Tetrahedron 65 (2009) 5680–5683

Contents lists available at ScienceDirect

Tetrahedron

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

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ARTICLE INFO

Article history:
Received 27 February 2009
Received in revised form 19 April 2009
Accepted 14 May 2009
Available online 21 May 2009

Keywords:
Biotransformation
(−)-Maaliol
(+)-7,8-Didehydro-9β-hydroxymaalioxide
(−)-7,8-Didehydro-1β-hydroxymaalioxide
Mucor plumbeus

ABSTRACT

Microbial transformation of the sesquiterpenoid (−)-maaliol by the fungus *Mucor plumbeus* gave two metabolites, (+)-7,8-didehydro-9β-hydroxymaalioxide and (−)-7,8-didehydro-1β-hydroxymaalioxide. Both metabolites are new and their structures were established on the basis of their spectroscopic properties and chemical reactions.

1. Introduction

Microorganisms are of interest to organic chemists, especially for the functionalization of unactivated carbon atoms. *Mucor plumbeus* has proved to be a useful organism for the biotransformation of many natural products.[1–8] In a continuation of our study of the biotransformation of natural products, we have carried out the biotransformation of (−)-maaliol (**1**), which possesses a rigid tricyclic ring system. Incubation of the natural product with *M. plumbeus* resulted in the formation of two new hydroxylated products, which also contained a carbon–carbon double bond and lacked the cyclopropane ring of the starting material.

2. Results and discussion

The structure of (−)-maaliol (**1**) was first confirmed by X-ray crystallographic analysis (Fig. 1). The organic-soluble material from the incubation of maaliol with *M. plumbeus* for 5 days was chromatographed to afford two novel metabolites **2** and **3**. It was immediately apparent from the spectroscopic properties of these metabolites that they were derivatives of maalioxide. (+)-7,8-Didehydro-9β-hydroxymaalioxide (**2**) ($C_{15}H_{24}O_2$, $[M]^+$ *m/z* 236.1768, ^1H and ^{13}C NMR spectroscopic data see Table 1) possessed a cis disubstituted double bond [δ_H 5.68 (dt, *J*=10.2, 2.3 Hz), 5.50 (dt, *J*=10.2, 2.3 Hz); δ_C 133.4, 126.1], an oxygenated methine [δ_H 3.94 (br s); δ_C 80.1], two fully substituted oxygenated carbons [δ_C 81.1,

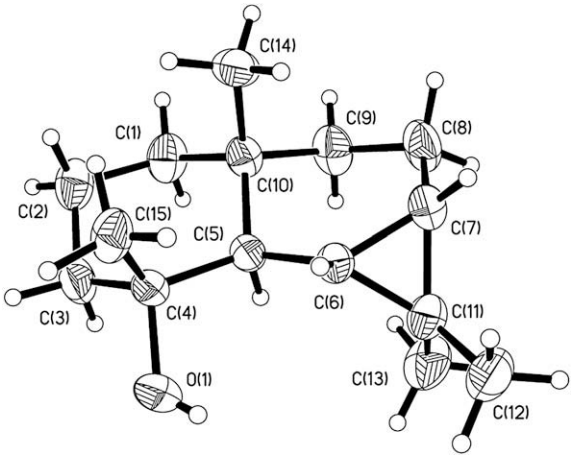

Figure 1. The ORTEP drawing of **1**, showing the atomic numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii at calculated positions.

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doi:10.1016/j.tet.2009.05.035

Table 1
^{1}H (500 MHz), HMBC and ^{13}C (125 MHz) NMR spectroscopic data for **2** in $CDCl_3$ (*J* in hertz in parentheses)

Position	δ_H	HMBC		δ_C
		^2J	^3J	
1α	1.13 (dt, 4.6, 8.4),	C-2, 10	C-3, 5, 9, 14	38.5 (CH_2)
1β	1.87 (m)			
2α	1.44 (m)	C-1, 3	C-4, 10	21.5 (CH_2)
2β	1.71 (m)			
3α	1.31 (dt, 3.2, 12.5)	C-4	C-1, 5, 15	39.8 (CH_2)
3β	1.87 (m)			
4	—			78.7 (C)
5	1.49 (d, 12.5)	C-4, 6	C-1, 3, 7, 9, 14, 15	53.7 (CH)
6	2.43 (m)	C-5, 7, 11	C-8, 13	44.1 (CH)
7	5.68 (dt, 10.2, 2.3)	C-6	C-5, 9, 11	126.4 (CH)
8	5.50 (dt, 10.2, 2.3)		C-6, 10	133.4 (CH)
9	3.94 (br s)	C-8	C-7	80.1 (CH)
10	—			38.2 (C)
11	—			81.1 (C)
12	1.36 (s)	C-11	C-6, 13	30.4 (CH_3)
13	0.99 (s)	C-11	C-6, 12	25.5 (CH_3)
14	0.84 (s)		C-1, 5, 9	11.2 (CH_3)
15	1.19 (s)	C-4	C-3, 5	22.9 (CH_3)

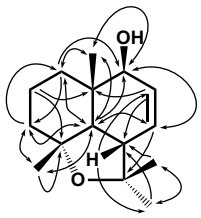


Figure 2. Important HMBC correlations of **2**.

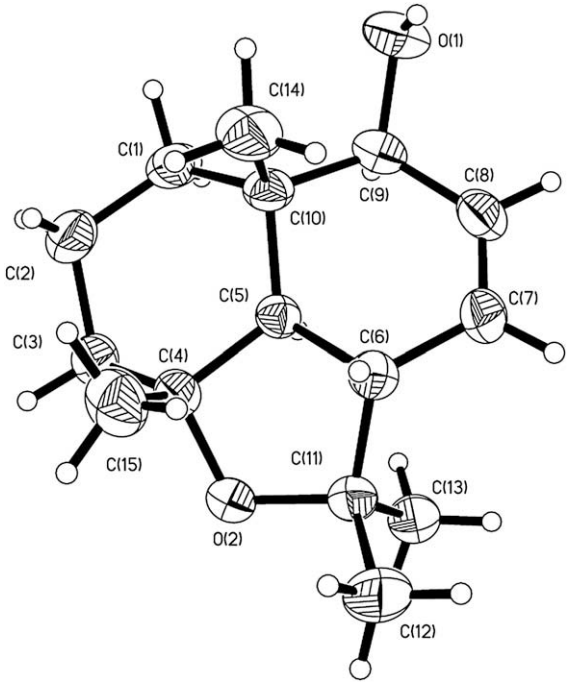
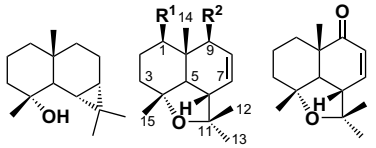
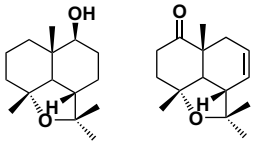


Figure 3. The ORTEP drawing of **2**, showing the atomic numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii at calculated positions.

78.7] and four tertiary methyl groups [δ_H 1.36, 1.19, 0.99, 0.84; δ_C 30.4, 25.5, 22.9, 11.2] and is tricyclic. Oxidation of **2** afforded the α,β-unsaturated ketone (**4**), (+)-7,8-didehydro-9-oxomaalioxide (see Experimental section), hence confirming the allylic alcohol nature of **2**. Complete analysis of the HMBC correlations (Fig. 2) determined the structure of **2** as 7,8-didehydro-9-hydroxymaalioxide. The relative stereochemistry of **2** was deduced from NOESY correlations of H-5 to H-9, H-1α, and H-3α, indicating that H-9 is α, and from H-6 to Me-14, and Me-15, indicating that the configuration of C-6 does not change during the biotransformation. The correlations between H-6 and Me-12 and H-5 and Me-13 were used to distinguish Me-12 from Me-13. The structure and the stereochemistry of **2** were confirmed by an X-ray crystallographic analysis (Fig. 3) and by hydrogenation to give the known (−)-9β-hydroxymaalioxide (**5**), which was obtained from our previous study of the biotransformation of maalioxide with *M. plumbeus*.[8] This chemical correlation established that (−)-maaliol and (−)-maalioxide belong to the same series of absolute configuration and confirmed the original correlation made by Büchi et al. and Narayanan et al.[9,10]



1 **2** R^1 = H, R^2 = OH; **3** R^1 = OH, R^2 = H **4**



5 **6**

The ^{1}H and ^{13}C NMR spectroscopic data (see Table 2) of (−)-7,8-didehydro-1β-hydroxymaalioxide (**3**) (3.1 mg) {$C_{15}H_{24}O_2$, $[M]^+$ *m/z* 236.1769 (calcd as: 236.1770)} are similar to those of **2** but the secondary alcohol [δ_H 3.53 (dd, *J*=10.6, 4.6 Hz); δ_C 81.6] is equatorial and no longer allylic. Oxidation of **3** afforded (−)-7,8-didehydro-1-oxomaalioxide (**6**), which lacks any UV absorption. The HMBC correlations of Me-14, H-5 and H-3 with the oxygenated C-1 and of H-1 with C-14 supported structure **3**, (−)-7,8-didehydro-1β-

Table 2
^{1}H (500 MHz), HMBC and ^{13}C (125 MHz) NMR spectroscopic data for **3** in $CDCl_3$ (*J* in hertz in parentheses)

Position	δ_H	HMBC		δ_C
		^2J	^3J	
1	3.53 (dd, 10.6, 4.6)	C-10	C-9, 14	81.6 (C)
2α	1.84 (m)	C-1, 3	C-4, 10	30.9 (CH_2)
2β	1.52 (m)			
3α	1.52 (dt, 3.2, 12.5)	C-2, 4	C-1, 5, 15	38.5 (CH_2)
3β	1.84 (m)			
4	—			77.9 (C)
5	1.59 (d, 12.9)	C-4, 6, 10	C-1, 3, 7, 9, 14, 15	53.7 (CH)
6	2.46 (ddd, 12.9, 4.2, 1.9)	C-11	C-8, 12, 13	42.9 (CH)
7	5.66 (br d, 10.1)	C-6	C-5, 9	124.6 (CH)
8	5.63 (ddd, 10.1, 4.2, 2.3)	C-9	C-6	127.6 (CH)
9α	2.16 (dt, 18.5, 3.2)	C-8, 10	C-1, 5, 7, 14	42.3 (CH_2)
9β	2.07 (br d, 18.5)			
10	—			37.1 (C)
11	—			82.3 (C)
12	1.39 (s)	C-11	C-6, 13	30.7 (CH_3)
13	1.02 (s)	C-11	C-6, 12	25.6 (CH_3)
14	0.91 (s)	C-10	C-1, 5, 9	13.3 (CH_3)
15	1.22 (s)	C-4	C-3, 5	23.1 (CH_3)

hydroxymaalioxide, for this metabolite. NOESY correlations confirmed the expected relative stereochemistry as in **3**.

The formation of these maalioxide derivatives can be explained by initial acid catalyzed cyclization of (−)-maaliol followed by dehydrogenation and hydroxylation. However, introduction of an alkene is rarely observed under incubation conditions and (−)-*ent*-maalioxide itself does not undergo such a reaction. It would thus appear that the cyclization and dehydrogenation steps are linked somehow although the precise mechanism for this process is still unclear.

3. Experimental

3.1. General experimental details

Mp uncorr.; ^{1}H NMR 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; 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}):[4,5] 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 mL). 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 mL conical flasks each containing 100 mL of medium for 24 h at 25 °C prior to the addition of the substrate.

3.3. Incubation of (−)-maaliol with *M. plumbeus*

(−)-Maaliol **1** (220 mg) was dissolved in ethanol (24 mL) and evenly distributed over 30 flasks containing *M. plumbeus* spores. The 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 methanol 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 904 mg) was chromatographed on silica gel (gradient elution, 0–100% EtOAc/hexane) to give the remaining starting material (14.3 mg) and five fractions, which were further purified. Fraction 4 (DIOL, 16% acetone/hexane) gave (+)-7,8-didehydro-9β-hydroxymaalioxide (**2**) (77.4 mg) and the fifth fraction (DIOL, 20% acetone/hexane) produced (−)-7,8-didehydro-1β-hydroxymaalioxide (**3**) (3.1 mg).

3.3.1. (−)-Maaliol (*1*)

The compound was previously isolated from a liverwort *Plagiochila cristata* (unpublished). Colourless crystals (from hexane); mp: 95.3–97.0 °C (lit. mp: 99–102 °C, 103–104 °C);[10–13] $[\alpha]_D^{20}$ −10.6 (*c* 3.6, EtOH) {lit. $[\alpha]_D^{20}$ −37.8 (*c* 1.48, $CHCl_3$), −20.3 (*c* 1.48, EtOH)}.[10]

3.3.2. (+)-7,8-Didehydro-9β-hydroxymaalioxide (*2*)

Colourless crystals from acetone; mp: 147.2–149.2 °C; $[\alpha]_D^{20}$ +31.8 (*c* 6.3, EtOH); FTIR ν_{max} (KBr, cm^{-1}): 3415.6, 3024.9, 2968.8, 2929.9, 2873.9, 2860.2, 1460.2, 1386.8, 1380.7, 1074.5, 1004.9, 786.7; ^{1}H NMR and ^{13}C NMR are listed in Table 1; HREI-MS 236.1768 ($C_{15}H_{24}O_2$ calcd as: 236.1770).

3.3.3. Oxidation of (+)-7,8-didehydro-9β-hydroxymaalioxide (*2*)

The alcohol (10.1 mg) was oxidized with Collins reagent[13] to give (+)-7,8-didehydro-9-oxomaalioxide (**4**) (6.3 mg). White solid; mp 102.2–105.4 °C; $[\alpha]_D^{20}$ +38.3 (*c* 0.63, EtOH); UV λ_{max} {EtOH, nm (log ε)} 224.0 (3.87); FTIR ν_{max} ($CHCl_3$, cm^{-1}): 3020.0, 2976.8, 2941.4, 1673.2, 1522.0, 1423.7, 1215.6; ^{1}H NMR 6.88 (dd, *J*=10.27 and 1.8 Hz), 5.94 (d, *J*=10.2 and 2.8 Hz), 2.73 (dt, *J*=12.9 and 2.3 Hz), 1.95 (d, *J*=12.5 Hz), 1.89 (m), 1.83 (2H, m), 1.55 (dt, *J*=14.3 and 3.8 Hz), 1.41 (m), 1.33 (dt, *J*=4.2 and 12.9 Hz), 1.48 (s), 1.24 (d, *J*=0.9 Hz), 1.09 (s), 1.05 (s); ^{13}C NMR 204.4 (C), 147.4 (CH), 131.0 (CH), 81.4 (C), 79.0 (C), 55.5 (CH), 44.5 (CH), 39.6 (CH_2), 32.6 (CH_3), 30.7 (CH_2), 30.3 (C), 25.8 (CH_3), 23.0 (CH_3), 21.1 (CH_2), 15.3 (CH_3); HREIMS 234.1613 ($C_{15}H_{22}O_2$ calcd as: 234.1614).

3.3.4. Hydrogenation of (−)-7,8-didehydro-9β-hydroxymaalioxide (*2*)

Compound **2** (5.5 mg) was dissolved in EtOAc using Pd/C (10%) as a catalyst in a long neck round-bottom flask. The reaction mixture was stirred for ca. 5 h with H_2 bubbling. The filtrate was concentrated to afford the product **5** (5.6 mg) as a colourless oil; $[\alpha]_D^{20}$ −36.3 (*c* 0.56, EtOH) {lit. $[\alpha]_D^{20}$ −30.9 (*c* 2.5, EtOH) in Ref. 8 the rotation was wrongly quoted as −0.9}.[8]

3.3.5. (−)-7,8-Didehydro-1β-hydroxymaalioxide (*3*)

Colourless oil; $[\alpha]_D^{20}$ −3.4 (*c* 0.38, EtOH); FTIR ν_{max} (CCl_4, cm^{-1}): 3421.4, 3014.7, 2972.3, 2935.7, 2872.4, 1381.8, 1361.1, 1217.2; ^{1}H NMR and ^{13}C NMR are listed in Table 2; HREIMS: 236. 1769 ($C_{15}H_{24}O_2$ calcd as: 236.1770).

3.3.6. Oxidation of (−)-7,8-didehydro-1β-hydroxymaalioxide (*3*)

(−)-7,8-Didehydro-1β-hydroxymaalioxide (**3**) (2.0 mg) was oxidized with Collins reagent as above to afford (−)-7,8-didehydro-1-oxomaalioxide (**6**) (2.0 mg). Colourless oil; $[\alpha]_D^{20}$ −39.0 (*c* 0.20, EtOH); FTIR ν_{max} (CCl_4, cm^{-1}): 3020.0, 2972.3, 2935.8, 2877.6, 1711.8, 1381.2, 1367.4, 1244.4, 1118.6, 1072.0. ^{1}H NMR 5.64 (m, 2H), 2.61 (dm, *J*=12.5 Hz), 2.55 (ddd, *J*=17.1, 12.9 and 6.5 Hz), 2.42 (dd, *J*=5.6 and 2.3 Hz), 2.41 (m), 2.10 (ddd, *J*=12.0, 6.9 and 2.3 Hz), 2.08 (d, *J*=13.0 Hz), 1.85 (dt, *J*=8.5 and 2.8 Hz), 1.84 (ddt, *J*=5.1, 0.9 and 13.4 Hz), 1.44 (s), 1.42 (s), 1.13 (s), 1.05 (s); ^{13}C NMR 214.6 (C), 127.7 (CH), 124.3 (CH), 83.1 (C), 76.7 (C), 55.3 (CH), 45.6 (C), 43.7 (CH), 39.4 (CH_2), 37.0 (CH_2), 36.8 (CH_2), 30.7 (CH_3), 25.6 (CH_3), 22.1 (CH_3), 17.9 (CH_3); HREIMS 234.1610 ($C_{15}H_{22}O_2$ calcd as: 234.1614).

3.4. Crystallographic analysis

All of the data were collected on Bruker AXS SMART APEX CCD diffractometer. Sadabs[14] was used for absorption corrections, λ=0.71073 Å. Tables of atomic co-ordinates, bonds lengths and angles, anisotropic displacement parameters and hydrogen atom co-ordinates are deposited with the Cambridge Crystallographic Data Centre.[†]

3.5. Crystallographic data for (−)-maaliol (1)

$C_{15}H_{26}O$, M_r 222.36, orthorhombic, space group $P2_12_12_1$, *a*=6.3930(6) Å, *b*=12.1787(12) Å, *c*=17.1488(17) Å, α=β=γ=90°,

[†] CCDC contains the supplementary crystallographic data for this paper (please refer to the following sections for the reference numbers). 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 336033; e-mail: deposit@ccdc.cam.ac.uk.

V=1335.2(2) Å^3, Z=4, density (calculated)=1.106 Mg/m^3, F(000)=496, λ=0.71073 Å, μ=0.066 mm^{-1}. Data were collected using a crystal of size ca. 0.70×0.60×0.32 mm^3. A total of 10,595 reflections were collected for $2.05^\circ<\theta<29.90^\circ$ and $-8<h<8$, $-17<k<17$ and $-23<l<18$. There were 3728 independent reflections were used in the refinement. The final R indices were R_1=0.0532, wR_2=0.1338 and R indices (all data) R_1=0.0577, wR_2=0.1373. The goodness-of-fit on F^2 was 1.077. 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 (CCDC 240736). The ORTEP drawing of the crystal structure is shown in Figure 1.

3.6. Crystallographic data and structure determination for (+)-7,8-didehydro-9β-hydroxymaalioxide (2)

$C_{15}H_{24}O_2$, M_r 236.34, orthorhombic, space group $P2_12_12_1$, a=7.6798(10) Å, b=12.1802(16) Å, c=14.5781(19) Å, $\alpha=\beta=\gamma=90^\circ$, V=1363.7(3) Å^3, Z=4, density (calculated)=1.151 Mg/m^3, F(000)=520, λ=0.71073 Å, μ=0.074 mm^{-1}. Data were collected using a crystal of size ca. 0.60×0.50×0.38 mm^3. A total of 20,250 reflections were collected for $2.79^\circ<\theta<29.97^\circ$ and $-10<h<10$, $-16<k<16$ and $-20<l<20$. There were 3873 independent reflections were used in the refinement. The final R indices were R_1=0.0549, wR_2=0.1313 and R indices (all data) R_1=0.0581, wR_2=0.1338. The goodness-of-fit on F^2 was 1.116. 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 (CCDC 242768). The ORTEP drawing of the crystal structure is shown in Figure 3.

Acknowledgements

We thank Miss Ling-Lih Chua for technical help and the National University of Singapore for financial assistance and the award of a research scholarship to Y.W.

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