Heterocycles, Vol. 62, 2004, pp. 655 - 666
Received, 8th September, 2003, Accepted, 24th October, 2003, Published online, 14th November, 2003

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

Toshihiro Hashimoto,[a)] Yoshiaki Noma,[b)] Yoshiaki Gotoh,[a)] Masami Tanaka, [a)] Shigeru Takaoka,[a)] and Yoshinori Asakawa[a)]*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University,[a] *Faculty of Human Life Sciences, Tokushima Bunri University,*[b] *Yamashiro-cho, Tokushima 770-8514, Japan*

Abstract -A sesquiterpene cyclic ether, (-)-maalioxide (**1**) from the liverwort *Plagiochila sciophila* was biotransformed by *Aspergillus niger* and *A. cellulosae* 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 microorganisms[2-4] and mammals[5-6] 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, dehydropinguisenol[8] from liverwort, (-)-ambrox[7] from animal origin by *Aspergillus niger A. cellulosae*, and *Botryospaeria dothidea* etc. (-)-Ambrox which possesses a powerful amber-type aroma was biotransformed by *A. niger* and *A. cellulosae* 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. cellulosae.* A metabolite, 1-oxoambrox by *A. cellulosae* indicated a good odor like (-)-ambrox.[7]

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

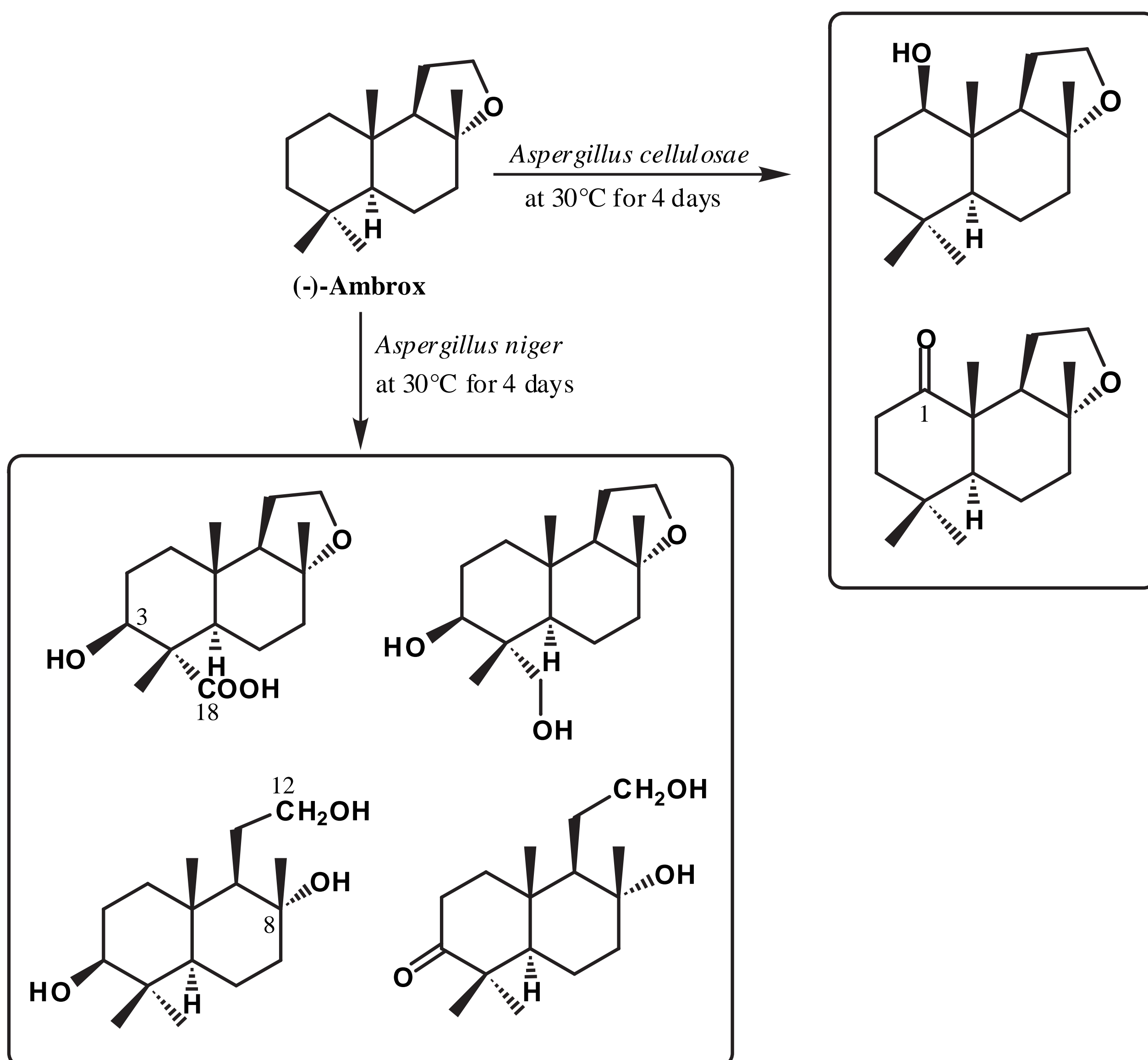

Figure 1. Biotransformation of (-)-ambrox by *Aspergillus niger* and *A. cellulosae*

The rare gorgonane-type sesquiterpene, (-)- and (+)-maalioxides were isolated from the liverwort *Plagiochila sciophila* [9-10] and *Valeriana wallichi*, [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.. cellulosae* IFO 4040. Compound (**1**) was easily converted to three metabolites (**2**～**4**) by *A. niger*, and a metabolite (**5**) by *A. cellulosae*. 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.

Biotaransformation 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 componds **2** (6.2%), **3** (53.6%) and **4** (11.0%), respectively (Figure 2).

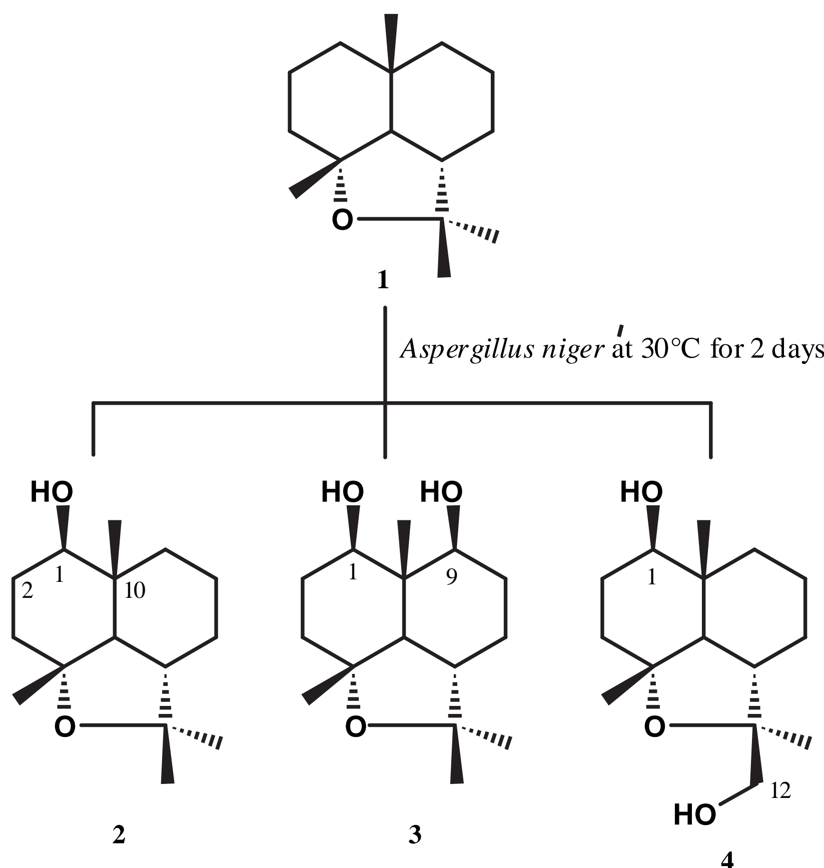

Figure 2. Biotransformation of (-)-maalioxide (**1**) by *Aspergillus niger*.

Compound (**2**) {$[\alpha]_D$ -21.9° ($CHCl_3$)} was obtained as a colorless needles (mp 130-133°), whose molecular formula, $C_{15}H_{26}O_2$ was established by high resolution electron impact mass spectroscopy (HR-EIMS)($[M]^+$ 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 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-2α, H-3α, H-5 and H-9α in the NOESY

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

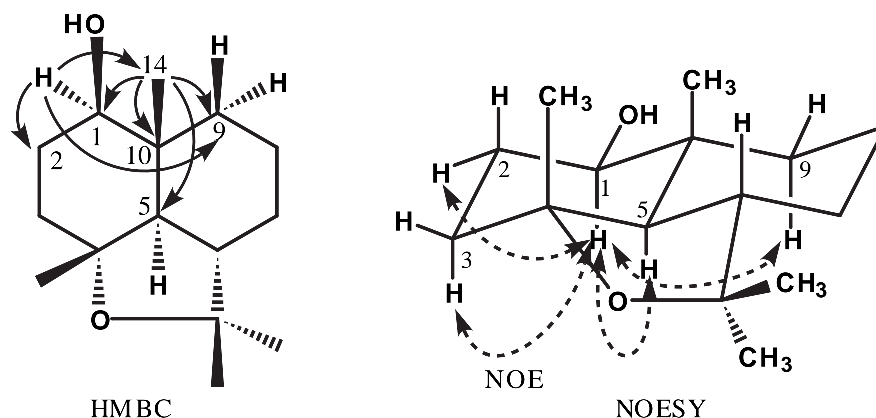

Figure 3. Important HMBC and NOESY correlations of compound (**2**).

Compound (**3**) {[α]$_D$ -28.6° ($CHCl_3$)} was obtained as a colorless prisms (mp 135-137°), whose molecular formula, $C_{15}H_{26}O_3$ was established by HR-EIMS ($[M]^+$ 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 [δ_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**) [δ_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-3α, H-5 and H-9; (ii) H-9 / H-1, H-5, H-7α and H-8α 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 1β, 9β -dihydroxymaalioxide.

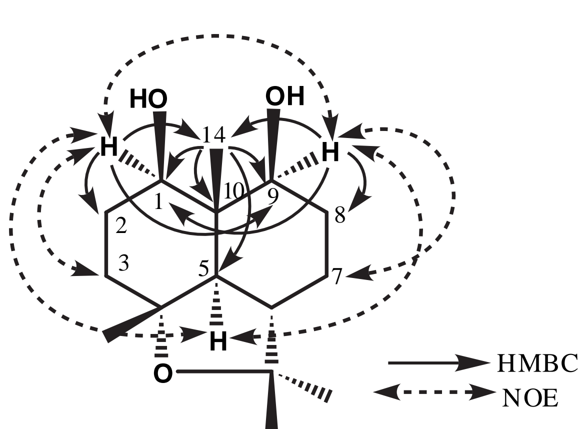

Figure 4. Important HMBC and NOESY correlations of compound (**3**).

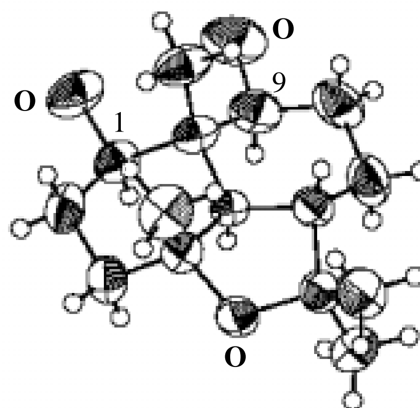

Figure 5. ORTEP drawing of **3**.

Compound (**4**) {$[\alpha]_D$ -16.7° ($CHCl_3$)} has the same molecular formula, $C_{15}H_{26}O_3$ (HR-EIMS; $[M]^+$ 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 [δ_H 3.48, 3.51(2H, each *d*, J=11.3 Hz); δ_C 70.4 (*t*)].and a secondary alcohol [δ_H 3.30 (1H, *dd*, J=4.9, 113 Hz); δ_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-2α, H-3α, H-5 and H-9α; (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 1β,12 -dihydroxymaalioxide.

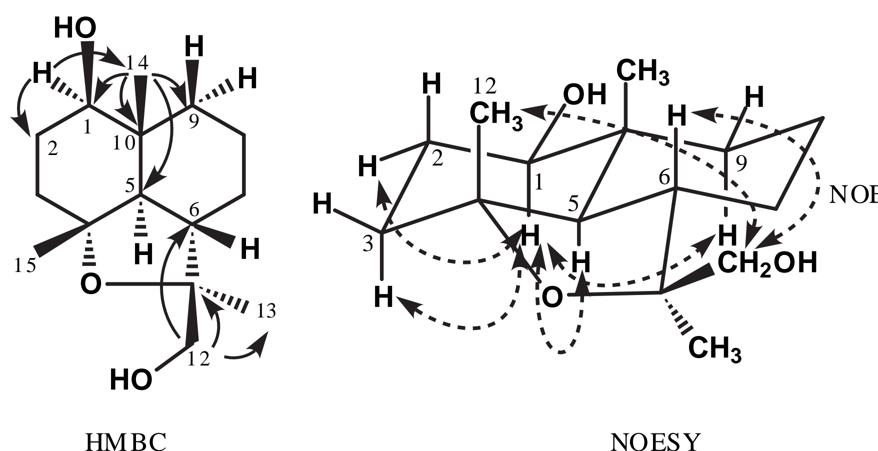

Figure 6. Important HMBC and NOESY correlations of compound (**4**).

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.

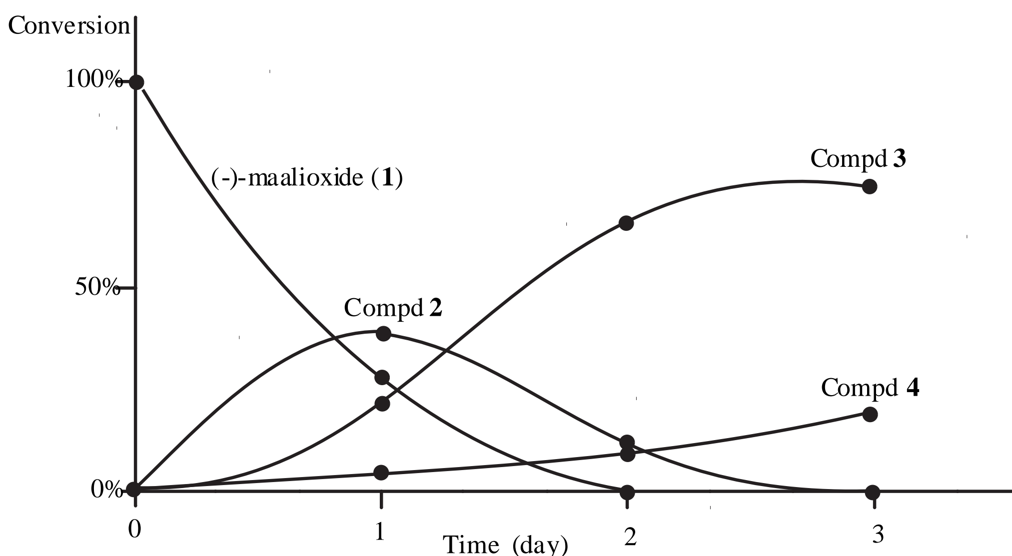

Figure 7. Time course of biotransformation of (-)-maalioxide (**1**) by *Aspergillus niger*

Biotaransformation of (-)-Maalioxide (1) by *Aspergillus cellulosae* *A. cellulosae* 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).

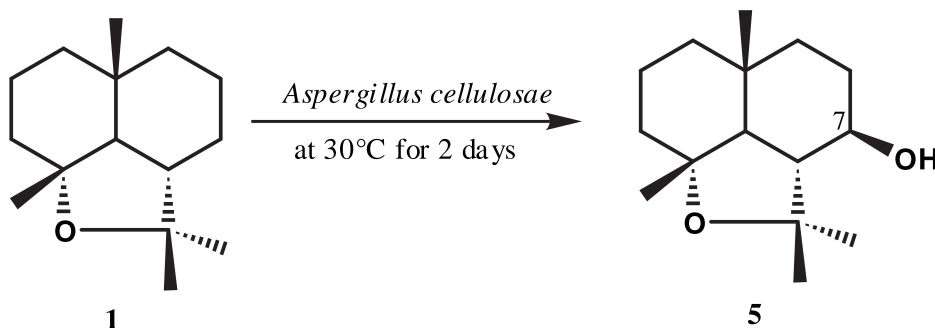

Figure 8. Biotransformation of (-)-maalioxide (**1**) by *A. cellulosae.*

Compound (**5**) {$[\alpha]_D$ -51.4° (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-8α 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 7β-hydroxymaalioxide.

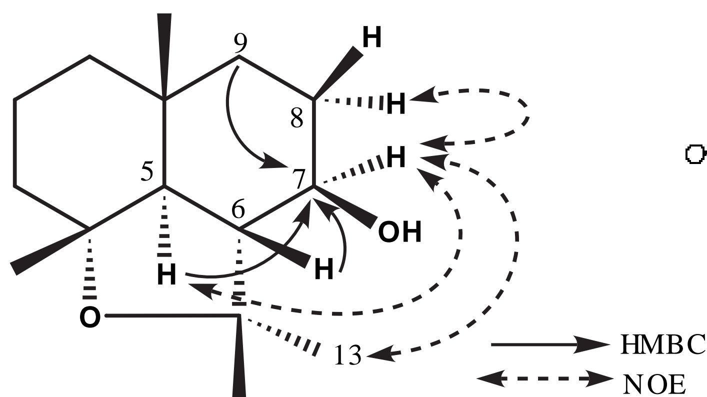

Figure 9. Important HMBC and NOESY correlations of **5**.

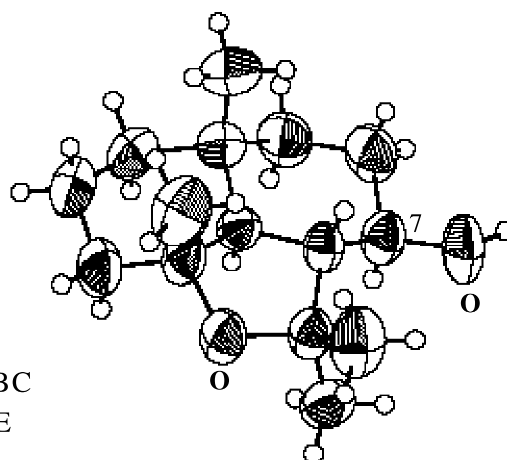

Figure 10. ORTEP drawing of **5**.

Table 1. 600 MHz ^{1}H NMR spectral ata of ompounds (**1-5**) in $CDCl_3$[a]

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

[a]Chemical shifts are in δ values. Coupling constants in Hz are in parenthesis.

Table 2 125 MHz ^{13}C NMR spectral data of compounds (**1**-**6**) in $CDCl_3$[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*)
OCO*CH*$_3$					21.2 (*q*)	
OCO*CH*$_3$					21.2 (*q*)	
O*CO*CH$_3$					171.5 (*s*)	
O*CO*CH$_3$					171.5 (*s*)	

[a] Chemical shifts from TMS in $CDCl_3$ and assignments from HMQC and HMBC spectra

We reported that (-)-maalioxide (**1**) was treated with m-chloroperbenzoic acid (mCPBA) in $CHCl_3$ under reflux to afford 2α–hydroxymaalioxide (2.0%), 7β-hydroxymaalioxide (**5**)(1.2%) and 8α-hydroxy-maalioxide (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-7β occurred in *A.cellulosae* to afford **5**. It is noteworthy that the position of oxidation of (-)-maalioxide (**1**) are strikingly different between *A. niger*, *A. cellulosae* 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.

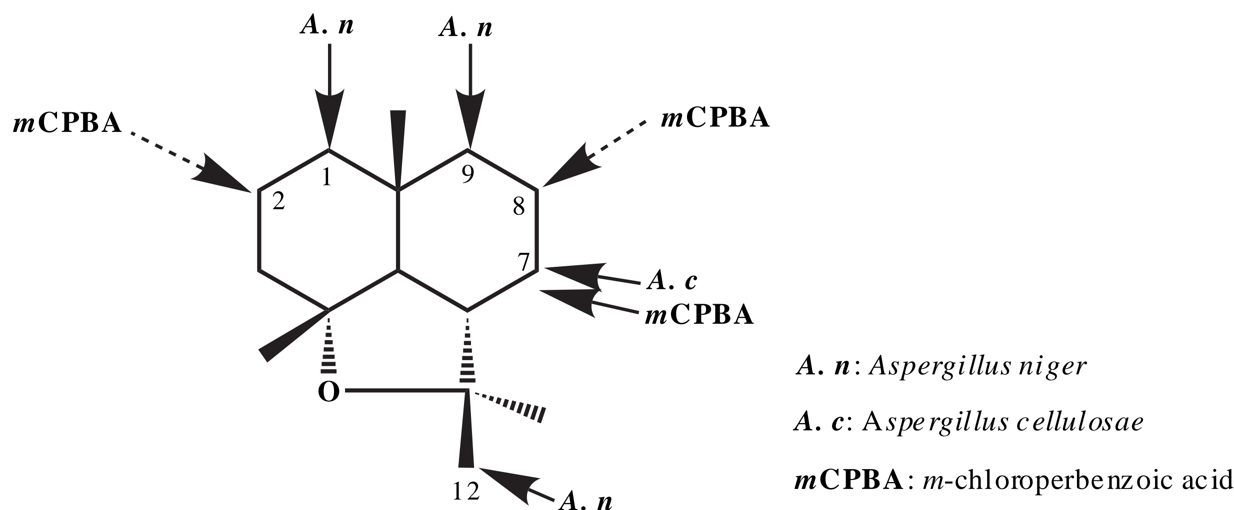

Figure 11. The position of oxidation of (-)-maalioxide (**1**) by *Aspergillus niger, A. cellulosae* and *m*CPBA

EXPERIMENTAL

General IR spectra were measured on a JASCO FT-IR 500 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian untiy 600 (^{1}H; 600 MHz, ^{13}C; 150 MHz) or a Varian Unity 200 (^{1}H; 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 cryrstallographic analysis was carried out by a Mac Science MXC18 diffractiotometer. Slica 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, $CHCl_3$)[lit.,[10] -34.5° (*c* 1.00, $CHCl_3$)]; EIMS: *m/z* 222 (M^+), 207 (100), 179, 149; HR-EIMS: *m/z* 222.1975 (M^+), $C_{15}H_{26}O$ requires 222.1984; FT-IR (KBr)cm^{-1}: 2932, 1377, 1050; ^{1}H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (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 cellulosae* 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% K_2HPO_4, 0.05% KCl and 0.001% $FeSO_4 \cdot 7H_2O$ 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℃ 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℃. 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 $MgSO_4$ 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, $CHCl_3$); EIMS: *m/z* 238 (M^+), 223 (100), 195, 162, 136; HR-EIMS: *m/z* 238.1918 (M^+), $C_{15}H_{26}O_2$ requires 238.1933; FT-IR (KBr)cm^{-1}: 3397 (OH), 2925, 1379, 1081; ^{1}H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (Table 2).

1β, 9β-Dihydroxymaalioxide (3) colorless prisms, mp 135-137°, $[\alpha]_D^{18}$ -28.6° (*c* 1.02, $CHCl_3$); EIMS: *m/z* 254 (M^+), 239 (100), 195; HR-EIMS: *m/z* 254.1882 (M^+), $C_{15}H_{26}O_3$ requires 254.1882; FT-IR (KBr)cm^{-1}: 3358 (OH), 2938, 2866, 1063; ^{1}H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz)

spectral data in $CDCl_3$ (Table 2).

1β, 12-Dihydroxymaalioxide (4) colorless oil, $[\alpha]_D^{18}$ -16.7° (*c* 0.87, $CHCl_3$); EIMS: *m/z* 254 (M^+), 239 , 223 (100), 205, 163; HR-EIMS: *m/z* 254.1868 (M^+), $C_{15}H_{26}O_3$ requires 254.1882; FT-IR (KBr)cm^{-1}: 3379 (OH), 2936, 1380, 1042; ^{1}H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (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 $CHCl_3$. The organic phase was washed with 1N HCl, 5% $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to give a residue. The residue was purified by a silica gel column chromatography with *n*-hexane-AcOEt gradient to afford 1β, 9β-diacetoxymaalioxide (**6**)(11 mg, 82.7%) as colorless oil; $[\alpha]_D^{21}$ -18.6° (*c* 1.01, $CHCl_3$); EIMS : *m/z* 338 (M^+), 323 (100), 263, 237; HR-EIMS: *m/z* 338.2079 (M^+), $C_{19}H_{30}O_5$ requires 338.2093; FT-IR (KBr)cm^{-1}: 2938, 1729 (C=O), 1372, 1026; ^{1}H NMR($CDCl_3$): 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); ^{13}C NMR (125 Hz) spectral data in $CDCl_3$ (Table 2).

The crystal data for 3 Orthorhombic, space group $P2_12_12_1$, *a*=6.299 (3)Å, *b*= 14.070 (9)Å, *c* = 16.324 (2)Å, *V* =1447 (2)$Å^3$, *Z* = 4, D_x= 1.094 Mg m^{-3}, D_x= 1.094 Mg m^{-3}, μ (Mo Kα) =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 diffractiotometer at rt. All diagrams and calculations were performed using maXus (Bruker Nonius, Delft & Mac Science, Japan).

Biotransformation of maalioxide (1) by *Aspergillus cellulosae* *A. cellulosae* 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 7β-hydroxymaalioxide (**5**)(32.1 mg; 29.9%) with the recovery of **1** (20.5 mg; 20.5%).

7β-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^+), $C_{15}H_{26}O_2$ requires 238.1933; FT-IR (KBr)cm^{-1}: 3392 (OH), 2926, 1461, 1082; ^{1}H-NMR (600 MHz) spectral data in $CDCl_3$ (Table 1); ^{13}C

NMR (125 Hz) spectral data in $CDCl_3$ (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)Å^3, Z = 4, D_x= 1.096 Mg m^{-3}, D_x= 1.096 Mg m^{-3}, δ (Mo Kα) =0.070 mm^{-1}, Final R and R_w were 0.107 and 0.208 for 1551 reflections.

REFERENCES

1. K. Kieslich, Microbial Transformation of Non-Steroid Cyclic Compounds, 1976, Georg Thieme Stuttgart, ermany, p. 1.
2. Y. Noma and Y. Asakawa, *Biotechnology in Agriculture and Forestry*, ed. by Y. P. S. Bajaj, 1994, Berlin, Springer, Vol. **28**, p. 185.
3. Y. Noma and Y. Asakawa, *Biotechnology in Agriculture and Forestry*, ed. by Y. P. S. Bajaj, 1995, Berlin, Springer, Vol. **33**, p. 62.
4. Y. Noma and Y. Asakawa, *Biotechnology in Agriculture and Forestry*, ed. by Y. P. S. Bajaj, 1998, Berlin, Springer, Vol. **41**, p. 194.
5. T. Matsumoto, N. Hayashi, T. Ishida, and Y. Asakawa, *J. Pharm. Sci.,* 1990, **79**, 540.
6. T. Matsumoto, T. Ishida, T. Yoshida, H. Terao, Y. Takeda, and Y. Asakawa, *Chem. Pharm. Bull*., 1992*,* **40**, 1721.
7. T. Hashimoto, Y. Noma, and Y.Asakawa, *Heterocycles,* 2001, **54**, 529.
8. E. H. Lahlou, Y. Noma, T. Hashimoto, Y. Noma, and Y. Asakawa, *Phytochemistry,* 2000, **54**, 455.
9. Y. Asakawa, in *Progress in the Chemistry of Organic Natural Products*, ed. by W. Herz, W. Kirby, R. E. Moore, W. Steglich, and Ch. Tamm, 1995, Wien, Springer, Vol. **65**, p. 1.
10. A. Matsuo, M. Nakayama, S. Sato, T. Nakamoto, S. Uto, and S. Hayashi, *Experientia,* 1974, **30**, 321.
11. C. S. Narayanan, K. S. Kulkarni, A. S. Vaidya, S. Kanthamani, G. L. Kumari, B. V. Bapat, S. N. Kulkarni, G. R. Kelkar, and S. C. Bhattacharyya, *Tetrahedron*, 1964, **20**, 963.
12. M. Tori, M. Sono, and Y. Asakawa, *Bull. Chem. Soc. Jpn*., 1990, **63**, 1770.