Biotransformation of Aristolane- and 2,3-Secoaromadendrane-Type Sesquiterpenoids Having a 1,1-Dimethylcyclopropane Ring by *Chlorella fusca* var. *vacuolata*, *Mucor* Species, and *Aspergillus niger*

Mai Furusawa,[a] Toshihiro Hashimoto,[a] Yoshiaki Noma,[b] and Yoshinori Asakawa*,[a]

[a] *Faculty of Pharmaceutical Science, Tokushima Bunri University; and* [b] *Faculty of Human Life Sciences, Tokushima Bunri University; Yamashiro-cho, Tokushima 770–8514, Japan.* Received January 30, 2006; accepted March 13, 2006

Biotransformation of the aristolane-type sesquiterpene hydrocarbon (+)-1(10)-aristolene (1) from the crude drug *Nardostachys chinensis* and of the 2,3-secoaromadendrane-type sesquiterpene lactone plagiochilide (2) from the liverwort *Plagiochila fruticosa* by three microorganisms, *Chlorella fusca* var. *vacuolata*, *Mucor* species, and *Aspergillus niger* was investigated. *C. fusca* var. *vacuolata* and *Mucor* sp. introduced oxygen function into the cyclohexane ring of aristolene while *A. niger* oxidized stereoselectively one methyl of the 1,1-dimethyl group on the cyclopropane ring of aristolanes and 2,3-secoaromadendrane to give C-12 primary alcohol and C-12 carboxylic acid. The possible metabolic pathway of the formation of new metabolites is discussed. The stereostructures of new metabolites were established by a combination of NMR spectroscopy including HMBC and NOESY, X-ray crystallographic analysis, and chemical reaction.

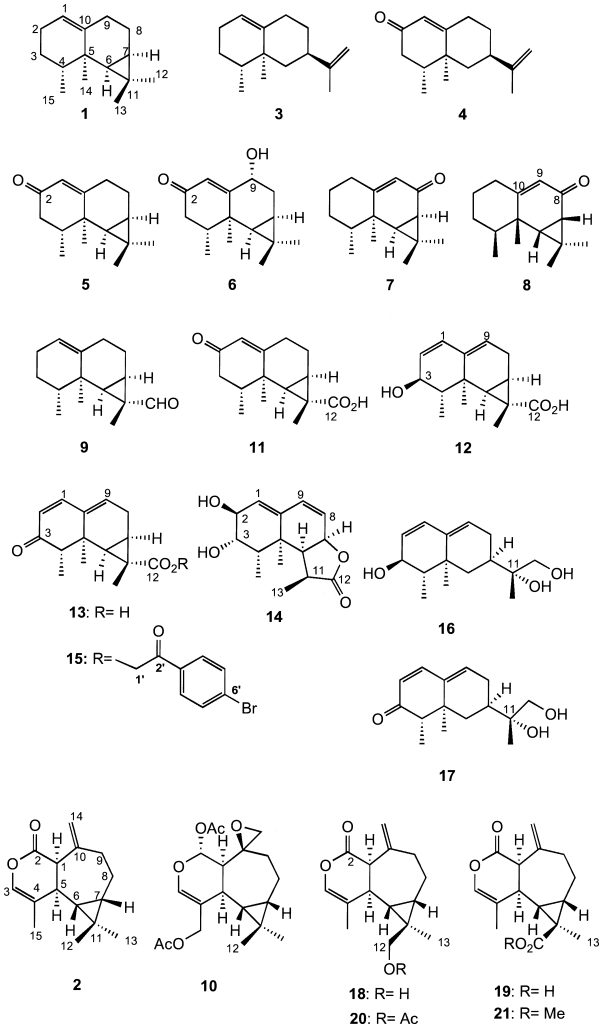
Key words aristolane; 2,3-secoaromadendrane; biotransformation; *Aspergillus niger*; *Chlorella fusca* var. *vacuolata*; *Mucor* species

We continue to study the biotransformation of terpenoids and aromatic compounds from crude drugs, liverworts and animal origin by microorganisms[1—8] and mammals[9,10] to obtain functional substances such as pheromones, aromas, and insecticides. Recently, we succeeded in highly efficient production of nootkatone, the most important and expensive grapefruit aroma that decreases somatic fat ratio[11] from the cheap and commercially available valencane-type sesquiterpene hydrocarbon valencene (**3**) from the valencia orange oil, by biotransformation using *Chlorella* and *Mucor* species.[12—14]

The structure of aristolane-type sesquiterpene, (+)-1(10)-aristolene (=β-gurjunene) (**1**)[15] whose structure resembles that of nootkatone (**4**) except for the presence of a 1,1-dimethylcyclopropane ring in the molecule, 1(10)-aristolen-2-one (**5**)[16,17] possessing excellent citrus scent and anti-microbial activity, and 9α-hydroxy-1(10)-aristolen-2-one (=debilon) (**6**)[18,19] having cytotoxic activity were isolated from *Nardostachys chinensis*. (−)-Aristolone (**7**)[20] and 1(10)-aristolen-12-al (**9**),[21] which inhibit melanin synthesis,[22,23] were also isolated from *Aristolochia debilis*, while the enantiomer (**8**) of **7** was obtained from the liverworts *Porella caespitans* var. *setigera*[24,25] and *Reboulia hemisphaerica*.[26]

We have previously reported the distribution of a number of novel terpenoids and aromatic compounds possessing biological activities such as antimicrobial, antitumor, and neural sprouting activities.[24,25] 2,3-Secoaromadendrane-type sesquiterpenoids such as plagiochilide (**2**) and plagiochiline A (**10**), which are distributed only in liverworts *Plagiochila* species, also possess a 1,1-dimethylcyclopropane ring.[24,25] Plagiochilide (**2**) elicits a nerve cell degeneration reparation activity,[27] and plagiochiline A (**10**) displays strong pungent tasting, intensive insect antifeedant and piscicidal acitivities.[24,25]

To obtain more biologically active compounds than the original aristolanes and 2,3-secoaromadendranes, biotransformation of (+)-1(10)-aristolene (**1**) and plagiochilide (**2**) by *Chlorella fusca* var. *vacuolata*, *Mucor* species, and *Aspergillus niger* was examined. This paper deals with the structure elucidation of metabolites obtained by biotransformation of **1** and **2** by these three microorganisms and their



* To whom correspondence should be addressed. e-mail: asakawa@ph.bunri-u.ac.jp

metabolic pathways.

Biotransformation of (+)-1(10)-Aristolene (1) by *Chlorella fusca* var. *vacuocola* and *Mucor* sp. Little attention has been paid to the biotransformation of terpenoids and aromatic compounds using the green algae *Chlorella* species. *C. fusca* var. *vacuolata* IAMC-28 was inoculated and cultivated stationary under illumination in Noro medium (pH 8.0) at 25 °C for 7 d. (+)-1(10)-Aristolene (**1**) was added to the medium and cultured by *C. fusca* var. *vacuolata* for 22 d to give three known compounds 1(10)-aristolone-2-one (**5**)[16,17] (18.7%, isolated yield), 9α-hydroxy-1(10)-aristolen-2-one (**6**)[18,19] (7.0%), and (−)-aristolone (**7**)[20] (7.1%). The stereostructures of their metabolites were established by high-resolution electron impact mass (HR-EI-MS) and NMR spectroscopy (Tables 1, 2), and by comparison of the spectral data *versus* authenticated samples.

Mucor species, a fungus strain from soil adhering to the liverwort *Pallavicinia subciliata*, converted valencene (**3**) into nootkatone (**4**) in very high yield (82%).[12—14] The same fungus was inoculated and cultivated stationary under illumination in Czapek-pepton medium (pH 7.0) at 30 °C for 5 d. (+)-1(10)-Aristolene (**1**) was added to the medium and biotransformed for 7 d to give 1(10)-aristolone-2-one (**5**) (0.7%) and (−)-aristolone (**7**) (0.6%) along with the starting material (**1**) (65%). It is noteworthy that the biochemical conversion ratio of **1** by *Chlorella* and *Mucor* species is poor. This phenomenon might be due to steric hindrance of the dimethylcyclopropane ring compared with the isopropenyl group of **3**. Plausible metabolic pathways of 1(10)-aristolene (**1**) by *C. fusca* var. *vacuolata* and *Mucor* species are shown in Fig. 1. (−)-Aristolone (**7**) might be obtained by isomerization of the double bond from 1(10) to 9(10), together with C-9 hydroxylation and elimination of a hydroxyl group followed by oxidation at C-8 (route a), by or C-8 oxidation then isomerization of the double bond (route b). Compound **1** was converted into 2-hydroxy-1(10)-aristolene followed by oxidation at C-2 to give 1(10)-aristolone-2-one (**5**), which was further converted into 9α-hydroxy-1(10)-aristolen-2-one (**6**) (route c).

Biotransformation of (+)-1(10)-Aristolene (1) by *Aspergillus niger* *A. niger* was inoculated and cultivated rotatory (100 rpm) in Czapek-pepton medium (pH 7.0) at 30 °C for 7 d. (+)-1(10)-Aristolene (**1**) (80 mg/200 ml) was added to the medium and further cultivated for 7 d. Crude ethyl acetate (EtOAc) extract obtained from the culture broth was chromatographed on silica gel (*n*-hexane–EtOAc gradient) to give small amounts of four new metabolites, **11** (1.4%), **12** (2.7%), **13** (0.8%), and **14** (2.1%).

Compound **11** {$[\alpha]_D$ +147.5° ($CHCl_3$)} was obtained as colorless prisms whose molecular formula $C_{15}H_{20}O_3$ was established by HR-EI-MS ($[M]^+$ *m/z* 248.1418). The FT-IR and UV spectra of **11** indicated the presence of carboxylic acid (3200—2800 cm^{-1}) and conjugated ketone (1669 cm^{-1}; λ_{max} 242.5 nm) groups. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectra of **11** showed the presence of two tertiary methyl [δ_H 1.20 (3H, s), 1.31 (3H, s)] and secondary methyl [δ_H 1.06

Table 1. 600 MHz ^{1}H-NMR Spectral Data of Compounds **1**, **5**—**7**, **11**, **12**, **14** and **15** in $CDCl_3$[a)]

H	1	5	6	7	11	12	14	15
1α	5.28 m	5.76 br s	5.81 br s	2.45 m	5.80 br s	5.94 dd	5.74 d	6.80 d
1β				2.26 m		(1.9, 9.9)	(4.4)	(9.9)
2α	1.93 m			1.38 m	2.31 m	5.60 d	4.21 br d	5.85 d
2β	1.93 m			1.83 m	2.27 m	(9.9)	(4.4)	(9.9)
3α	1.40 m	2.28 m	2.35 m	1.43 m	2.31 m	3.94 d	3.88 br s	
3β	1.40 m	2.28 m		1.56 m	2.31 m	(9.6)		
4	1.76 m	2.28 m	2.30 m	1.83 m	2.27 m	1.57 m	2.17 m	2.76 q
								(6.9)
6	0.56 d	0.70 d	0.76 d	1.39 d	1.79 d	1.76 d	2.84 dd	1.91 d
	(9.1)	(9.6)	(9.1)	(8.0)	(9.9)	(10.2)	(8.0, 8.0)	(10.2)
7	0.74 ddd	0.92 ddd	0.95 ddd	1.74 dd	1.89 ddd	1.86 dd	5.13 m	2.03 dd
	(3.6, 9.1, 9.1)	(3.6, 9.6, 9.6)	(3.8, 9.1, 9.1)	(1.4, 8.0)	(3.0, 3.0, 9.9)	(1.4, 8.0)		(6.9, 10.2)
8α	1.97 m	2.18 m	2.28 ddd		2.29 m	2.67 m	5.86 dd	2.80 m
			(2.2, 9.1, 15.9)				(2.5, 10.2)	
8β	1.38 m	1.54 m	1.74 ddd		1.63 m	2.28 dd		2.48 dd
			(3.8, 3.8, 15.9)			(5.2, 15.9)		(5.2, 21.4)
9α	2.22 m	2.44 m		5.73 dd	2.53 m	5.40 m	6.26 dd	5.92 dd
				(1.4, 1.6)			(1.4, 10.2)	(4.1, 4.1)
9β	1.73 m	2.04 m	4.22 dd		2.14 m			
			(2.2, 3.8)					
11							2.62 m	
12	1.02 s	1.03 s	1.08 s	1.21 s				
13	0.98 s	0.96 s	0.92 s	1.26 s	1.20 s	1.30 s	1.27 d	1.45 s
							(7.7)	
14	1.07 s	1.24 s	1.45 s	1.20 s	1.31 s	1.09 s	1.25 s	1.21 s
15	0.97 d	1.08 d	1.07 d	1.07 d	1.06 d	1.13 d	1.19 d	1.24 d
	(6.9)	(6.6)	(6.6)	(6.9)	(6.3)	(6.6)	(7.4)	(6.9)
1′								5.29 s
4′, 8′								7.77 d
								(8.8)
5′, 7′								7.64 d
								(8.8)

a) Chemical shifts from TMS (multiplicity, *J* in Hz) in $CDCl_3$.

Table 2. 150 MHz ^{13}C-NMR Spectral Data of Compounds **1**, **5**—**7**, **11**, **12**, **14** and **15** in $CDCl_3$[a)]

C	1	5	6	7	11	12	14	15
1	120.3	125.0	126.4	33.1	125.6	129.4	124.7	144.7
2	25.7	199.1	200.0	26.1	198.5	129.1	68.9	125.2
3	27.2	42.5	42.7	30.6	42.2	72.0	75.5	201.0
4	36.7	36.4	37.0	38.7	35.9	42.3	32.9	47.4
5	36.8	38.6	37.3	39.6	38.2	35.9	37.9	38.3
6	33.5	33.3	31.7	39.2	35.0	33.7	44.4	33.2
7	19.6	19.3	16.3	35.5	23.0	23.6	76.4	22.2
8	20.8	20.2	27.9	196.3	18.8	21.6	125.0	22.4
9	29.9	30.6	72.5	124.3	29.5	123.2	132.7	131.4
10	144.1	174.2	170.4	167.6	171.1	139.6	142.4	139.8
11	18.5	19.1	19.1	24.4	26.0	25.9	37.3	26.3
12	29.9	29.2	29.1	29.7	181.4	181.7	180.8	174.6
13	16.5	17.2	17.5	16.5	15.2	8.8	12.5	9.7
14	23.0	21.7	23.9	16.3	21.4	21.9	22.9	23.4
15	16.1	15.4	14.9	22.6	15.2	11.0	11.6	7.3
1′								66.0
2′								191.4
3′								132.9
4′, 8′								129.2
5′, 7′								132.2
6′								129.1

a) Chemical shifts from TMS in $CDCl_3$.

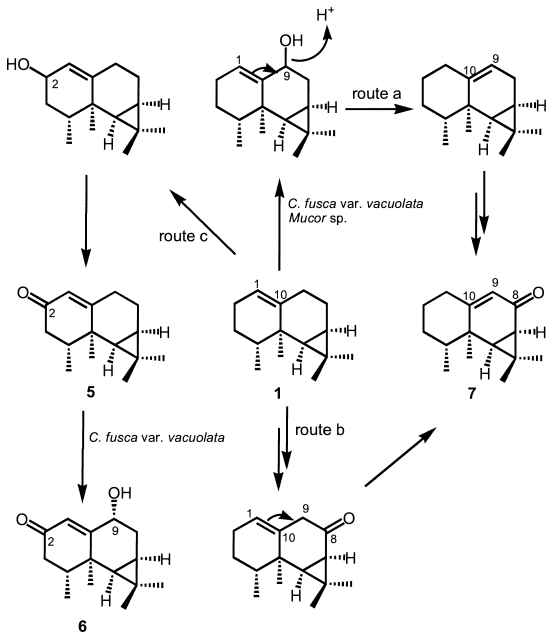


Fig. 1. Possible Metabolic Pathways of 1(10)-Aristolene (**1**) by *Chlorella fusca* var. *vacuolata* and *Mucor* Species

(3H, d, J=6.3 Hz)], carboxyl [δ_C 181.4 (s)], and ketone [δ_C 198.5 (s)] groups. The similarity of ^{1}H- and ^{13}C-NMR spectral data with those of 1(10)-aristolone-2-one (**5**) indicated that one carboxylic acid was introduced to one of the four tertiary methyl groups. This prediction and the relative structure of compound **11** were confirmed by X-ray crystallographic analysis as shown in Fig. 2. Thus the structure of **11** was established as 2-oxo-1(10)-aristolen-12-oic acid.

Compound **12**: {[α]$_D$ −3.1° ($CHCl_3$)} has the same molecular formula $C_{15}H_{20}O_3$ (HR-EI-MS; $[M]^+$ m/z 248.1418) as that of **11**. The FT-IR and UV spectra of **12** indicated the presence of carboxylic acid (3400—2400 cm^{-1}) and conjugated diene (λ_{max} 240.0 nm) groups. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectra of **12** showed the presence of

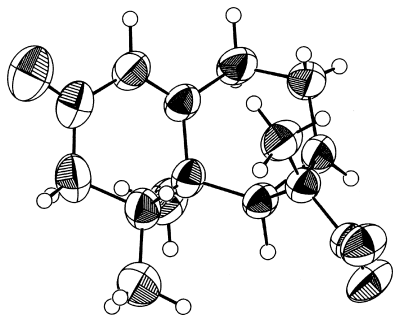


Fig. 2. ORTEP Drawing of Compound **11**

conjugated diene [δ_H 5.40 (1H, m), 5.60 (1H, d, J=9.9 Hz), 5.94 (1H, dd, J=1.9, 9.9 Hz)], secondary alcohol [δ_H 3.94 (1H, d, J=6.3 Hz); δ_C 72.0 (d)], and carboxyl [δ_C 181.7 (s)] groups. Compound **12** showed correlation between (i) H-1/C-3, and C-9, (ii) H-2/C-3, and C-10, (iii) H-9/C-1, and C-7, (iv) H-13/C-6, C-7, and C-12 in HMBC spectrum (Fig. 3), and NOEs between (i) H-1/H-2, and H-9, (ii) H-3/H-14, and H-15, (iii) H-13/H-4, and H-8β in the NOESY spectrum (Fig. 3). Based on the above spectral evidence, the relative structure of **12** was deduced as 3β-hydroxy-1(2),9(10)-aristoladien-13-oic acid.

Compound **13** contained a little impurity, the purification of which was very difficult by any separation method. Thus **13** was converted to a *p*-bromophenacyl ester (**15**). Compound **15** {[α]$_D$ −52.1° ($CHCl_3$)} has the molecular formula $C_{23}H_{23}O_4Br$ (HR-EI-MS; $[M]^+$ m/z 442.0775). The IR and UV spectra of **15** indicated the presence of ester (1722 cm^{-1}) and conjugated ketone (1704, 1674 cm^{-1}; λ_{max} 257 nm) groups. The ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2) of **15** showed the presence of a similar conjugated diene [δ_H 5.85 (1H, d, J=9.9 Hz), 5.92 (1H, t, J=4.1 Hz), 6.80 (1H, d, J=9.9 Hz)] as seen in **12**, and ester [δ_C 174.67 (s)] and two ketones [δ_C 191.4 (s) and 201.0 (s)] groups. Compound (**15**) showed correlation between (i) H-1/C-3 and C-9, (ii) H-2/C-4 and C-10, (iii) H-13/C-6, C-11, and C-12, (iv) H-1′/C-12 in

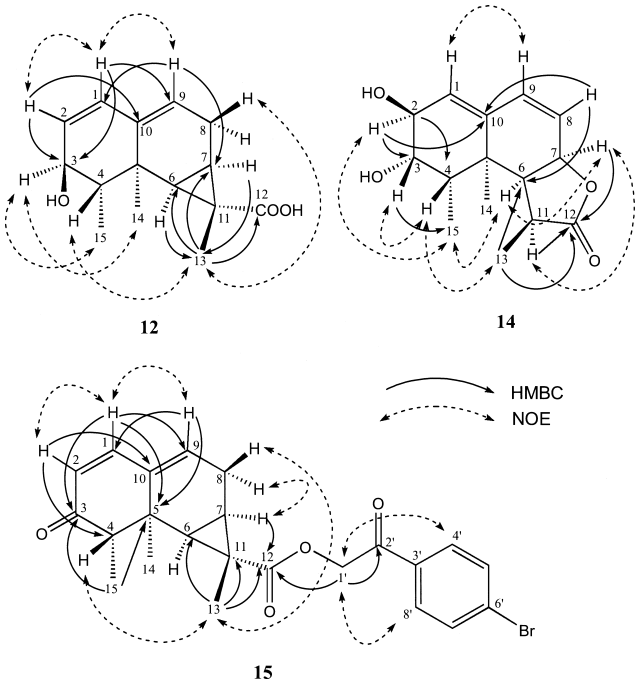


Fig. 3. Important HMBC and NOESY Spectra of Compounds **12**, **14** and **15**

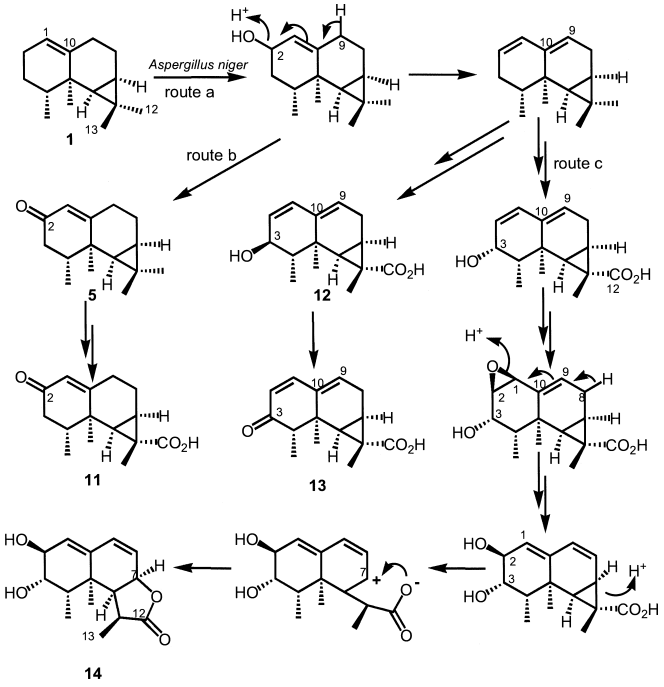


Fig. 4. Possible Metabolic Pathways of 1(10)-Aristolene (**1**) by *Aspergillus niger*

HMBC spectrum (Fig. 3), and the NOEs between (i) H-1/H-2 and H-9; (ii) H-4/H-13, (iii) H-13/H-4 and H-8β in the NOESY spectrum (Fig. 3). From the above spectral evidence, the relative structure of **13** was deduced as 3-oxo-1(2),9(10)-aristoladien-13-oic acid.

Compound **14**: {$[\alpha]_D$ −61.4° ($CHCl_3$)} has the molecular formula $C_{15}H_{20}O_4$ (HR-CI-MS; $[M+H]^+$ *m/z* 265.1414). The FT-IR spectrum of **14** indicated the presence of hydroxyl (3419 cm^{-1}) and ester (1747 cm^{-1}) groups. The UV, IR, ^{1}H- (Table 1), and ^{13}C-NMR (Table 2) spectra of **14** contained signals corresponding to conjugated diene [λ_{max} 237 nm; δ_H 5.74 (1H, d, *J*=4.4 Hz), 5.86 (1H, dd, *J*=2.5, 10.2 Hz), 6.26 (1H, dd, *J*=1.4, 10.2 Hz)], two secondary alcohol [δ_H 3.88 (1H, br s), δ_C 68.9 (d); 4.21 (1H, br d, *J*=4.4 Hz), δ_C 75.5 (d)], and lactone carbonyl [1747 cm^{-1}; δ_C 180.8 (s)] groups. Compound **14** showed correlations between (i) H-1/C-3, and C-9 (ii) H-2/C-3, and C-10 (iii) H-3/C-2, C-14, and C-5, (iv) H-13/C-6, and C-12 in HMBC spectrum (Fig. 3), and the NOEs between (i) H-2/H-14, (ii) H-3/H-4, (iii) H-4/H-3, and H-13, (iv) H-6/H-7, H-11, and H-14 in the NOESY spectrum (Fig. 3). Thus the relative structure of **14** was deduced as 2β,3α-dihydroxynardosinan-1(10),8(9)-dien-11β-methyl-12,7-olide.

Possible metabolic pathways of 1(10)-aristolene (**1**) by *A. niger* are shown in Fig. 4. 2-Hydroxy-1(10)-aristolene, obtained from hydroxylation of **1** at C-2, probably gives 1(2),9(10)-aristoladiene by elimination of a hydroxyl group at C-2 followed by oxidation to give compounds **12** and **13** (route a). Similar reaction of **1** to compounds **12** and **13** has been found in valencene (**3**), which gives conjugated dienes **16** and **17**.[28] The same type oxidation fashion of valencene (**3**) to nootkatone (**4**)[14] has been recognized in compound **1**, which afforded compound **5**, followed by stereospecific oxidation at C-13 to furnish carboxylic acid (**11**) (route c). Compound **14** might be formed through a complex process with

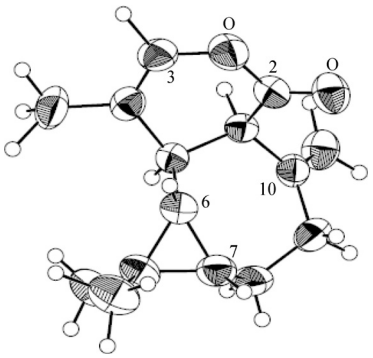


Fig. 5. ORTEP Drawing of Plagiochilide (**2**)

cleavage of the cyclopropane ring as shown in Fig. 4.

Biotransformation of Plagiochilide (2) by *Aspergillus niger* Ether extract of *Plagiochila fruticosa* was chromatographed on silica gel to afford plagiochilide (**2**) and plagiochiline A (**10**), the absolute configuration of which was established by CD spectrum.[29,30] The relative structure of plagiochilide was first confirmed by X-ray crystallographic analysis as shown Fig. 5. Plagiochilide (**2**) was treated in the same manner as mentioned in the biotransformation of **1** by *A. niger* for 2 d to give 12-hydroxyplagiochilide (**18**) (40.9%) and plagiochilide-12-oic acid (**19**) (12.2%). Biotransformation of **2** by the same fungus for 7 d by *A. niger* gave **18** (5.8%) and **19** (74.4%).

Compound (**18**): {$[\alpha]_D$ −50.1° ($CHCl_3$)} has the molecular formula $C_{15}H_{20}O_3$ (HR-EI-MS; $[M]^+$ *m/z* 248.1416). FT-IR spectra of **3** indicated the presence of hydroxyl (3408 cm^{-1}) and lactone (1764 cm^{-1}) groups. The ^{1}H- (Table 3) and ^{13}C-NMR spectra (Table 4) of **18** indicated the presence a new hydroxylmethyl group [δ_H 3.25, 3.39 (each 1H, d, *J*=11.0 Hz); δ_C 72.6 (t)] in place of one dimethyl group on the cyclopropane ring, indicating that **18** was C-12 or C-13

Table 3. 600 MHz ^{1}H-NMR Spectral Data of Compounds **2**, **18**—**21** in $CDCl_3$[a)]

H	**2**	**18**	**19**	**20**	**21**
1	3.53 d (4.7)	3.55 d (4.7)	3.71 d (4.7)	3.55 d (4.7)	3.55 d (4.8)
3	6.23 q (1.6)	6.25 q (1.6)	6.36 q (1.6)	6.26 q (1.6)	6.27 q (1.6)
5	1.98 dd (4.7, 10.4)	2.03 dd (4.7, 10.4)	1.98 dd (4.7, 10.4)	2.07 dd (4.7, 9.9)	2.04 dd (4.8, 9.9)
6	0.43 dd (8.8, 10.4)	0.59 dd (9.6, 10.4)	1.30 dd (10.2, 10.2)	0.66 dd (9.9, 9.9)	1.30 dd (10.2, 10.2)
7	0.86 m	0.97 m	1.71 m	0.98 m	1.70 m
8α	0.89 m	0.92 m	1.01 m	0.90 m	0.98 m
8β	2.08 m	2.08 m	2.11 m	2.08 m	2.15 m
9α	2.46 dd (6.6, 13.5)	2.48 dd (6.6, 12.6)	2.48 dd (6.3, 13.5)	2.49 dd (6.6, 12.6)	2.53 dd (6.3, 13.5)
9β	2.55 ddd (1.9, 13.5, 13.5)	2.75 ddd (1.9, 12.6, 12.6)	2.57 ddd (1.9, 13.5, 13.5)	2.58 m	2.63 m
12	1.04 s	3.25 d (11.0) 3.39 d (11.0)		3.65 d (11.0) 3.93 d (11.0)	
13	1.05 s	1.17 s	1.30 s	1.14 s	1.30 s
14α	4.76 d (1.9)	4.79 d (1.9)	4.85 d (1.9)	4.79 d (1.9)	4.82 d (1.9)
14β	4.93 d (1.6)	4.95 d (1.9)	4.95 d (1.6)	4.95 d (1.9)	4.98 d (1.9)
15	1.74 d (1.6)	1.76 d (1.6)	1.74 d (1.6)	1.76 d (1.6)	1.72 d (1.6)
OAc				2.05 s	
$COOCH_3$					3.65 s

a) Chemical shifts from TMS (multiplicity, *J* in Hz) in $CDCl_3$.

Table 4. 150 MHz ^{13}C-NMR Spectral Data of Compounds **2**, **18**—**20** in $CDCl_3$[a)]

C	**2**	**18**	**19**	**20**
1	53.6	53.3	53.8	53.2
2	169.8	169.7	171.4	169.4
3	134.9	135.1	136.7	135.2
4	124.2	123.9	125.3	123.6
5	38.7	38.1	38.8	38.1
6	27.5	24.6	31.2	25.3
7	29.2	25.9	31.3	26.2
8	25.3	24.8	25.5	24.7
9	35.1	34.8	35.4	34.7
10	147.1	146.7	148.0	146.7
11	19.4	26.3	27.4	23.3
12	28.6	72.6	178.9	73.7
13	16.1	11.8	10.7	12.2
14	116.8	117.2	117.9	117.3
15	15.4	15.5	15.5	15.4
$OCO\underline{C}H_3$				20.9
$O\underline{C}OCH_3$				171.0

a) Chemical shifts from TMS in $CDCl_3$.

hydroxylated product. This assumption was further confirmed by acetylation of **18** to give an acetate (**20**) [1739 cm^{-1}; δ_H 2.05 (s)]. Compound **18** showed correlations between H-1/C-6, C-7, C-11, and C-13 in HMBC spectrum (Fig. 6), and NOEs between (i) H-12/H-6, H-7 and H-13, (ii) H-13 H-5, H-8α and H-12 in the NOESY spectrum (Fig. 6). Consequently, the structure of compound **18** was established as 12-hydroxyplagiochilide.

The molecular formula $C_{15}H_{18}O_4$ of **19** {$[\alpha]_D$ +2.5° ($CHCl_3$)} was deduced by (HR-EI-MS; $[M]^+$ *m/z* 262.1191). IR spectra of **19** indicated the presence of a lactone

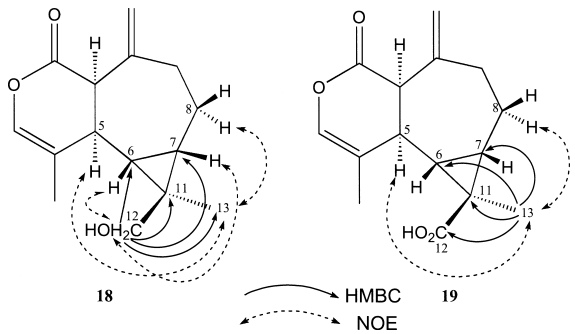


Fig. 6. Important HMBC and NOESY Spectra of Compounds **18** and **19**

(1756 cm^{-1}) and conjugated carboxyl (3200—2400, 1675 cm^{-1}) group, which was confirmed by methylation with trimethylsilyl diazomethane {$(CH_3)_3$–$SiCHN_2$} to afford a methyl ester (**21**) [1719 cm^{-1}; δ_H 3.65 (3H, s)]. Similarity of ^{1}H- (Table 3) and ^{13}C-NMR (Table 4) spectra to those of **18** except for the presence of a carboxyl group showed that **19** was plagiochilide-12-oic acid. This presumption was further supported by correlation between (i) H-6/C-11 and C-12, (ii) H-13/C-6, C-7, and C-12 in HMBC spectrum (Fig. 6), and NOEs between (i) H-13/H-5, and H-8α in the NOESY spectrum (Fig. 6). From the above spectral evidence, the relative structure of compound **19** was deduced as plagiochilide-12-oic acid.

Plagiochilide (**2**) was cultivated with *A. niger* for 7 d with 1-aminobenzotriazole, an inhibitor of Cytochrome P-450, to afford only compound **18** (61.7% isolated yield). 1-Aminobenzotriazole inhibited the oxidation process of **18** to **19**. In the time course (Fig. 7) of the biotransformation of

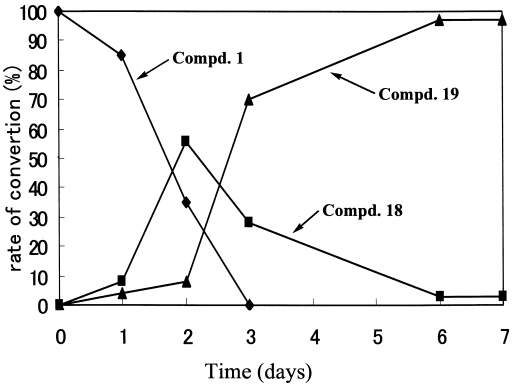


Fig. 7. Time Course Change for the Biotransformation of Plagiochilide (**2**) by *Aspergillus niger*

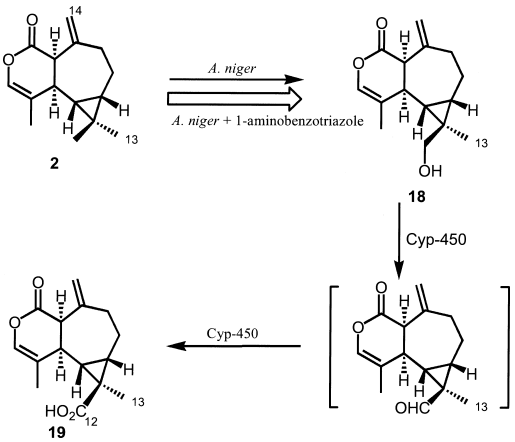


Fig. 8. Possible Metabolic Pathways of Plagiochilide (**2**) by *Aspergillus niger*

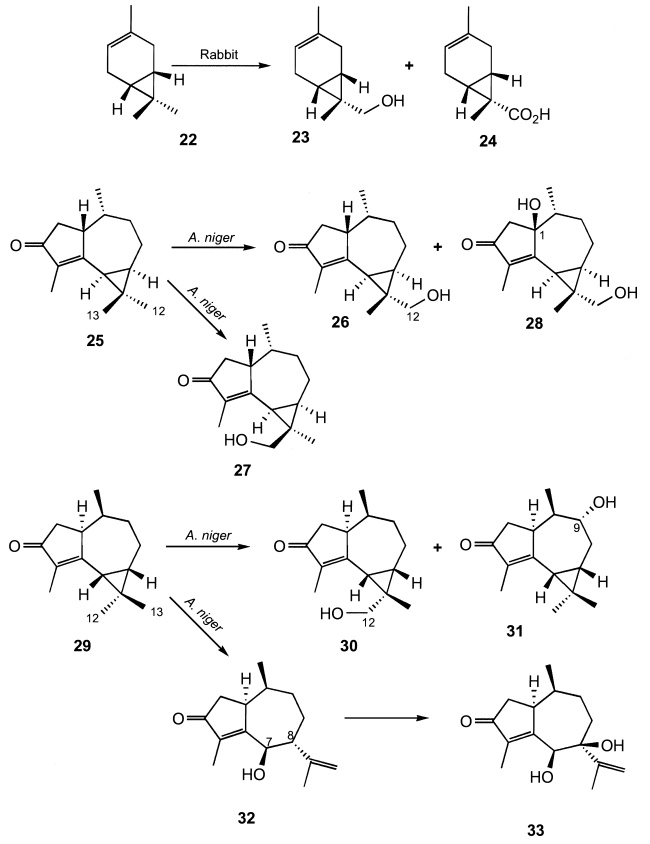


Fig. 9. Biotransformation of Δ^3-Carene (**22**) by Rabbit and (−)-Cyclocolorenone (**25**) and its Enantiomer (**29**) by *Aspergillus niger*

plagiochilide (**2**) by *A. niger*, the yield of **2** increased with decreasing that of **18**, subsequently the yield of **19** increased with decreasing that of **18**. From the above results, plausible metabolic pathways of plagiochilide (**2**) are shown in Fig. 8.

Δ^3-Carene (**22**), a monoterpene hydrocarbon with the same 1,1-dimethylcyclopropane moiety as that of aristrane-, aromadendrane-, and secoaromadendrane-type sesquiterpenoids, was biosynthesized by rabbit to give 8-hydroxy-Δ^3-carene (**23**) and Δ^3-carene-8-oic acid (**24**).[31] As far as we are aware, this is the first example of stereospecific oxidation of one of the gemdimethyl groups by animal. Microbial biotransformation of aromadendrane, (−)-cyclocolorenone (**25**) isolated from *Solidago canadensis* and its enantiomer (**29**) from the liverwort *Plagiochila sciophyla* by *A. niger* gave C-12-hydroxy (**26**), C-13-hydroxy (**27**), and 1,12-dihydroxy products (**28**) from the former compound, while *ent*-C-12-hydroxy (**30**) together with *ent*-9β-hydroxycyclocololenone (**31**) and two cyclopropane-cleaved guaiane-type sesquiterpene alcohol (**32**, **33**) were obtained from the metabolites of *ent*-cyclocolorenone (**29**) by *A. niger*.[32] Four aromadendranes, (−)-globulol, (+)-ledol, myli-4(15)-en-9-one, and myliol were converted by the plant pathogenic fungus *Glomerella cingulata*[33] and *A. niger*[34] to give C-12 hydroxy or C-12 carboxylic products. *A. niger* converted *ent*-maaliane-type sesquiterpenoid with the same 1,1-dimethylcyclopropane ring to form C-12-primary alcohol.[35] Thus oxidation of one 1,1-dimethyl group on the cyclopropane ring in fungal and mammalian biotransformation seems a common phenomenon.

In summary, (+)-1(10)-aristolene (**1**) from the crude drug *N. chinensis* was biotransformed by *C. fusca* var. *vacuolata* and *Mucor* sp. to afford 1(10)-aristolen-2-one (**5**) with citrus aroma[16,17] and 9α-hydroxy-1(10)-aristolen-2-one (**6**),[18,19] which was also obtained from *N. chinensis*, and (−)-aristolone (**7**), which was isolated from *A. debilis* and inhibits melanin synthesis.[23] In the biotransformation of (+)-1(10)-aristolene (**1**) and plagiochilide (**2**) by *Chlorella*, *Mucor* species and *A. niger*, clearly different oxidation occurred; stereospecific oxidation of one of the methyl groups of 1,1-dimethylcyclopropane ring mainly proceeded to afford carboxylic acids **11**—**13** and **19** by *A. niger*, while an active methylene group of cyclohexane ring in (−)-1(10)-aristolene (**1**) was oxidized to give an α,β-unsaturated ketone by *Chlorella* and *Mucor* species. It is noteworthy that in (+)-1(10)-aristolene (**1**) having a 6β,7β-1,1-dimethylcyclopropane ring, the C-11α methyl group was oxidized to give C-11α carboxylic acid while in plagiochilide (**2**) having a 6α,7α-1,1-dimethylcyclopropane ring, the C-11β methyl group was oxidized stereoselectively by *A. niger* to give C-11β primary alcohol (**12**) and C-11 carboxylic acid (**19**), respectively.

Experimental

General Procedures IR spectra were measured by JASCO FT-IR 500 spectrophotometer. ^{1}H- and ^{13}C-NMR were recorded on a Varian unity 600 (^{1}H; 600 MHz, ^{13}C; 150 MHz) or Varian Unity 200 (^{1}H; 200 MHz, ^{13}C; 50 MHz) spectrometer. The solvent used for NMR spectra was $CDCl_3$. MS spectra including CI-MS, HR-EI-MS, and HR-CI-MS were measured on a

JEOL JMS HX-100 or JEOL AX-500 spectrometer. Optical rotation was taken on a JASCO DIP-140 POLARIMETER. X-Ray crystallographic analysis was carried out by Mac Science MXC18 diffractometer. The structure was solved by direct method (Monte-Carlo Multan) and refined by full-matrix least squares refinement. Diffraction data were obtained by Mac Science MXC18 diffractometer at rt. All diagrams and calculations were performed using maXux (Bruker Nonius, Delft & Mac Science, Japan). Silica gel 60 for column chromatography was purchased from Merck. TLC was carried out on silica gel 60 F_{254} pre-coated (layer thickness 0.25 mm, Merck) with *n*-hexane–EtOAc (1 : 1). High-performance liquid chromatography (HPLC) was carried out by Shimadzu LC-6A Liquid Chromatograph.

Plant Material *N. chinensis* (甘松香) was collected in China in 2000. The imported plant was purchased from the Nakai Pharmacy in Kobe, Japan. The liverwort *P. fruticosa* was collected in May 1999 at Aioi, Tokushima, Japan and identified by Y. A. and confirmed by Dr. M. Mizutani. The voucher specimen (H9905001) has been deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Isolation of (+)-1(10)-Aristolene (1) and 1(10)-Aristolen-2-one (5) Dried powder (5.0 kg) of the crude drug *N. chinensis* was extracted with Et_2O (6 l) for 1 month. A part (181 g) of Et_2O extract (353 g) was chromatographed on silica gel (500 g) with a gradient solvent system of *n*-hexane–EtOAc increasing the amount of 5% portion EtOAc stepwise to give crude 1(10)-aristolene (**1**) (5.265 g) from Fr. 9. A mixture containing 1(10)-aristolene (**1**) was further rechromatographed on SiO_2 impregnated with 10% $AgNO_3$ to afford (+)-1(10)-aristolene (**1**) (2.787 g) as pure state. Oil (5.0455 g) from Fr. 49 was chromatographed on Sephadex LH-20 with $CHCl_3$–MeOH (1 : 1) and silica gel with *n*-hexane–EtOAc, and finally subjected to preparative HPLC (SiO_2: 10% EtOAc–*n*-hexane) to afford 1(10)-aristolen-2-one (**3**) (17.1 mg).

(+)-1(10)-Aristolene (**1**)[15]: Colorless oil; $[\alpha]_D$ +76.8° (c=1.3, MeOH); IR (KBr) cm^{-1}: 2927, 2871, 1455, 1376, 834; EI-MS; *m/z* 204 (M^+, 21%), 189 (22), 161 (100), 147 (14), 105 (24), 91 (16); HR-EI-MS; [M^+] Found 204.1880 $C_{15}H_{24}$ requires 204.1878; ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 1); ^{13}C-NMR (150 MHz) in $CDCl_3$ (Table 2).

1(10)-Aristolen-2-one (**3**)[16,17]: Colorless oil; $[\alpha]_D$ +133.5° (c=1.7, MeOH); IR (KBr) cm^{-1}: 2938, 1669 (C=O), 1617 (C=C), 1456, 1282; UV (MeOH) λ_{max} nm (log ε): 243.4 (3.99); EI-MS: *m/z* 218 (M^+, 45%), 203 (42), 176 (100), 175 (69), 161 (82), 105 (64), 91 (61), 69 (35), 41 (60); HR-EI-MS: [M^+] Found 218.1665 $C_{15}H_{22}O$ requires 218.1671; ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 1): ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 2).

Isolation of Plagiochilide (2) Dried powder (1.09 kg) of the liverwort *P. fruticosa* was extracted with Et_2O (3 l) for 1 month. The Et_2O extract (19.33 g) was chromatographed on silica gel (200 g) with *n*-hexane–EtOAc gradient to afford plagiochilide (**2**)[29] (1.133 g) from Fr. 18 and plagiochiline A (**3**)[29,30] (1.360 g) from Fr. 31. The crystal data for **2** are as follows: Orthorhombic, space group $P2_12_12_1$, a=6.4980(3) Å, b=9.6070(7) Å, c=21.257(2) Å, V=1327.0(2) Å^3, α=90.00°, β=90.00°, γ=90.00°, Z=4, Dx=1.163 Mg m^{-3}, μ(Mo$K\alpha$)=0.075 mm^{-1}, λ=0.71073. Final R was 0.0786 for 2338 reflections.

Microorganisms and Media Noro medium [$MgCl_2 \cdot 6H_2O$ (1.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.2 g), $CaCl_2 \cdot 2H_2O$ (0.2 g), KNO_3 (1.0 g), $NaHCO_3$ (0.43 g), TRIS (2.45 g), K_2HPO_4 (0.045 g), Fe-EDTA (3.64 mg), EDTA-2Na (1.89 mg), $ZnSO_4 \cdot 7H_2O$ (1.5 g), H_3BO_2 (0.61 mg), $CoCl_2 \cdot 6H_2O$ (0.015 mg), $CuSO_4 \cdot 5H_2O$ (0.06 mg), $MnCl_2 \cdot 4H_2O$ (0.23 mg), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.38 mg) in distilled water 1 l (pH 8.0)] was used for the biotransformation of (+)-1(10)-aristolene (**1**) by *C. fusca* var. *vacuocola*. Czapek-pepton medium [1.5% sucrose, 1.5% glucose, 0.5% polypepton, 0.1% K_2HPO_4, 0.05% KCl and 0.001% $FeSO_4 \cdot 7H_2O$ in distilled water (pH 7.0)] was prepared for the biotransformation of **1** and plagiochilide (**2**) by *A. niger* and *Mucor* species. *A. niger* was isolated in our laboratories from soil in Osaka prefecture and identified according to its physiological and morphological characteristics. A new fungus *Mucor* sp. was isolated from the soil adhering to the liverwort *P. subcilita* and was identified by NCIMB Japan CO., LTD.

Biotransformation of 1(10)-Aristolene (1) by *Chlorella fusca* var. *vacuocola* IAMC-28 An Erlenmeyer flask (100 ml) containing 50 ml Noro medium was inoculated with a suspension of *C. fusca* var. *vacuoloca* and incubated at 25 °C for 7 d in a rotary shaker operating at 100 rpm under light illumination (*ca.* 3000 lux). After full growth of *C. fusca* var. *vacuolata*, (+)-1(10)-aristolene (**1**) (19 mg×6; 114 mg) was added to the culture medium of the microorganism. Incubation was then continued at 25 °C for a further 22 d. After completion of incubation time, the culture was filtered *in vacuo* and broth was extracted with Et_2O (500 ml) twice. The Et_2O layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo* to give the crude extract (201.4 mg), which was chromatographed on silica gel (50 g) with a gradient solvent system of *n*-hexane–Et_2O increasing the amount of 5% portions Et_2O stepwise to afford 1(10)-aristolen-2-one (**5**)[16,17] (22.8 mg; 18.7%) from Fr. 38, (−)-aristolone (**7**)[20] (8.6 mg; 7.1%) from Fr. 50 and 9α-hydroxy-1(10)-aristolen-2-one (**6**)[18,19] (9.2 mg; 7.0%) from Fr. 101.

(−)-Aristolone (**7**): Colorless crystal, mp 99—101 °C, $[\alpha]_D$ −268.3° (c=1.20, $CHCl_3$); EI-MS: *m/z* 218 (M^+, 100%), 203 (84), 161 (36), 147 (46), 119 (51), 91 (52), 77 (30), 41 (49); HR-EI-MS: [M^+] 218.1672, $C_{15}H_{22}O$ requires 218.1671; IR (KBr) cm^{-1}: 2931, 1653 (C=O), 1460, 1360, 1070; UV (MeOH) λ_{max} nm (log ε): 235.6 (3.95); ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 1); ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 2).

9α-Hydroxy-1(10)-aristolen-2-one (**6**): Colorless oil, $[\alpha]_D$ +9.0° (c=1.08, $CHCl_3$); EI-MS: *m/z* 234 (M^+, 36%), 216 (100), 192 (96), 191 (76), 147 (81), 105 (89), 91 (77), 41 (60); HR-EI-MS: [M^+] 234.1610, $C_{15}H_{22}O_2$ requires 234.1620; IR (KBr) cm^{-1}: 3408 (OH), 2932, 1657 (C=O), 1284, 1190, 1048; UV (MeOH) λ_{max} nm (log ε): 233.0 (4.12); ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 1); ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 2).

Biotransformation of 1(10)-Aristolene (1) by *Mucor* sp. An Erlenmeyer flask (500 ml) containing 200 ml Czapek-pepton medium was inoculated with a suspension of *Mucor* sp. and incubated at 30 °C for 5 d in a rotary shaker operating at 100 rpm. After full growth of the microorganism, 1(10)-aristolene (**1**) (100 mg) was added to the culture medium of *Mucor* sp. Incubation was then continued at 30 °C for 7 d. The cultured medium was filtered *in vacuo* and the broth extracted with Et_2O (200 ml) under stirring for 1 h twice. The Et_2O layer was dried over $MgSO_4$ and evaporated *in vacuo* to give the crude extract (105 mg), which was chromatographed on silica gel with a gradient solvent system of $CHCl_3$–EtOAc to afford two metabolites **3** (0.8 mg; 0.7%) and **4** (0.6 mg; 0.6%) together with the starting material **1** (65 mg).

Biotransformation of 1(10)-Aristolene (1) by *Aspergillus niger* An Erlenmeyer flask (500 ml) containing 200 ml Czapek-pepton medium was inoculated with a suspension of *A. niger* and incubated at 30 °C for 3 d in a rotary shaker operating at 100 rpm. (+)-1(10)-Aristolene (**1**) (100 mg×10; 1.00 g) was added to the culture medium of *A. niger*. Incubation was then continued at 30 °C for a further 6 d. After completion of incubation time, the cultured medium was filtered *in vacuo* and broth was extracted with EtOAc (200 ml) under stirring for 1 h twice. The EtOAc layer was dried over $MgSO_4$ and evaporated *in vacuo* to give crude extract (791.5 mg), which was chromatographed on silica gel (50 g) with a gradient solvent system of *n*-hexane–EtOAc increasing the amount of 5% portions EtOAc stepwise to afford four new compounds **11** (16.4 mg; 1.4%), **12** (32.3 mg; 2.7%), **13** containing a small impurity (9.8 mg; 0.8%) and **14** (27.6 mg; 2.1%).

2-Oxo-1(10)-aristolen-13-oic Acid (**11**): Colorless crystals, mp 190—193 °C; $[\alpha]_D^{22}$ +147.5° (c=1.7, $CHCl_3$); EI-MS: *m/z* 248 (M^+, 26%), 233 (50), 202 (96), 174 (100), 159 (61), 105 (65), 91 (72), 77 (47), 41 (41); HR-EI-MS: *m/z* 248.1418 [M^+], $C_{15}H_{20}O_3$ requires 248.1412; IR (KBr) cm^{-1}: 3200—2800 (COOH), 1669 (C=O), 1621, 1467, 1419, 1282; UV (MeOH) λ_{max} nm (log ε): 242.5 (4.22); ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 1); ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 2). The crystal data for **11** are as follows: Orthorhombic, space group $P2_12_12_1$, a=6.6610(2) Å, b=13.8290(7) Å, c=29.1040(2) Å, V=2680.9(2) Å^3, α=90.00°, β=90.00°, γ=90.00°, Z=8, Dx=1.230 Mg m^{-3}, μ(Mo$K\alpha$)=0.084 mm^{-1}, λ=0.71073. Final R was 0.0497 for 4259 reflections.

3β-Hydroxy-1(2),9(10)-aristoladien-13-oic Acid (**12**): Colorless oil; $[\alpha]_D$ −3.13° (c=1.6, $CHCl_3$); EI-MS: *m/z* 248 (M^+, 70%), 230 (37), 176 (62), 149 (100), 131 (57), 91 (76), 77 (47), 43 (51); HR-EI-MS: *m/z* 248.1393 [M^+], $C_{15}H_{20}O_3$ requires 248.1412; IR (KBr) cm^{-1}: 3200—2800 (COOH), 1684 (C=O), 1462, 1418, 1014; UV (MeOH) λ_{max} nm (log ε): 240.0 (3.97); ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 1); ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 2).

Compound **14**: Colorless oil; $[\alpha]_D$ −61.4° (c=1.0, $CHCl_3$); CI-MS: *m/z* 265 [$(M+H)^+$, 80%], 247 (76), 201 (100), 173 (59), 132 (22), 119 (5), 105 (16), 58 (41), 56 (37); HR-CI-MS: *m/z* 265.1414 $[M+H]^+$, $C_{15}H_{21}O_4$ requires 265.1439; IR (KBr) cm^{-1}: 3419 (OH), 2970, 2941, 1747 (C=O); UV (MeOH) λ_{max} nm (log ε): 236.8 (4.16); ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 1); ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 2).

Preparation of *p*-Bromophenacyl Ester (15) To a solution of 3-oxo-1(2),9(10)-aristoladien-13-oic acid (**13**) (9.8 mg) in acetone (5 ml) was added *p*-bromophenacyl bromide (33 mg) and K_2CO_3 (54.6 mg) with stirring at rt for 4 h. The reaction mixture was filtered over Celite and evaporated to give a residue that was purified by silica gel column chromatography with *n*-hexane–EtOAc gradient to afford *p*-bromophenacyl derivative (**15**) (14.3 mg).

p-Bromophenacyl Derivative (**15**): Colorless oil; $[\alpha]_D$ −52.1° (*c*=1.0, $CHCl_3$); EI-MS: *m/z* 442 (M^+, 3%), 309 (6), 294 (4), 228 (100), 213 (49), 185 (53), 173 (43), 145 (25), 91 (23), 77 (14), 41 (8); HR-EI-MS: *m/z* 442.0775 [M^+], $C_{23}H_{23}O_4Br$ requires 442.0780; IR (KBr) cm^{-1}: 3451 (OH), 3102, 2973, 1733 (C=O), 1668 (C=O), 1632 (C=C), 1547, 1168; UV (MeOH) λ_{max} nm (log ε): 256.6 (4.34), 205.2 (4.27); ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 1); ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 2).

Biotransformation of Plagiochilide (2) by *Aspergillus niger* *A. niger* was cultivated rotatory (100 rpm) in Czapek-pepton medium at 30 °C for 2 d. Plagiochilide (**2**) (100.6 mg) was added to the medium and further cultivated for 2 d. The cultured medium was worked up in the same manner as described above to give EtOAc extract (75.2 mg), which was chromatographed on silica gel (*n*-hexane–EtOAc gradient) to afford two new metabolites 12-hydroxyplagiochilide (**18**) (44.0 mg; 40.9%) and plagiochilide-12-oic acid (**19**) (11.0 mg; 12.2%). In the same condition, plagiochilide (**2**) (50.0 mg) was biotransformed by *A. niger* for 7 d to afford the same metabolites **18** (3.1 mg; 5.8%) and **19** (42.0 mg; 74.4%).

12-Hydroxyplagiochilide (**18**): Colorless oil, $[\alpha]_D^{20}$ −50.1° (*c*=1.1, $CHCl_3$); EI-MS: *m/z* 248 (M^+, 50%), 217 (38), 189 (26), 147 (36), 120 (94), 111 (44), 107 (100), 91 (80); HR-EI-MS: *m/z* 248.1416 [M^+], $C_{15}H_{20}O_3$ requires 248.1413; IR (KBr) cm^{-1}: 3408 (OH), 2937, 1764 (C=O), 1178, 1112; ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 3): ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 4).

Plagiochilide-12-oic Acid (**19**): Colorless crystal; mp 205—206 °C; $[\alpha]_D$ +2.5° (*c*=1.0, MeOH); EI-MS: *m/z* 262 (M^+, 50%), 244 (12), 216 (17), 189 (30), 163 (39), 145 (42), 135 (39), 120 (100), 201, 175 (100 %), 173, 105, 91; HR-EI-MS: *m/z* 262.1191 [M^+], $C_{15}H_{18}O_4$ requires 262.1205; IR (KBr) cm^{-1}: 3200—2400 (OH), 1756 (C=O), 1675 (C=O), 1110; ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 3); ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 4).

Acetylation of Compound 18 A solution of **18** (10 mg) in pyridine (1 ml) was treated with Ac_2O (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 1 N HCl, 5% $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated to give a residue that was purified by silica gel column chromatography with *n*-hexane–EtOAc gradient to afford 12-acetoxyplagiochilide (**20**) (11 mg, 94.1%).

12-Acetoxyplagiochilide (**20**): Colorless oil; $[\alpha]_D^{20}$ −12.5° (*c*=1.0, $CHCl_3$); EI-MS: *m/z* 290 (M^+, 10%), 230 (20), 202 (20), 180 (24), 149 (26), 120 (67), 91 (41), 79 (28), 43 (100); HR-EI-MS: *m/z* 290.1521 [M^+], $C_{17}H_{22}O_4$ requires 290.1518; IR (KBr) cm^{-1}: 3076, 2932, 1766 (C=O), 1739 (C=O), 1638, 1229, 1113, 1020; ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 3); ^{13}C-NMR (150 Hz) in $CDCl_3$ (Table 4).

Methylation of Compound 19 To a solution of **19** (8.5 mg) in MeOH (3 ml) was added $(CH_3)_3$–$SiCHN_2$ (1.0 ml). The reaction mixture was stirred at 0—5 °C for 1 h. One drop of AcOH was added and the mixture evaporated to give a residue (10.5 mg) that was purified by silica gel column chromatography with *n*-hexane–EtOAc gradient to afford **21** (4.8 mg).

Plagiochilide-12-oic Acid Methyl Ester (**21**): Colorless oil; $[\alpha]_D$ +4.9° (*c*=0.40, $CHCl_3$); EI-MS: *m/z* 276 (M^+, 42), 245 (21), 216 (28), 189 (45), 145 (36), 120 (83), 107 (100), 91 (83), 80 (65), 41 (56); HR-EI-MS: *m/z* 276.1349 [M^+], $C_{16}H_{20}O_4$ requires 276.1362; IR (KBr) cm^{-1}: 2948, 2973, 1766 (C=O), 1719 (C=O), 1204, 1111, 1019; ^{1}H-NMR (600 MHz) in $CDCl_3$ (Table 3).

Biotransformation of Plagiochilide (2) with Cytochrome P_{450} Inhibitor In the same condition as described above, plagiochilide (**2**) (10.0 mg) was biotransformed by *A. niger* with 1-aminobenzotriazole (10.0 mg) for 7 d to give compound **18** (6.6 mg; 61.7%) together with the substrate **2** (0.8 mg; 8.0%).

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