11.7412(6)$ Å, $c = 12.1048(7)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 1349.25(13)$ Å^3, $Z = 4$, density (calculated) = 1.095 Mg/m^3, $F(000) = 496$, $\lambda = 0.71073$ Å, $\mu = 0.066$ mm^{-1}. Data were collected using a crystal of size ca. $0.60 \times 0.60 \times 0.40$ mm^3 on a Bruker AXS SMART APEX CCD X-ray diffractometer. A total of 9647 reflections were collected for $2.42° < \theta < 27.50°$ and $-12 < h < 10$, $-10 < k < 15$ and $-15 < l < 15$. There were 3086 independent reflections used in the refinement. SADABS (Sheldrick, 2004)

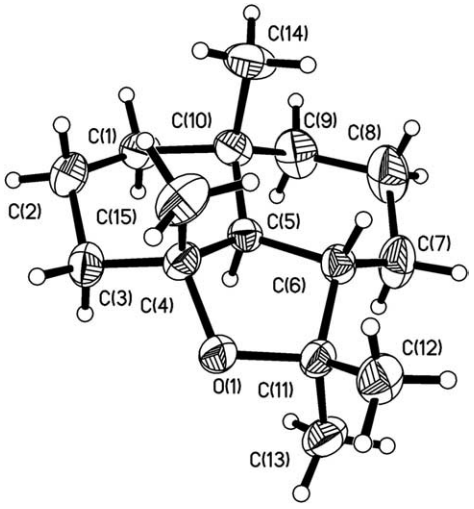


Fig. 3. ORTEP drawing of **1**, showing the atomic numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii at calculated positions.

was used for absorption corrections. The final R indices were $R_1 = 0.0440$, $wR_2 = 0.1108$ and R indices (all data) $R_1 = 0.0463$, $wR_2 = 0.1133$. The goodness-of-fit on F^2 was 1.054. 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.[1] The ORTEP drawing of the crystal structure is shown in Fig. 3.

Acknowledgements

We thank Miss L.-L. Chua for technical help and the National University of Singapore for financial support as well as the award of a research scholarship to Y. Wang.

References

Arantes, S.F., Hanson, J.R., Mobbs, D.J., 1999a. The microbiological hydroxylation of some 2,9-dioxygenated clovanes by *Mucor plumbeus*. Phytochemistry 52, 631–634.

Arantes, S.F., Hanson, J.R., Hitchcock, P.B., 1999b. The microbiological hydroxylation of the sesquiterpenoid patchoulol by *Mucor plumbeus*. Phytochemistry 52, 635–638.

Buchanan, G.O., Williams, L.A., Reese, P.B., 2000. Biotransformation of cadinane sesquiterpenes by *Beauveria bassiana* ATCC 7159. Phytochemistry 54, 39–45.

Büchi, G., Wittenau, M.S., White, D.M., 1959. Terpenes. X. The constitution of maaliol. J. Am. Chem. Soc. 81, 1968–1980.

Hashimoto, T., Noma, Y., Gotoh, Y., Tanaka, M., Takaoka, S., Asakawa, Y., 2004. Biotransformation of (−)-maalioxide by *Aspergillus niger* and *Aspergillus cellulosae*. Heterocycles 62, 655–666.

Huneck, S., Schreiber, K., Connolly, J.D., Harrison, L.J., Rycroft, D.S., 1984. Sesquiterpenoids from the liverwort *Lophozia ventricosa*. Phytochemistry 23, 1792.

Matsuo, A., Nakayama, M., Sato, S., Nakamoto, T., Uto, S., Hayashi, S., 1974. (−)-Maalioxide and (+)-cyclocolorenone, enantiomeric sesquiterpenoids from the liverwort, *Plagiochila acanthophylla* subspecies *japonica*. Experientia 30, 321–322.

Narayanan, C.S., Kulkarni, K.S., Vaidya, A.S., Kanthamani, S., Kumari, G.L., Bapat, B.V., Paknikar, S.K., Kulkarni, S.N., Kelkar, G.R., Bhattacharyya, S.C., 1964. Terpenoids – XLVI. Components of Indian Valerian root oil. Tetrahedron 20, 963–968.

Peterson, D.H., Murray, H.C., Epstein, S.H., Reineke, L.M., Weintraub, A., Meister, P.D., Leigh, H.M., 1952. Microbiological transformations of steroids. I. Introduction of oxygen at carbon-11 of progesterone. J. Am. Chem. Soc. 74, 5933–5936.

Ratcliffe, R.W., 1976. Oxidation with the chromium trioxide-pyridine complex prepared in situ: 1-decanal. Org. Synth. 55, 84–86.

Sheldrick, G.M., 2004. SADABS. University of Göttingen, Germany.

Suhara, Y., Itoh, S., Ogawa, M., Yokose, K., Sawada, T., Sano, T., Ninomiya, R., Maruyama, H.B., 1981. Regioselective 10-hydroxylation of patchoulol, a sesquiterpene, by *Pithomyces* species. Appl. Environ. Microbiol. 42, 187–191.

Tori, M., Sono, M., Asakawa, Y., 1990. The reaction of three sesquiterpene ethers with *m*-chloroperbenzoic acid. Bull. Chem. Soc. Jpn. 63, 1770–1775.

[1] CCDC 240736 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html, or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).