

BIOTRANSFORMATION OF (-)-MAALIOXIDE BY *ASPERGILLUS NIGER* AND *ASPERGILLUS CELLULOSAE*[#]

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Abstract -A sesquiterpene cyclic ether, (-)-maalioxide (**1**) from the liverwort
Plagiochila sciophila was biotransformed by *Aspergillus niger* and *A. cellulosa*
to afford the structurally interesting compounds. The stereostructures of the
metabolites were established by a combination of high resolution NMR
spectrum, X-Ray crystallographic analysis and the chemical reaction.

INTRODUCTION

Microorganisms are able to transform a huge variety of organic compounds, such as terpene hydrocarbons, alkaloids, steroids, antibiotics and aminoacid.¹ We are continuing to study the biotransformation of the secondary metabolites from crude drugs and liverworts by microorganisms²⁻⁴ and mammals⁵⁻⁶ to obtain some functional substances such as pheromones and perfumes. Recently, we reported the biotransformation of sesquiterpenoids such as dehydrocostuslactone, costunolide, α -, β -, and γ -cyclocostunolides, α -santonin and atractylon from crude drugs, a sesquiterpene, dehydropinguisenol⁸ from liverwort, (-)-ambrox⁷ from animal origin by *Aspergillus niger*, *A. cellulosa*, and *Botryosphaeria dothidea* etc. (-)-Ambrox which possesses a powerful amber-type aroma was biotransformed by *A. niger* and *A. cellulosa* to afford the structurally interesting compounds as shown in Figure 1.⁷ It is noteworthy that the metabolic pathways of (-)-ambrox are strikingly different between *A. niger* and *A. cellulosa*. A metabolite, 1-oxoambrox by *A. cellulosa* indicated a good odor like (-)-ambrox.⁷

[#]Dedicated to 70 th birthday of Professor L. A. Paquette

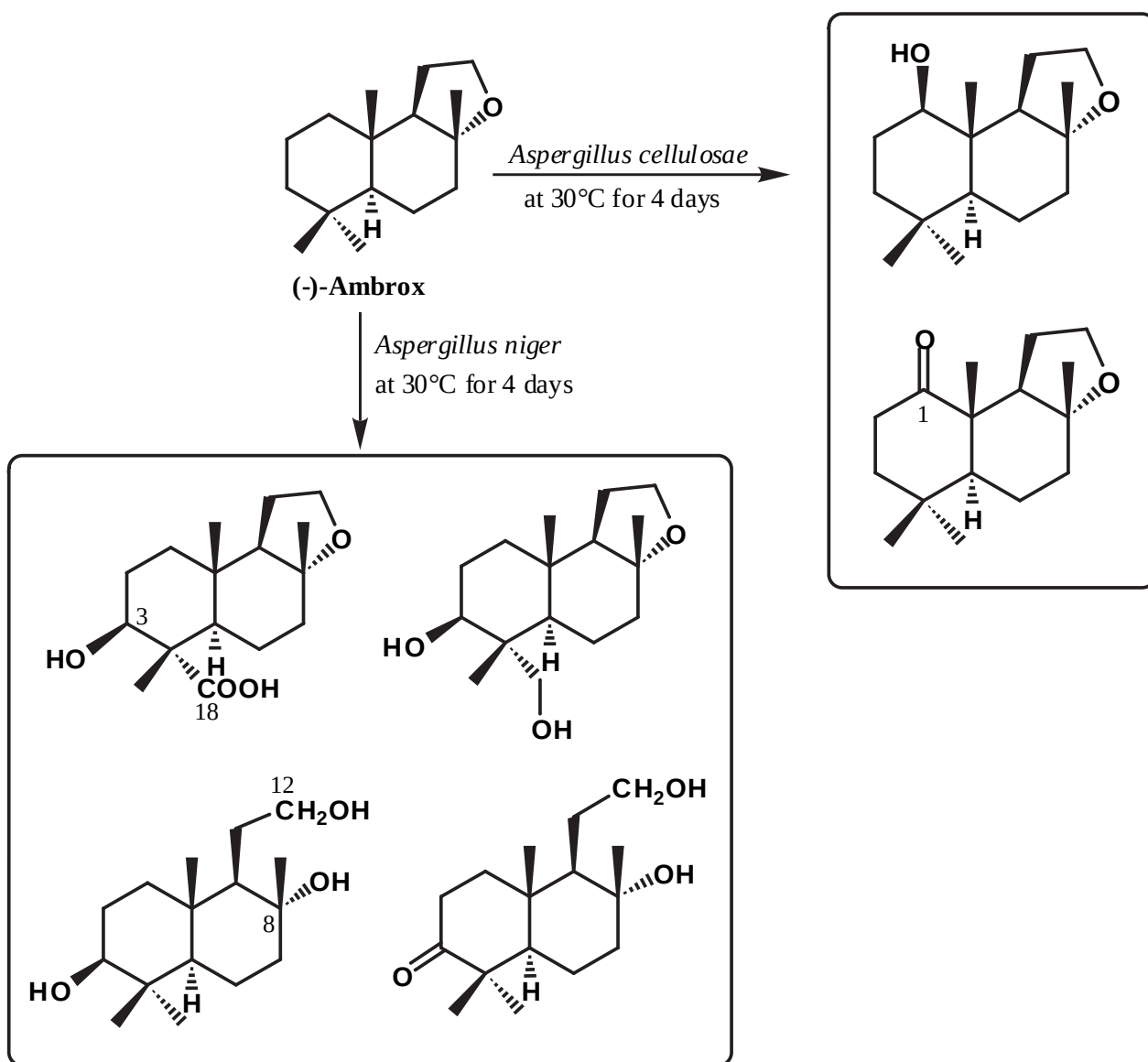


Figure 1. Biotransformation of (-)-ambrox by *Aspergillus niger* and *A. cellulosa*

The rare gorgonane-type sesquiterpene, (-)- and (+)-maalioxides were isolated from the liverwort *Plagiochila sciophila*⁹⁻¹⁰ and *Valeriana wallichii*,¹¹ respectively. (-)-Maalioxide (**1**) have a five member ether linkage and a good odor as well as (-)-ambrox. In continuation of the biotransformation studies of the chemical constituents isolated from liverworts into biologically active compounds, the biotransformation of (-)-maalioxide (**1**) was carried out by *Aspergillus niger* and *A. cellulosa* IFO 4040. Compound (**1**) was easily converted to three metabolites (**2**~**4**) by *A. niger*, and a metabolite (**5**) by *A. cellulosa*. This paper deals with the structure elucidation of the four metabolites (**2**~**5**) of **1**. Their stereostructures were established by a combination of high-resolution NMR spectrum, X-Ray crystallographic analysis and chemical reaction.

Biotransformation of (-)-Maalioxide (1) by *Aspergillus niger* *A. niger* was inoculated and cultivated rotatory (100 rpm) in Czapek-pepton medium (pH 7.0 at 30°C for 2 days. (-)-Maalioxide (**1**)(100 mg/200 mL) was added to the medium and further cultivated for 2 days. The crude metabolites obtained from the culture broth by EtOAc extraction were chromatographed on silica gel (*n*-hexane-EtOAc gradient) to give three new compounds **2** (6.2%), **3** (53.6%) and **4** (11.0%), respectively (Figure 2).

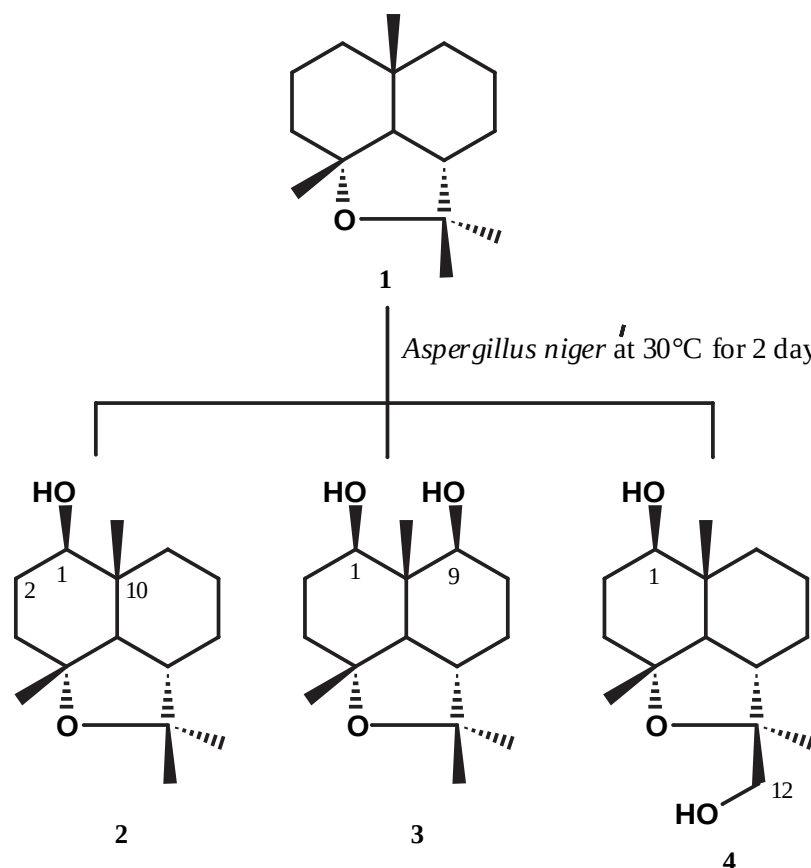


Figure 2. Biotransformation of (-)-maalioxide (**1**) by *Aspergillus niger*.

Compound (**2**) $\{[\alpha]_D -21.9^\circ (\text{CHCl}_3)\}$ was obtained as a colorless needles (mp 130-133°), whose molecular formula, $\text{C}_{15}\text{H}_{26}\text{O}_2$ was established by high resolution electron impact mass spectroscopy (HR-EIMS)($[\text{M}]^+$ m/z 238.1918). The FT-IR spectrum of **2** indicated the presence of a hydroxyl (3397 cm^{-1}) group. The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of **2** showed the presence of four tertiary methyl [δ_{H} 0.89, 1.04, 1.18, 1.32 (each 3H, s)] groups, a secondary alcohol [δ_{H} 3.28 (1H, *dd*, $J=3.8, 11.5\text{ Hz}$); δ_{C} 81.1 (*d*)] and a ether linkage [δ_{C} 77.8 (*s*), 80.7 (*s*)]. The carbon signals at C-1, C-2 and C-10 positions of **2** appeared at lower field (+37.9, +9.4 and +4.8 ppm) in comparison with that of **1** as shown in Table 2. Compound (**2**) showed the correlations between (i) H-1 / C-2, C-9 and C-14 ; (ii) H-14 / C-1, C-5, C-9 and C-10 in HMBC spectrum (Figure 3), and the NOEs between H-1 / H-2 α , H-3 α , H-5 and H-9 α in the NOESY

spectrum (Figure 3). From the above careful analysis of its 2D NMR spectrum, the structure of metabolite **2** was formulated as 1 β -hydroxymaalioxide.

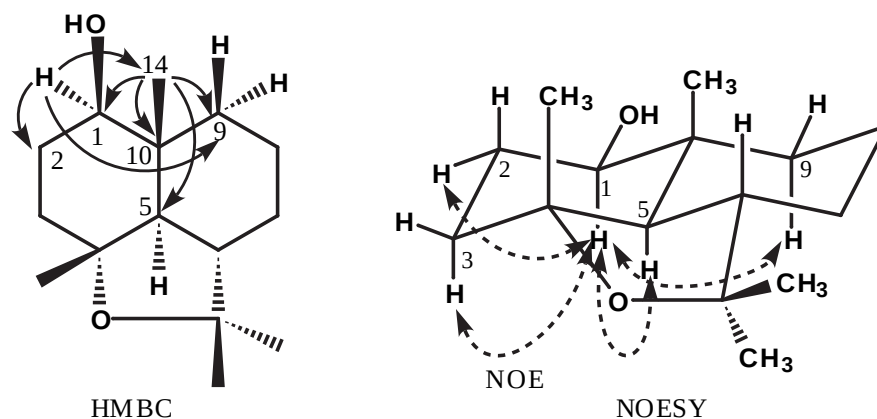


Figure 3. Important HMBC and NOESY correlations of compound (**2**).

Compound (**3**) $\{[\alpha]_D -28.6^\circ (\text{CHCl}_3)\}$ was obtained as a colorless prisms (mp 135-137 $^\circ$), whose molecular formula, $\text{C}_{15}\text{H}_{26}\text{O}_3$ was established by HR-EIMS ($[\text{M}]^+ m/z$ 254.1882). The FT-IR spectrum of **3** indicated the presence of a hydroxyl (3379 cm^{-1}) group. The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of **3** showed the presence of two secondary alcohol $[\delta_{\text{H}}$ 3.49 (1H, *dd*, $J=3.8, 11.3$ Hz), 3.64 (1H, *dd*, $J=4.9, 11.3$ Hz); δ_{C} 81.4 (*d*), 82.7 (*d*)]. Acetylation of **3** afforded a diacetate (**6**) $[\delta_{\text{H}}$ 2.00 (6H, *s*, 2xOAc); 1729 cm^{-1} (COO)] indicating that compound (**3**) has two hydroxyl groups. Compound (**3**) showed the correlations between (i) H-1 / C-2, C-9 and C-14; (ii) H-9 / C-1, C-14 and C-8; (iii) H-14 / C-1, C-5, C-9 and C-10 in HMBC spectrum, (Figure 4), and the NOEs between (i) H-1/ H-2 α , H-3 α , H-5 and H-9; (ii) H-9 / H-1, H-5, H-7 α and H-8 α in the NOESY spectrum (Figure 4). From the above spectral and chemical evidence, the relative structure of compound (**3**) was deduced and finally established by X-Ray crystallographic analysis (Figure 5) as 1 β ,9 β -dihydroxymaalioxide.

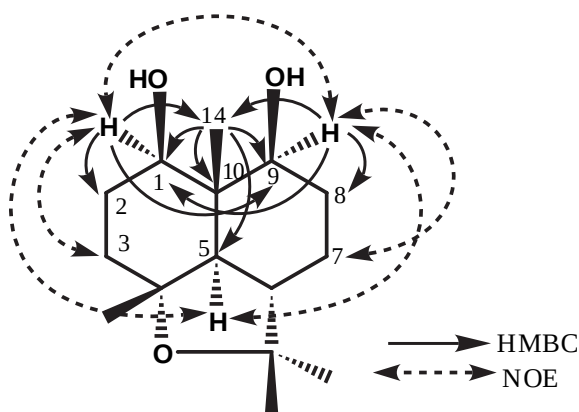


Figure 4. Important HMBC and NOESY correlations of compound (**3**).

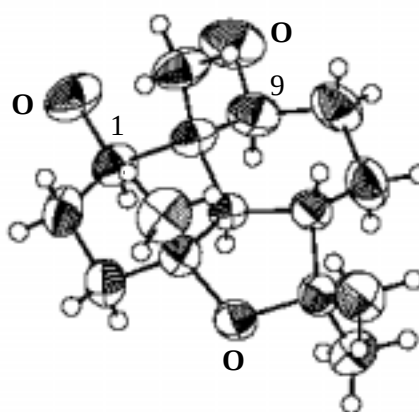


Figure 5. ORTEP drawing of **3**.

Compound (**4**) $\{[\alpha]_D -16.7^\circ (\text{CHCl}_3)\}$ has the same molecular formula, $\text{C}_{15}\text{H}_{26}\text{O}_3$ (HR-EIMS; $[\text{M}]^+ m/z$ 254.1868) with that of compound (**3**). The ^1H (Table 1) and ^{13}C (Table 2) NMR spectrum of **4** showed the presence of a primary alcohol $[\delta_{\text{H}} 3.48, 3.51(2\text{H, each } d, J=11.3 \text{ Hz}); \delta_{\text{C}} 70.4 (t)]$ and a secondary alcohol $[\delta_{\text{H}} 3.30 (1\text{H, } dd, J=4.9, 11.3 \text{ Hz}); \delta_{\text{C}} 81.0 (d)]$. Compound (**4**) showed the correlations between (i) H-1 / C-2 and C-14; (ii) H-12 / C-6, C-11 and C-13 in HMBC spectrum (Figure 6), and the NOEs between (i) H-1 / H-2 α , H-3 α , H-5 and H-9 α ; (ii) H-12 / H-6 and H-15 in the NOESY spectrum (Figure 6). From the above spectral evidence, the relative structure of compound (**4**) was deduced as 1 β ,12-dihydroxymaalioxide.

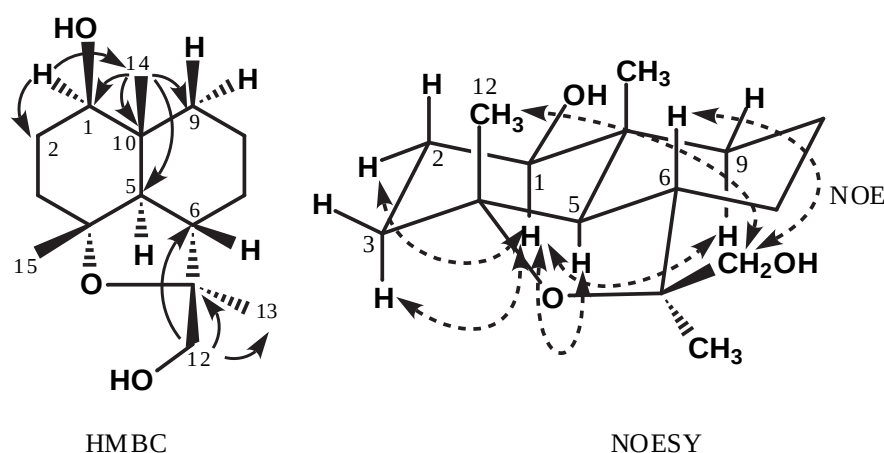


Figure 6. Important HMBC and NOESY correlations of compound (**4**).

In time course (Figure 7) of biotransformation of (-)-maalioxide (**1**) by *A. niger*, the yield of **2** increased with decreasing that of **1**, subsequently the yields of **3** and **4** increased with decreasing that of **2**. 1-Aminobezotriazole, an inhibitor of cytochrome P-450 inhibited the oxidation process of **1** into **2-4**. For the oxidation from **1** to **2-4**, cytochrome P-450 may be concerned.

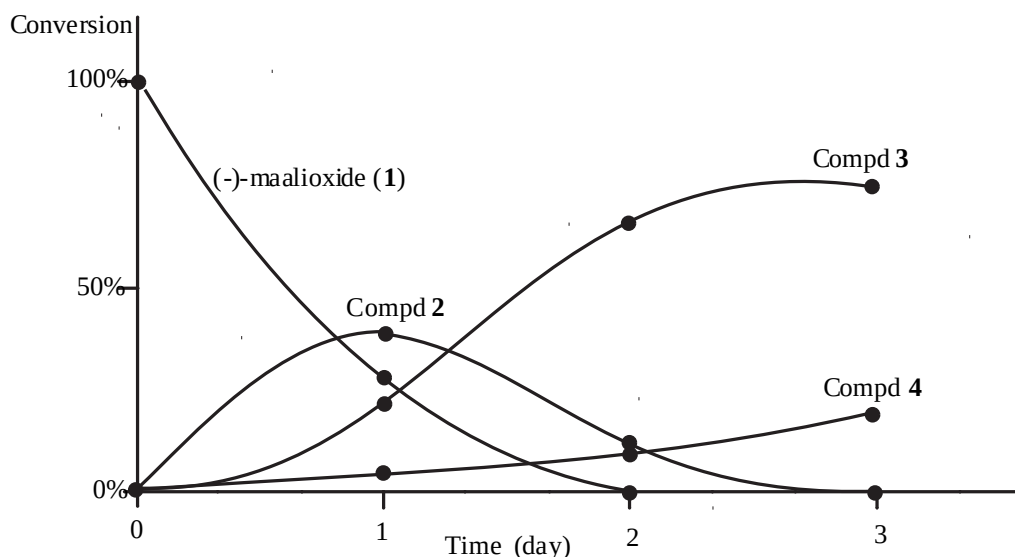


Figure 7. Time course of biotransformation of (-)-maalioxide (**1**) by *Aspergillus niger*

Biotransformation of (-)-Maalioxide (1) by *Aspergillus cellulosa* *A. cellulosa* was inoculated and cultivated rotatory (100 rpm) in Czapek-pepton medium (pH 7.0 at 30°C for 2 days. (-)-Maalioxide (1)(100 mg/200 mL) was added to the medium and further cultivated for 2 days. The crude metabolites obtained from the culture broth by EtOAc extraction were chromatographed on silica gel (*n*-hexane-EtOAc gradient) to give a metabolite (5)(29.9%) (Figure 8).

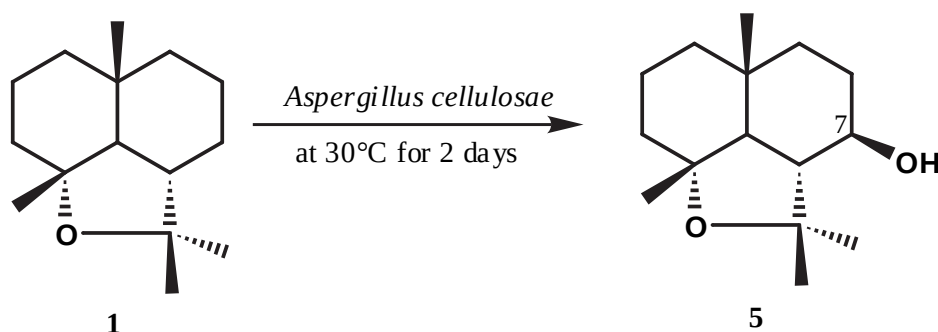


Figure 8. Biotransformation of (-)-maalioxide (1) by *A. cellulosa*.

Compound (5) $\{[\alpha]_D -51.4^{\circ}$ (MeOH) $\}$ has the same molecular formula, $C_{15}H_{26}O_2$ (HR-EIMS)($[M]^+$ m/z 238.1923) with that of compound (2). The FT-IR spectrum of 5 indicated the presence of a hydroxyl (3392 cm^{-1}) group. The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of 5 showed a secondary alcohol [δ_H 3.61 (1H, *ddd*, $J=4.4, 9.3, 9.3$ Hz); δ_C 72.6 (*d*)] and a ether linkage [δ_C 78.8 (*s*), 81.3 (*s*)]. The carbon signals at C-6, C-7 and C-8 positions of 5 appeared at lower field in comparison with that of 1 as shown in Table 2. Compound (5) showed the correlations between H-5, H-6, H-8 and H-9 / C-7 in HMBC spectrum, (Figure 9), and the NOEs between (i) H-7 / H-5, H-13, H-8 α in the NOESY spectrum (Figure 9). From the above spectral evidence, the relative structure of compound (5) was deduced and finally established by X-Ray crystallographic analysis (Figure 10) as 7 β -hydroxymaalioxide.

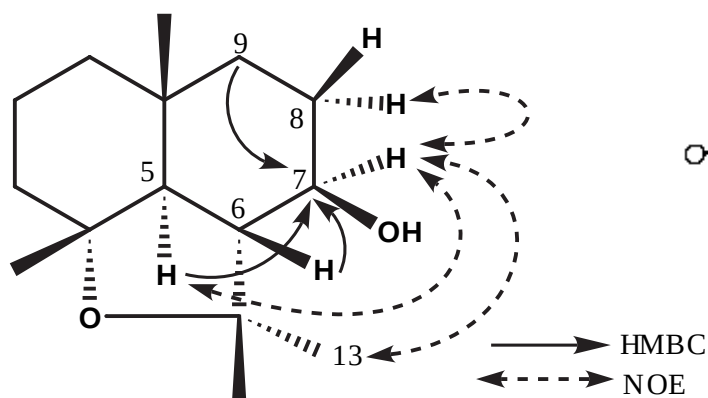


Figure 9. Important HMBC and NOESY correlations of 5.

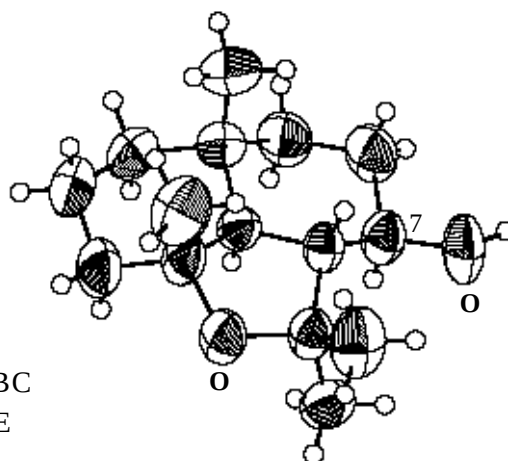


Figure 10. ORTEP drawing of 5.

Table 1. 600 MHz ¹H NMR spectral data of compounds (**1-5**) in CDCl₃^a

Position	1	2	3	4	5
1 α -H	0.99 <i>m</i>	3.28 <i>dd</i> (3.8, 11.5)	3.64 <i>dd</i> (4.9, 11.3)	3.30 <i>dd</i> (4.9, 11.3)	1.13 <i>m</i>
1 β -H	1.44 <i>m</i>				1.53 <i>m</i>
2 α -H	1.66 <i>m</i>	1.71 <i>m</i>	1.79 <i>m</i>	1.69 <i>m</i>	1.69 <i>m</i>
2 β -H	1.54 <i>m</i>	1.53 <i>m</i>	1.56 <i>m</i>	1.51 <i>m</i>	1.53 <i>m</i>
3 α -H	1.30 <i>m</i>	1.30 <i>m</i>	1.45 <i>m</i>	1.35 <i>m</i>	1.27 <i>m</i>
3 β -H	1.86 <i>m</i>	1.87 <i>m</i>	1.84 <i>m</i>	1.89 <i>m</i>	1.86 <i>m</i>
5-H	1.14 <i>d</i> (13.2)	1.17 <i>d</i> (13.8)	1.15 <i>d</i> (13.8)	1.25 <i>d</i> (13.7)	1.16 <i>d</i> (13.7)
6-H	1.75 <i>m</i>	1.84 <i>ddd</i> (4.7, 11.8, 13.8)	1.91 <i>ddd</i> (4.7, 11.8, 13.8)	1.92 <i>m</i>	1.79 <i>dd</i> (9.3, 13.7)
7 α -H	1.09 <i>m</i>	1.26 <i>m</i>	1.25 <i>m</i>	1.33 <i>m</i>	3.61 <i>ddd</i> (4.4, 9.3, 9.3)
7 β -H	1.75 <i>m</i>	1.73 <i>m</i>	1.72 <i>m</i>	1.71 <i>m</i>	
8 α -H	1.61 <i>m</i>	1.71 <i>m</i>	1.67 <i>m</i>	1.75 <i>m</i>	1.78 <i>m</i>
8 β -H	1.41 <i>m</i>	1.51 <i>m</i>	1.52 <i>m</i>	1.56 <i>m</i>	1.49 <i>m</i>
9 α -H	1.03 <i>m</i>	1.05 <i>m</i>	3.49 <i>dd</i> (3.8, 11.3)	1.06 <i>m</i>	0.99 <i>m</i>
9 β -H	1.51 <i>m</i>	1.77 <i>m</i>		1.79 <i>m</i>	1.56 <i>m</i>
12-H	1.31 <i>s</i>	1.32 <i>s</i>	1.33 <i>s</i>	3.48, 3.51 (each <i>d</i> , 11.3)	1.4 <i>s</i>
13-H	1.03 <i>s</i>	1.04 <i>s</i>	1.04 <i>s</i>	1.07 <i>s</i>	1.21 <i>s</i>
14-H	0.89 <i>s</i>	0.89 <i>s</i>	0.95 <i>s</i>	0.89 <i>s</i>	0.92 <i>s</i>
15-H	1.21 <i>s</i>	1.18 <i>s</i>	1.22 <i>s</i>	1.19 <i>s</i>	1.14 <i>s</i>

^aChemical shifts are in δ values. Coupling constants in Hz are in parenthesis.

Table 2 125 MHz ¹³C NMR spectral data of compounds (**1-6**) in CDCl₃^a

position	1	2	3	4	5	6
C-1	43.2 (t)	81.1 (d)	81.4 (d)	81.0 (d)	79.9 (d)	42.3 (t)
C-2	21.3 (t)	30.7 (t)	29.5 (t)	30.5 (t)	26.8 (t)	21.5 (t)
C-3	40.7 (t)	40.3 (t)	38.6 (t)	40.3 (t)	38.4 (t)	40.4 (t)
C-4	78.5 (s)	77.8 (s)	77.5 (s)	78.8 (s)	77.7 (s)	78.8 (s)
C-5	58.4 (d)	57.6 (d)	55.7 (d)	56.8 (d)	55.7 (d)	56.5 (d)
C-6	43.1 (d)	42.6 (d)	42.0 (d)	38.0 (d)	42.2 (d)	50.5 (d)
C-7	27.5 (t)	24.9 (t)	24.7 (t)	24.9 (t)	24.4 (t)	72.6 (d)
C-8	22.3 (t)	21.1 (t)	30.1 (t)	21.3 (t)	27.6 (t)	32.4 (t)
C-9	41.0 (t)	38.0 (t)	81.7 (d)	37.7 (t)	80.7 (d)	40.4 (t)
C-10	34.1 (s)	38.9 (s)	41.6 (s)	39.1 (s)	41.4 (s)	33.4 (s)
C-11	81.4 (s)	80.7 (s)	82.7 (s)	83.8 (s)	82.1 (s)	81.3 (s)
C-12	30.7 (q)	30.7 (q)	30.7 (q)	70.4 (t)	30.7 (q)	32.4 (q)
C-13	25.8 (q)	26.0 (q)	25.9 (q)	21.0 (q)	25.9 (q)	25.3 (q)
C-14	17.9 (q)	10.9 (q)	6.7 (q)	10.9 (q)	8.5 (q)	17.8 (q)
C-15	22.8 (q)	23.2 (q)	23.3 (q)	23.7 (q)	23.3 (q)	22.8 (q)
OCOCH ₃					21.2 (q)	
OCOCH ₃					21.2 (q)	
OCOCH ₃					171.5 (s)	
OCOCH ₃					171.5 (s)	

^aChemical shifts from TMS in CDCl₃ and assignments from HMQC and HMBC spectra

We reported that (-)-maalioxide (**1**) was treated with m-chloroperbenzoic acid (mCPBA) in CHCl₃ under reflux to afford 2α-hydroxymaalioxide (2.0%), 7β-hydroxymaalioxide (**5**)(1.2%) and 8α-hydroxymaalioxide (1.5%) in the low yield.¹² In *Aspergillus niger*, hydroxylation at C-1β, C-9β and C-12

occurred to afford 2~4, while hydroxylation at C-7 β occurred in *A. cellulosa*e to afford 5. It is noteworthy that the position of oxidation of (-)-maalioxide (1) are strikingly different between *A. niger*, *A. cellulosa*e and mCPBA as shown in Figure 11. Metabolites (2- 5) obtained by biotransformations of (-)-maalioxide (1) by two fungi possessing a good odor did not show an effective odor.

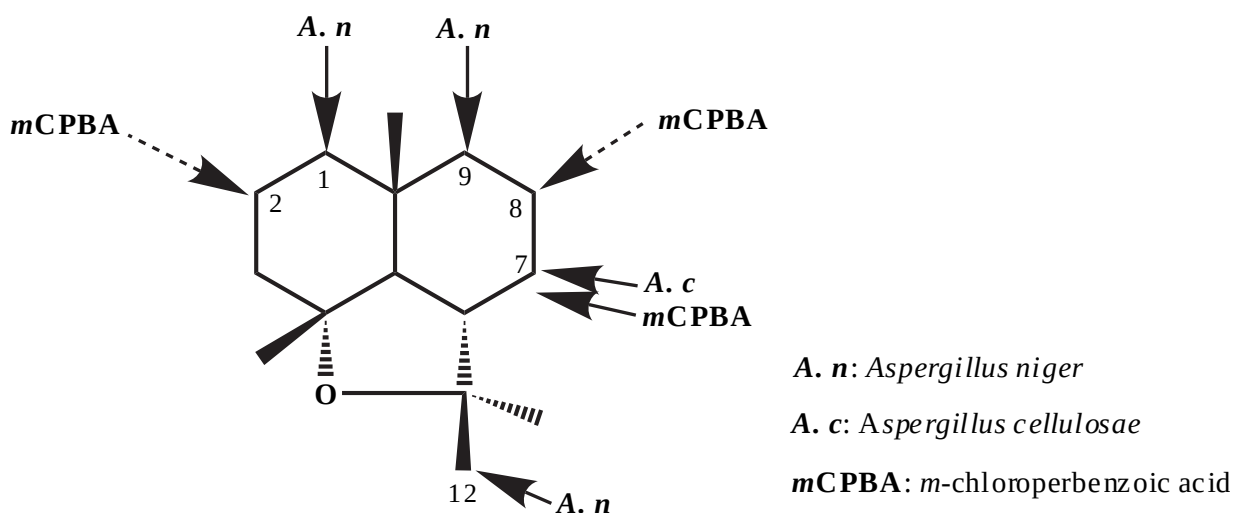


Figure 11. The position of oxidation of (-)-maalioxide (1) by *Aspergillus niger*, *A. cellulosa*e and mCPBA

EXPERIMENTAL

General IR spectra were measured on a JASCO FT-IR 500 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Varian unity 600 (^1H ; 600 MHz, ^{13}C ; 150 MHz) or a Varian Unity 200 (^1H ; 200 MHz, ^{13}C ; 50 MHz) spectrometer. The solvent used for NMR spectra was CDCl_3 unless otherwise stated. MS spectra were measured on a JEOL JMS HX-100 or a JEOL AX-500 spectrometer. The specific rotation was taken on a JASCO DIP-140 polarimeter. X-Ray crystallographic analysis was carried out by a Mac Science MXC18 diffractometer. Silica gel 60 for column chromatography was purchased from Merk.

Isolation of maalioxide (1) Dried powders (869 g) of *Plagiochila sciophila* collected in kamiyama-cho, Tokushima, Japan, in March 2001, was extracted with Et_2O (5 L) for 1 week at rt. The Et_2O extract (24.789 g) was chromatographed on silica gel (500 g) with a gradient solvent system of *n*-Hexane-AcOEt increasing the amount of 5% portions AcOEt stepwise to give a number of fractions. 20% AcOEt-*n*-Hex. eluate (Fr. 28-35) was evaporated in *vacuo* to afford the crude oil (3.0278 g), which was rechromatographed on silica gel (100 g) with a gradient solvent system of *n*-Hexane- Et_2O increasing the amount of 5% portions Et_2O stepwise to give (-)-maalioxide (1) (0.291 g) from 30% Et_2O -*n*-Hex. eluate (fr. 56-67) as

colorless needles. mp 65-66° (lit.,¹⁰ mp 66°); $[\alpha]_D^{21}$ -34.4° (c 1.02, CHCl₃)[lit.,¹⁰ -34.5° (c 1.00, CHCl₃)]; EIMS: m/z 222 (M⁺), 207 (100), 179, 149; HR-EIMS: m/z 222.1975 (M⁺), C₁₅H₂₆O requires 222.1984; FT-IR (KBr)cm⁻¹: 2932, 1377, 1050; ¹H-NMR (600 MHz) spectral data in CDCl₃ (Table 1); ¹³C NMR (125 Hz) spectral data in CDCl₃ (Table 2).

Microorganism and media *Aspergillus niger* was isolated in our laboratories from soil in Osaka prefecture, and was identified according to its physiological and morphological characters. *Aspergillus cellulosa* IFO 4040 was obtained from the Department of Microbiology, Osaka University, Osaka prefecture, Japan. A Czapek-pepton medium [1.5% sucrose, 1.5% glucose, 0.5% polypeptone, 0.1% K₂HPO₄, 0.05% KCl and 0.001%FeSO₄ · 7H₂O in distilled water (pH 7.0)] was used for the biotransformation of substrate by microorganism.

Biotransformation of maalioxide (1) by *Aspergillus niger*

An Erlenmyer flask (500 mL) containing 200 mL medium was inoculated with a suspension of *A. niger* and incubated at 30°C for 2 days in a rotary shaker operating at 100 rpm. After full growth of the microorganism, solution of (-)-maalioxide (1)(100 mg) in EtOH (0.5 mL) was added to the media culture of *A. niger*. The incubation was then continued for a further 2 days at 30°C. After the completion of the incubation time, the culture was filtered *in vacuo* and the broth was extracted with EtOAc (3x100 mL). The extracts were dried over MgSO₄ and the solvent was evaporated *in vacuo* to give the crude extract (145.6 mg) as an oil. The crude extract was chromatographed on silica gel (10 g) with a gradient solvent system of *n*-Hexane-AcOEt increasing the amount of 5% portions AcOEt stepwise to afford 1β-hydroxymaalioxide (2)(6.6 mg; 6.2%) from fr. 25-28, 1β, 9β-dihydroxymaalioxide (3)(61.3 mg; 53.6%) from fr. 44-48 and 1β, 12-dihydroxymaalioxide (4)(12.6 mg; 11.0%) from fr. 51-54, respectively.

1β-Hydroxymaalioxide (2) colorless needles, mp 130-133°, $[\alpha]_D^{18}$ -21.9° (c 0.67, CHCl₃); EIMS: m/z 238 (M⁺), 223 (100), 195, 162, 136; HR-EIMS: m/z 238.1918 (M⁺), C₁₅H₂₆O₂ requires 238.1933; FT-IR (KBr)cm⁻¹: 3397 (OH), 2925, 1379, 1081; ¹H-NMR (600 MHz) spectral data in CDCl₃ (Table 1); ¹³C NMR (125 Hz) spectral data in CDCl₃ (Table 2).

1β, 9β-Dihydroxymaalioxide (3) colorless prisms, mp 135-137°, $[\alpha]_D^{18}$ -28.6° (c 1.02, CHCl₃); EIMS: m/z 254 (M⁺), 239 (100), 195; HR-EIMS: m/z 254.1882 (M⁺), C₁₅H₂₆O₃ requires 254.1882; FT-IR (KBr)cm⁻¹: 3358 (OH), 2938, 2866, 1063; ¹H-NMR (600 MHz) spectral data in CDCl₃ (Table 1); ¹³C NMR (125 Hz)

spectral data in CDCl₃ (Table 2).

1 β , 12-Dihydroxymaalioxide (4) colorless oil, $[\alpha]_D^{18}$ -16.7° (*c* 0.87, CHCl₃); EIMS: *m/z* 254 (M⁺), 239, 223 (100), 205, 163; HR-EIMS: *m/z* 254.1868 (M⁺), C₁₅H₂₆O₃ requires 254.1882; FT-IR (KBr)cm⁻¹: 3379 (OH), 2936, 1380, 1042; ¹H-NMR (600 MHz) spectral data in CDCl₃ (Table 1); ¹³C NMR (125 Hz) spectral data in CDCl₃ (Table 2).

Acetylation of 3 A solution of **3** (10 mg) in pyridine (1 mL) was treated with acetic anhydride (1 mL). The mixture was stirred overnight at rt. Water was added and the mixture was extracted with CHCl₃. The organic phase was washed with 1N HCl, 5% NaHCO₃ and brine, dried (MgSO₄), and evaporated to give a residue. The residue was purified by a silica gel column chromatography with *n*-hexane-AcOEt gradient to afford 1 β , 9 β -diacetoxymaalioxide (**6**)(11 mg, 82.7%) as colorless oil; $[\alpha]_D^{21}$ -18.6° (*c* 1.01, CHCl₃); EIMS : *m/z* 338 (M⁺), 323 (100), 263, 237; HR-EIMS: *m/z* 338.2079 (M⁺), C₁₉H₃₀O₅ requires 338.2093; FT-IR (KBr)cm⁻¹: 2938, 1729 (C=O), 1372, 1026; ¹H NMR(CDCl₃): δ 1.05 (3H, s, H-13), 1.08 (3H, s, H-14), 1.24 (3H, s, H-15), 1.33 (3H, s, H-12), 2.00 (6H, s, 2xOAc), 4.64 (1H, *dd*, *J*= 3.9, 11.2 Hz, H-9), 4.84 (1H, *dd*, *J*= 4.9, 11.3 Hz, H-1); ¹³C NMR (125 Hz) spectral data in CDCl₃ (Table 2).

The crystal data for 3 Orthorhombic, space group *P2₁2₁2₁*, *a*=6.299 (3)Å, *b*= 14.070 (9)Å, *c* = 16.324 (2)Å, *V*=1447 (2)Å³, *Z* = 4, *D_x* = 1.094 Mg m⁻³, *D_x* = 1.094 Mg m⁻³, μ (Mo K α) =0.070 mm⁻¹, Final *R* and *R_w* were 0.084 and 0.198 for 1488 reflections. The structure was solved by direct method (Monte-Carlo Multan) and refined by full-matrix least-squares techniques. Diffraction data were obtained using a Mac Science MXC18 diffractometer at rt. All diagrams and calculations were performed using maXus (Bruker Nonius, Delft & Mac Science, Japan).

Biotransformation of maalioxide (1) by *Aspergillus cellulosa* *A. cellulosa* was rotatory cultivated (100 rpm) in Czapek-pepton medium at 30°C for 2 days. (-)-maalioxide (**1**)(100 mg / 200 mL) was added to the medium and further cultivated for 2 days. The crude metabolites obtained from the culture broth by EtOAc extraction were chromatographed on silica gel (*n*-hexane-EtOAc gradient) to give 7 β -hydroxymaalioxide (**5**)(32.1 mg; 29.9%) with the recovery of **1** (20.5 mg; 20.5%).

7 β -Hydroxymaalioxide (5) colorless needles, mp 134-136°, $[\alpha]_D^{18}$ -51.4° (*c* 1.05, MeOH); EIMS: *m/z* 238 (M⁺), 223 (100), 195, 187, 147; HR-EIMS: *m/z* 238.1923 (M⁺), C₁₅H₂₆O₂ requires 238.1933; FT-IR (KBr)cm⁻¹: 3392 (OH), 2926, 1461, 1082; ¹H-NMR (600 MHz) spectral data in CDCl₃ (Table 1); ¹³C

NMR (125 Hz) spectral data in CDCl₃ (Table 2).

The crystal data for 5 Orthorhombic, space group $P2_12_12_1$, $a=9.439$ (7)Å, $b=11.045$ (7)Å, $c=13.858$ (13)Å, $V=1445$ (2)Å³, $Z=4$, $D_x=1.096$ Mg m⁻³, $D_c=1.096$ Mg m⁻³, δ (Mo K α) = 0.070 mm⁻¹, Final R and R_w were 0.107 and 0.208 for 1551 reflections.

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