Isolation and Structures of New Cyclomyltaylane and *ent*-Chamigrane-Type Sesquiterpenoids from the Liverwort *Reboulia hemishaerica* and Their Biotransformation by the Fungus *Aspergillus niger*

Mai FURUSAWA,[a] Toshihiro HASHIMOTO,[a] Yoshiaki NOMA,[b] and Yoshinori ASAKAWA*,[a]

[a] *Faculty of Pharmaceutical Sciences, Tokushima Bunri University; and* [b] *Faculty of Human Life Sciences, Tokushima Bunri University; Yamashiro-cho, Tokushima 770–8514, Japan.* Received January 25, 2006; accepted March 23, 2006

***Reboulia hemisphaerica*, the thalloid liverwort, contained four new cyclomyltaylane- and two new *ent*-β-chamigrane-type sesquiterpenoids of which the absolute stereostructures were established by a combination of two-dimensional NMR spectroscopy, X-ray crystallographic analysis, and the modified Mosher's method. Cyclomyltaylan-5α-ol and *ent*-β-chamigren-1α-ol were biotransformed by the fungus *Aspergillus niger* to afford new oxygenated matabolites. Their structures were also elucidated in the same manner as described above.**

Key words liverwort; *Reboulia hemisphaerica*; cyclomyltaylane; *ent*-β-chamigrane; biotrasformation; *Aspergillus niger*

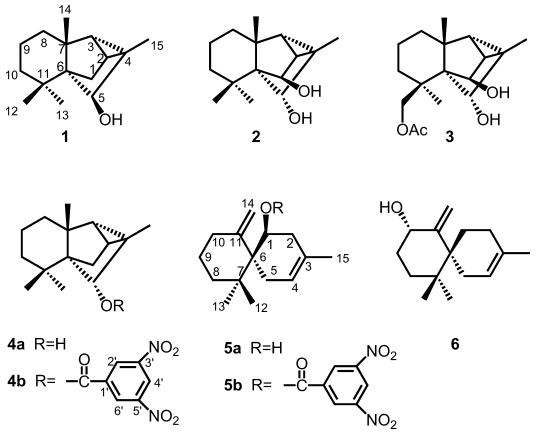
We are continuing to study the chemical constituents of Hapaticae which produce a large number of new lipophilic terpenoids and phenolics.[1—3] Various sesqui- and diterpenoids, bibenzyls, and bisbibenzyls isolated from several liverworts have a characteristic fragrant odor, intensely hot and bitter taste, and muscle-relaxing, antimicrobial, antifungal, allergenic contact dermatitis, antitumor, insect antifeedant, superoxide anion release-inhibitory, piscicidal, and neuritic-sprouting activity.[1—5] On the other hand, the biotransformation of terpenoids and aromatic compounds from crude drugs, liverworts, and animal origin has been carried out by microorganisms and mammals to obtain functional substances such as pheromones, aromatic agents, and insecticides.[6—8] Recently, we have succeeded in highly efficient production of nootkatone, the most important grapefruit aroma, using this technique.[9—11]

The liverwort *Reboulia hemisphaerica* has three chemotypes, the: 1) aristrane,[12] 2) cyclomyltaylane-bisbibenzyl,[13] and 3) gymnomitrane-cuparane types.[14] Further fractionation of the crude extract of *R. hemispaherica* resulted in the isolation of four new cyclomyltaylane- and two new *ent*-β-chamigrane-type sesquiterpenoids. In this paper, we report the isolation and structural elucidation of the new myltaylane- and *ent*-β-chamigrane-type sesquiterpenoids and their biotransformation using the fungus *Aspergillus niger* together with the possible biotransformation pathway of each metabolite.

Isolation and Structural Determination *R. hemisphaerica* collected on the campus of Tokushima Bunri University was extracted with ether, and the crude extract was chromatographed on silica gel using an *n*-hexane and ethyl acetate gradient to afford three new cyclomyltaylane- (**1**—**3**) and two new β-chamigrane-type sesquiterpenoids (**5**, **6**), along with the previously known cyclomyltaylane-5α-ol (**4a**)[13] and the bisbibenzyl marchantin C.[15] However, neither aristolene nor aristolone has been isolated from the present species. Thus the species belongs to the second chemotype (cyclomyltaylane-bisbibenzyl type) of *R. hemisphaerica*, which is closely related chemically to the Taiwanese species.[16]

The stereochemistry of cyclomyltaylane-5α-ol (**4a**) has been elucidated by a combination of spectral data without the absolute configuration.[13] The relative stereostructure was further established by X-ray crystallographic analysis of 3,5-dinitrobenozate (**4b**) prepared from **4a**, as shown in Fig. 1. The absolute configuration of **4a** has not been determined at this stage, although it was established by application of the modified Mosher's method[16] for one (**7a**) of the metabolites obtained from the biotransformation of compound **4a** (see below).

The molecular formula $C_{15}H_{24}O$ of compound **1** was established in high-resolution electron-impact mass spectroscopy (HR-EI-MS) ([M]$^+$ *m*/*z* 220.1804)). The IR and NMR spectra showed the presence of a secondary hydroxyl group (3454 cm^{-1}; δ_H 4.08, 1H, s; δ_C 78.9) and four tertiary



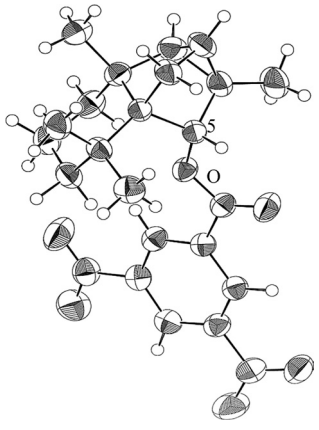


Fig. 1. ORTEP Drawing of Compound **4b**

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

Table 1. ^{1}H-NMR Spectral Data (600 MHz) of Compounds (**1**—**4a**) in $CDCl_3$

	1	**2**	**3**	**4a**
1α	1.15 (d, 10.7)[a)]	3.85 (s)	3.86 (s)	1.22 (d, 10.7)
1β	1.83 (d, 10.7)			1.36 (dd, 1.6, 10.7)
2	1.02 (dd, 1.1, 4.9)	1.23 (m)	1.23 (dd, 1.4, 5.2)	0.89 (dd, 1.6, 10.7)
3	0.96 (d, 10.7)	1.27 (d, 4.9)	1.27 (d, 5.2)	0.93 (d, 5.2)
5	4.08 (s)	3.48 (s)	3.59 (s)	3.64 (s)
8α	1.56 (ddd, 3.8, 3.8, 13.5)	2.12 (ddd, 4.1, 4.1, 12.6)	2.16 (ddd, 4.4, 4.4, 12.9)	1.99 (ddd, 4.4, 4.4, 12.4)
8β	1.32 (m)	1.33 (m)	1.32 (m)	1.16 (m)
9α	1.47 (m)	1.50 (m)	1.50 (m)	1.47 (m)
9β	1.65 (m)	1.67 (m)	1.60 (m)	1.60 (m)
10α	1.41 (m)	1.98 (ddd, 4.7, 4.7, 13.7)	1.96 (ddd, 4.9, 4.9, 13.9)	1.87 (ddd, 4.7, 4.7, 13.7)
10β	1.12 (m)	1.11 (m)	1.48 (m)	1.16 (m)
12	1.20 (s)	1.20 (s)	4.36, 4.41 (d, 11.3)	0.89 (s)
13	0.93 (s)	1.07 (s)	1.06 (s)	0.98 (s)
14	1.43 (s)	1.47 (s)	1.46 (s)	1.01 (s)
15	1.10 (s)	1.15 (m)	1.16 (s)	1.13 (s)
$C\underline{H}_3COO$			2.09 (s)	

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

Table 2. ^{13}C-NMR Spectral Data (150 MHz) of Compounds (**1**—**4a**) in $CDCl_3$

	1	**2**	**3**	**4a**
1	39.7	74.9	73.5	28.0
2	25.6	25.8	24.9	17.8
3	34.6	36.6	36.2	34.4
4	18.2	24.2	24.0	23.3
5	78.9	83.1	82.5	86.0
6	53.7	56.0	55.4	51.9
7	45.2	45.9	45.7	45.3
8	34.1	34.2	33.5	32.6
9	19.4	19.1	18.8	19.2
10	39.2	38.7	32.0	37.2
11	32.9	33.0	37.1	32.1
12	24.6	26.4	68.9	25.7
13	29.5	29.2	22.3	29.3
14	23.0	24.4	23.6	23.2
15	16.6	12.4	12.3	12.7
$C\underline{H}_3COO$			21.0	
$CH_3\underline{C}OO$			171.2	

methyl groups. The ^{1}H- and ^{13}C-NMR spectra were similar to those of cyclomylytaylane-5α-ol (**4a**), as shown in Tables 1 and 2, except for the difference in the chemical shift at C-1, C-2, C-4, and C-5, indicating that **1** might be a stereoisomer of **4a** at C-5. The above assumption was confirmed by the NOESY correlations for H-5α/H-10α and H-5α/H-13. Thus the structure of **1** was established to be cyclomyltaylane-5β-ol.

Compound **2**, $C_{15}H_{24}O_2$ (HR-EI-MS $[M]^+$ *m/z* 235.1780), was obtained as colorless crystals, mp 154—155 °C. The NMR data were closely related to those of compound **4a**, as shown in Tables 1 and 2, except for the presence of an additional secondary hydroxyl group (δ_H 3.85, 1H, s; δ_C 74.9) in place of the absence of one methylene group, showing that compound **2** had the same cyclomyltaylane-5α-ol skeleton. The presence of the newly introduced secondary hydroxyl group at C-1 and its stereochemistry were determined by correlations for i) H-1α/C-5 and C-7; ii) H-5α/C-1, C-4, and C-7; and iii) H-15/C-5, in HMBC and NOE spectra of H-1α/H-13, H-5β/H-13, and H-5β/H-15 in NOESY, respectively. Furthermore, the structure was determined to be cyclomyltaylane-1β,5α-diol in the X-ray crystallographic analysis of **2**, as shown in Fig. 2.

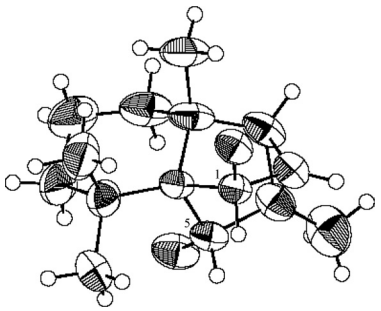


Fig. 2. ORTEP Drawing of Compound **2**

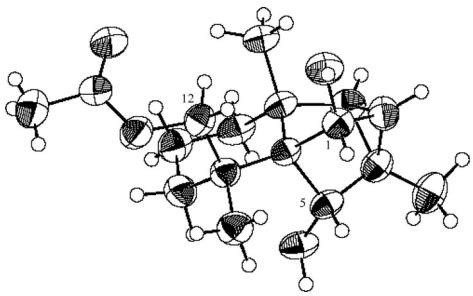


Fig. 3. ORTEP Drawing of Compound **3**

The IR and NMR spectra of compound **3**, $C_{17}H_{26}O_4$ (HR-EI-MS $[M]^+$ *m/z* 294.183), showed that it contained an acetoxyl (1699 cm^{-1}; δ_H 2.09, 3H, s, δ_C 171.2, 21.0) and two secondary hydroxyl groups (3422 cm^{-1}; δ_H 3.50, 3.86 each 1H, s, δ_C 82.5, 73.5), acetoxymethyl (δ_H 4.36, 4.41, each 1H, d, J=11.3 Hz; δ_C 68.9) and three tertiary methyl group. The NMR spectral data of **3** are similar to those of compound **2**, as shown in Tables 1 and 2, except for the presence of an acetoxymethyl, suggesting that one of the four tertary methyls in **2** was acetoxylated. The HMBC and NOESY spectra showed the same correlations as seen in compound **2**, except for the presence of new correlations for H-10/C-12 and H-13/C-12 in HMBC and NOEs between H-12/H14 in NOESY, suggesting that the acetoxyl group was located at C-12. This prediction was confirmed by X-ray crystallographic analysis of **3**, as indicated in Fig. 3. Consequently the struc-

Table 3. ^{1}H- (600 MHz) and ^{13}C-NMR Spectra Data (150 MHz) of Compounds (**5a**, **6**)

	5a		**6**	
	H	C	H	C
1α	4.08 (dd, 9.6, 9.6)[a]	72.6	1.54 (m)	27.1
1β			2.23 (m)	
2α	1.96 (m)	39.2	1.78 (m)	29.1
2β			1.85 (m)	
3		131.3		133.6
4	5.25 (m)	120.4	5.33 (m)	120.1
5α	2.03 (m)	32.1	1.90 (m)	30.0
5β			2.14 (m)	
6		49.4		44.2
7		37.8		37.2
8α	1.21 (m)	39.8	2.02 (m)	32.1
8β	2.28 (ddd, 4.4, 4.4, 13.5)		1.13 (m)	
9α	1.54 (m)	23.7	1.66 (m)	29.8
9β	1.62 (m)		1.81 (m)	
10α	2.20 (m)	35.7	4.35 (t, 3.6)	74.8
10β	2.56 (ddd, 5.2, 5.2, 13.2)			
11		148.6		150.2
12	1.06 (s)	24.9	0.90 (s)	24.7
13	0.82 (s)	25.0	0.79 (s)	23.0
14	4.63 (s), 4.93 (t, 1.6)	111.0	4.89 (d, 1.6), 5.14 (d, 1.9)	114.9
15	1.59 (s)	22.5	1.59 (s)	23.2

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

ture of **3** was established to be 12-acetoxy-myltaylane-1α,5β-diol.

The molecular formula $C_{15}H_{24}O$ ([M]$^+$ *m/z* 220.1827) of compound **5a** was determined by HR-EI-MS. The IR, ^{1}H- and ^{13}C-NMR of **5a** indicated the presence of a secondary hydroxyl group (3479 cm^{-1}; δ_H 4.08, 1H, dd, *J*=9.6 Hz, δ_C 72.6), an exomethylene, a trisubstituted double bond, two tertiary methyl, and one vinyl methyl groups as well as five methylenes and two *sp*^{3} quaternary carbon atoms (Table 3). On the basis of above spectroscopic evidence, compound **5a** was a bicyclic compound. These spectral data resembled those of β-chamigrene,[17] indicating that **5a** is a β-chamigrene alcohol. The location and relative stereochemistry of **5a** were further confirmed by HMBC and NOESY spectra in which correlations for i) H-1/C-2, C-5, C-6, and C-11, ii) H-14/C-6, C-10, and C-11; and iii) H-15/C-2, C-3, and C-4 in HMBC and NOEs between H-1α/H-13, and H-9β/H12 in NOESY spectra were observed, respectively. Finally, the stereochemistry of **5a** was established to be β-chamigrene-1β-ol in X-ray crystallographic analysis of 3,5-dinitrobenzoate (**5b**) prepared from **5a**, as shown in Fig. 4. The absolute configuration of **5a** was determined using the modified Mosher's method[16] on the metabolite (**12a**) prepared from **5a** (see below).

The IR and NMR data of compound **6**, $C_{15}H_{24}O$ (HR-EI-MS [M]$^+$ *m/z* 220.1823), showed the presence of an allylic secondary hydroxyl group (3392 cm^{-1}; δ_H 4.35, 1H, t, *J*=3.6 Hz; δ_C 74.8). The NMR spectral data of **6** resembled those of compound **5a**, except for the existence of the lower chemical shift at C-10, showing that compound **6** was β-chamigrene-10-ol. This assumption and stereochemisty at C-10 was further confirmed by the coupling constant of H-10 (δ_H 4.35, t, *J*=3.6 Hz) and the correlations for i) H-14/C-10 and C-11 and ii) H-10/C-6 in HMBC and NOE spectra between i) H-10β/H-9α,β and ii) H-10b/H-14 in the NOESY spectrum. Thus the structure of **6** was deremined to be β-chamigrene-10α-ol.

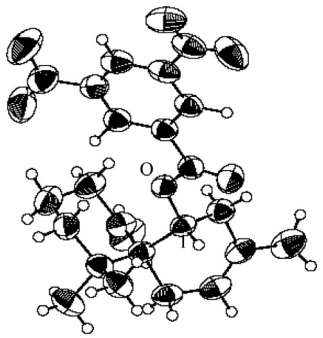


Fig. 4. ORTEP Drawing of Compound **5b**

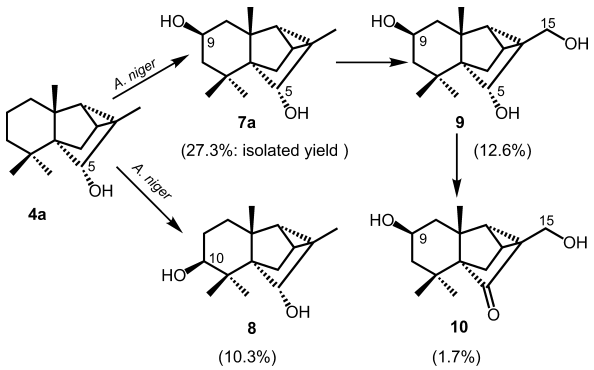


Fig. 5. Biotransformation of Cyclomyltaylane-5α-ol (**4a**) by *Aspergillus niger*

Biotransformation of Cyclpomyltaylane-5α-ol (4a) by *Aspergillus niger* We carried out the biotransformation of the major compound **4a** with a monomethylcyclopropane ring to compare the reactivity with aristolene, which also has a 1,1-dimethylcylcopropane ring in the molecule. One of the most common fungi, *A. niger* was inoculated in Czapek pep-

Table 4. ^{1}H-NMR Spectral Data (600 MHz) of Compounds (**7a**—**10**) in $CDCl_3$

	7a	**8**	**9**	**10**
1α	1.29 (d, 10.7)[a)]	1.24 (d, 10.7)	1.32 (d, 10.7)	1.98 (m)
1β	1.48 (dd, 1.6, 11.0)	1.42 (dd, 1.4, 10.7)	1.54 (dd, 1.4, 10.9)	
2	0.93 (d, 5.5)	0.94 (d, 5.2)	1.15 (d, 5.5)	2.08 (m)
3	1.00 (br d, 3.3)	0.91 (d, 5.8)	1.18 (br d, 5.5)	1.96 (d, 5.2)
5	3.65 (s)	3.67 (s)	3.93 (s)	
8α	2.35 (dd, 4.4, 14.8)	1.36 (m)	2.44 (dd, 4.7, 14.3)	1.35 (dd, 3.6, 14.8)
8β	1.61 (br d, 14.3)	2.20 (ddd, 4.4, 4.4, 13.5)	1.60 (br d, 14.3)	1.92 (m)
9α	4.36 (m)	1.60 (m)	4.24 (m)	4.34 (m)
9β		1.68 (m)		
10α	2.13 (dd, 4.4, 14.8)	4.24 (dd, 5.2, 11.8)	2.12 (dd, 4.4, 14.3)	2.10 (dd, 0.5, 4.7)
10β	1.45 (m)		1.47 (m)	1.48 (m)
12	0.99 (s)	0.86 (s)	1.11 (s)	1.11 (s)
13	1.30 (s)	1.08 (s)	1.01 (s)	1.07 (s)
14	1.25 (s)	1.04 (s)	1.28 (s)	1.39 (br s)
15	1.12 (s)	1.13 (s)	3.69, 3.74 (d, 11.8)	3.66, 3.77 (d, 12.4)

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

tone medium and cultivated in a rotary (100 rpm) at 30 °C for 3 d. Compound **4a** (300 mg) was added and further cultured under the same conditions described above for 5 d. The culture broth was extracted with ethyl acetate, and the crude extract was further chromatographed on silica gel (*n*-hexane/ethyl acetate gradient) to afford four new metabolites (**7a**—**9**), as shown in Fig. 5.

The IR and ^{13}C-NMR spectra of the major metabolite (**7a**, 27.3% in isolated yield), $C_{15}H_{24}O_2$ (HR-EI-MS $[M]^+$ *m/z* 236.1775), showed the presence of two secondary hydroxyl groups (δ_H, 4.36, 1H, m, 3.65, s, 1H; δ_C 65.9, 85.5), suggesting that one hydroxyl group was introduced to the substrate (**4a**). The position of the newly introduced hydroxyl group was determined to be at C-9, based on the NMR spectral data (see Tables 4, 5) and HMBC correlations for i) H-8/C-9 and ii) H-10/C-9) and NOE for i) H-9α/H-8α,β and ii) H-9α/H-10α,β. To determine the absolute configuration of this hydroxyl group, **7a** was converted to *R*-(+)- and *S*-(−)-α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA) esters **7b** and **7c**, respectively. The $\Delta\delta$ values between **7b** and **7c** are shown in Fig. 6. The values were subjected to the modified Mosher's method[16)] and the results indicated that C-9 had the *R* configuration. Thus the structure of **7a** was confirmed to be cyclomyltaylane-5α,9β-diol.

The second metabolite (**8**) had the same molecular formula, $C_{15}H_{24}O_2$ (HR-EI-MS $[M]^+$ *m/z* 236.1778) as that of **7a**, indicating that **8** was the isomer of **7a** with an additional secondary hydroxyl group. This assumption and the stereochemistry of the questionable hydroxyl group at C-10β were confirmed by a combination of the ^{1}H- and ^{13}C-NMR data (Tables 4, 5) as well as the coupling constant of H-10α (δ_H, 4.24, dd, *J*=11.8, 5.2 Hz). The relative stereostructure of **8** was confirmed by HMBC correlations for i) H-10α/C-11, C-12, and C-13; ii) H-8/C-10; iii) H-9/C-10; iv) H-12/C-10; and v) H-13/C-10 and NOESY correlations for i) H-10α/H8α and H-9α/H-13. Based on the above spectroscopic evidence, the structure of **8** was determined to be cyclomyltaylane-5α,10β-diol.

The third metabolite (**9**), $C_{15}H_{24}O_3$ (HR-EI-MS $[M]^+$ *m/z* 251.1740), had an additional secondary hydroxyl (δ_H 4.24 m, 1H, δ_C 68.9) and a primary hydroxyl group (δ_H 3.64, 3.74; each 1H, d, *J*=11.8 Hz; δ_C 61.2), which were confirmed by the ^{1}H- and ^{13}C-NMR spectra. The NMR spectral data of **9** were closely related to those of compound **7a**, as shown in Tables 4 and 5, except for the presence of a primary alcohol, indicating that **9** was the C-12, 13, 14, or 15 hydroxylated product of **7a**. The location of the primary hydroxyl group at C-15 was established by HMBC correlations for i) H-15/C-3, C-4, and C-5 and ii) H-9 and H-10/C-9 and by the same NOE correlations as observed in compound **7a**. The final structure was determined to be cyclomyltaylane-5α,9β,15-triol using the X-ray crystallographic technique, as shown in Fig. 7.

Table 5. ^{13}C-NMR Spectral Data (150 MHz) of Compounds (**7a**—**10**)

	7a	**8**	**9**	**10**
1	27.8	28.1	27.8	28.3
2	18.8	19.0	15.5	24.0
3	35.3	34.1	33.2	38.3
4	23.1	23.2	29.2	33.8
5	85.5	85.3	82.7	215.9
6	52.1	54.3	52.0	56.5
7	44.5	45.4	43.9	43.7
8	39.1	30.9	38.0	39.1
9	69.5	28.3	68.9	68.6
10	43.9	75.1	43.1	42.0
11	31.3	37.1	31.0	30.7
12	29.3	17.8	27.6	27.4
13	28.1	24.6	28.7	27.4
14	25.7	23.1	25.0	23.4
15	12.5	12.6	61.2	57.1

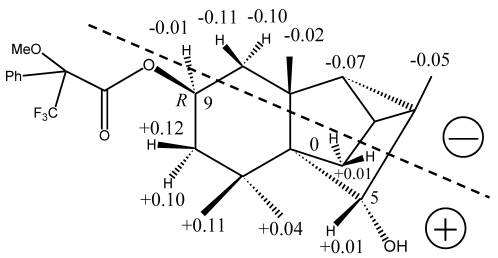


Fig. 6. Modified Mosher's Method of MTPA Esters of **7a**

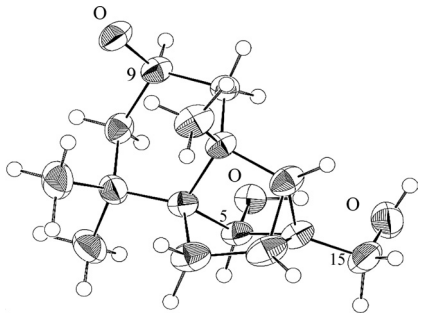


Fig. 7. ORTEP Drawing of Compound **9**

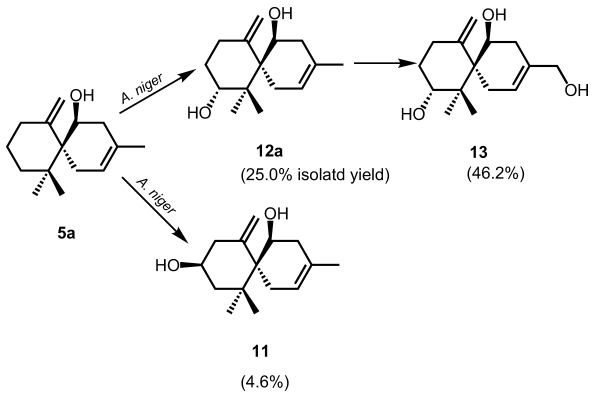
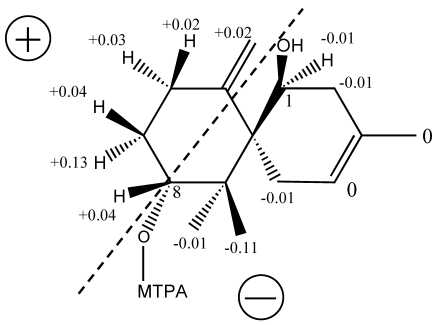


Fig. 8. Biotransformation of *ent*-β-Chamigrene-1α-ol (**5a**) by *Aspergillus niger*



in 600MHz ^{1}H-NMR $\Delta\delta$ values [δ(-)-MTPA-δ(+)MTPA]

Fig. 9. Modified Mosher's Method of MTPA Esters of **12c**

The IR and NMR spectra of the fourth metabolite (**10**), $C_{15}H_{22}O_3$ (HR-EI-MS $[M]^+$ *m/z* 250.1572), indicated the presence one secondary hydroxyl (δ_H 4.33, 1H, m, δ_C 68.6), a primary hydroxyl (δ_H 3.66, 3.77, each 1H, d, J=12.4 Hz; δ_C 57.1), and a saturated ketone (1732 cm^{-1}; δ_C 215.9) group. The similarity of the NMR spectroscopic data (Tables 4, 5) of **10** to those of the third metabolite (**9**) indicated that one of two hydroxyl groups in the molecule of **9** was oxidized. This is supported by the HMBC spectra, in which correlations for H-15/C3, C-4, and C-5 were observed. The stereochemistry of C-9 was also established by the presence of the same NOEs as seen in compound **7a**. Based on the above spectroscopic evidence, the structure of the final metabolite was established to be 5-oxomyltaylane-9β,15-diol.

Cyclomyltaylane-5α-ol (**4a**) was converted by *A. niger* to 9- and 10-hydroxy products (**7a**, **8**), and then former diol was further oxidized to give triol (**9**), followed by oxidation at C-10 to afford **10**, as shown in Fig. 5. *A. niger* easily introduced an oxygen function to the cyclohexane ring of the cyclomyltaylane skeleton since these positions are not hindered. Such oxygenation has been found in eudesmane-type sesquiterpene lactone by the same fungus.[7] However, the cyclopentane group was not converted by this fungus although the liverwort *R. hemisphaerica* elaborates two 1β-hydroxycyclomyltaylanes (**2**, **3**). *A. niger* also oxidized the cyclopropylmethyl group at C-15 to afford two metabolites (**9**, **10**), while liverwort biosynthesizes C-1 oxygenated cyclomyltaylane (**3**). *A. niger* biotransformed one of methyl groups of the 1,1-diimethylcyclopropane ring in the molecule of aristolone to give a carboxylic acid.[18,19] However, neither a C-15 aldehyde nor a C-15 carboxylic product from **9** or **10** has been found in the metabolites of **4a**.

Biotransformation of β-Chamigrene-1β-ol (5a) by *Aspergillus niger* As far we are aware, the biotransformation of spirostructural terpenoids has not been carried out, thus we carried out the biotransformation of β-chamigrene-1β-ol (**5a**) by *A. niger*. *A. niger* was inoculated in Czapek peptone medium and cultivated in a rotary (100 rpm) at 30 °C for 3 d. β-Chamigrene-1β-ol (**5a**) (192.3 mg) was added and treated in the same manner as described above. After the culture broth was extracted with ethyl acetate, the crude was purified on silica gel chromatography to give three new metabolites (**11**, **12a**, **13**), of which **12a** was the major product (46.2% in isolated yield) (Fig. 8).

Their structures were characterized by comparison of the NMR spectroscopic data (Table 5) to those of the substrate (**5a**). From the molecular formula, $C_{15}H_{24}O_2$ ($[M]^+$ *m/z* 236.1771) obtained from HR-EI-MS and NMR spectra of **11**, it was clear that one more secondary hydroxyl group (δ_H 4.09; 1H, m; δ_C 69.0) was introduced to the substrate. The location of this alcohol was established to be C-9β by the careful analysis of the HMBC spectrum in which correlations between i) H-9β/C-11, ii) H-8/C-9, and iii) H-10/C-9 were observed. The relative stereochemisry of the β-hydroxyl group at C-9 was also determined based on NOESY spectrum in which NOEs were observed between i) H-9β/H-8α,β and ii) H-9β/H-10α,β. Based on the above spectral evidence, the structure of **11** was established to be β-chamigren-1β,9α-diol.

The NMR data of the second metabolite (**12a**), which had the same molecular formula, $C_{15}H_{24}O_2$ (HR-EI-MS $[M]^+$ *m/z* 236.1779), as that of **11**, had an additional hydroxyl group (δ_H 4.61 1H, dd, J=12.1, 4.9 Hz; δ_C 74.3) in the molecule. The position and stereochemistry of the questionable hydroxyl group was determined to be C-8 based on the presence of correlations between i) H-8β/C-12 and C-13, ii) H-10/C-8, and iii) H-13/C-8 in HMBC and NOEs between i) H-8β/H-13 and ii) H-8β/H-10β in NOESY. From the above spectroscopic evidence, the structure of **12a** was characterized to be β-chamigren-1β,8α-diol. The absolute configuration of **12a** was established by application of the modified Mosher's method[16] as for compound **7a**, as shown in Fig. 9. Thus the β-chamigrene series discussed in the present paper was established as enantiomers of those found in higher plants.[17,20] Many liverworts produce enantiomeric sesqui- and diterpenoids to those found in higher plants, but this is not a general phenomenon, since germacrane, eudesmane, guaiane, drimanes *etc.* have the same absolute configurations

Table 6. ^{1}H- (600 MHz) and ^{13}C-NMR Spectral Data (150 MHz) of Compounds (**11**—**13**)

	11		**12a**		**13**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	4.10 (dd, 6.0, 6.0)[a]	72.3	4.04 (dd, 5.5, 10.2)	71.8	3.98 (dd, 4.4, 10.7)	72.4
2α 2β	1.98 (m)	39.0	1.93 (m)	39.0	1.87 (m) 2.11 (m)	35.3
3		131.5		131.2		126.7
4	5.27 (m)	120.0	5.27 (m)	120.3	5.53 (m)	122.5
5α 5β	2.07 (m)	31.5	2.02 (m) 2.09 (m)	32.3	2.07 (br d, 3.8)	33.4
6		49.1		50.2		51.9
7		37.5		43.0		44.2
8α 8β	1.50 (dt, 2.4, 14.3) 2.39 (br d, 12.9)	46.2	4.53 (dd, 4.7, 11.8)	73.9	4.61 (dd, 4.9, 12.1)	74.3
9α 9β	4.09 (m)	69.0	1.45 (m) 1.81 (m)	31.6	1.45 (m) 1.75 (m)	32.8
10α 10β	2.22 (dt, 2.7, 13.5) 3.13 (br d, 12.9)	42.4	2.21 (m) 2.66 (m)	33.6	2.17 (m) 2.78 (ddd, 4.9, 4.9, 13.5)	34.6
11		143.6		146.6		148.8
12	1.05 (s)	25.0	1.13 (s)	19.8	1.13 (s)	20.3
13	0.98 (s)	28.2	0.73 (s)	15.2	0.72 (s)	15.9
14	4.82 (s), 4.97 (t,1.6)	114.3	4.66 (t, 1.4), 4.95 (t, 1.9)	111.9	4.62 (s), 4.91 (t, 1.9)	111.9
15	1.60 (s)	22.5	1.59 (s)	22.4	3.81, 3.86 (d, 12.9)	66.2

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

as those isolated from higher plants. More interestingly, several liverworts biosynthesize both enantiomeric sesquiterpenoids, and the different species of the same genera produce the same compound with different absolute configurations.[1—3] In the case of chamigrane-type sesquiterpenoids isolated from liverworts, only enantiomers to those found in higher plants have been reported previously.[1—3]

The NMR spectra of the major metabolite (**13**), $C_{15}H_{23}O_3$ (HR-CI-MS *m/z* 251.1663) resembled those of **11** and showed the presence of a hydroxymethyl group (δ_H 3.81, 3.86, each 1H, J=12.9 Hz; δ_C 66.2) replacing a vinyl methyl. These data indicate that **13** is β-chamigren-1β,8α,15-triol. This was further confirmed by HMBC and NOESY spectra, in which correlations between i) H-8/C-7, C-10, C-12, and C-13 were observed in HMBC and the same NOEs as seen in **11** in NOESY.

An exomethylene group on a seven-membered sesquiterpene lactone or isoprepenyl group was easily converted to epoxide or diol by *A. niger*,[7,11] while this organism converts neither the exomethylene group nor the cyclohexane ring with the vinyl methyl group of compound **5a** but hydroxylated another cyclohexane ring to give 8α- and 9α-alcohol. The hydroxylation of the vinyl methyl group has been known to be very common in cases of microbial and mammalian biotransformation.[6,21]

Experimental

General Experimental Procedures Melting points are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian unity 600 (^{1}H; 600 MHz, ^{13}C; 150 MHz) spectrometer. The solvent used for NMR spectra was $CDCl_3$. IR spectra were recorded on a Jasco FT-IR spectrophotometer. UV spectra were run on a Hitachi U-300. MS spectra including high-resolution mass spectra were recorded on a Jeol AX-500 spectrometer at 70 eV. The optical rotation was measured on a Jasco DIP 140 polarimeter. X-ray reflection data were collected with a Mac Science MXC18 diffractometer using Mo$K\alpha$ radiation (λ=0.7103 Å). Preparative HPLC was carried out on a Shimadzu LC-6A liquid chromatograph. TLC was carried out on silica gel 60 F_{254} plates (Merck) and visualized by spraying with Godin reagent, followed by heating at 120 °C. Column chromatography was performed on silica gel 60 (0.2—0.5 mm, Merck) and Sephadex LH-20 (Pharmacia).

Plant Material The liverwort *R. hemisphaerica* (Aytoniaceae, Marchantiales) with sporophytes was collected on the campus of Tokushima Bunri University, Yamashiro-cho, Tokushima, Japan, in March 2003 by Y.A. and identified by Y.A. A boucher specimen (#033017) was deposited in the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and Isolation After purification, the plant material was dried for 1 week at room temperature. The dried materials (558.9 g) were mechanically ground and extracted with ether for 1 month. The crude extract (20.3 g), after filtration and evaporation of the solvent *in vacuo*, was chromatographed on silica gel using an *n*-hexane–ethyl acetate gradient to give seven fractions. The first fraction was rechromatographed on Sephadex LH-20 ($CHCl_3$/MeOH 1 : 1) and then on silica gel (*n*-hexane/ether 1 : 1) to give cyclomyltaylane-5α-ol (**4a**) (1.243 g). Fractions 2, 3, and 5 were treated in the same manner to afford β-chamigrene-1β-ol (=*ent*-β-chamigrene-1α-ol) (**5a**) (289 mg), cyclomyltaylane-5β-ol (**1**) (28 mg), and *ent*-β-chamigrene-10β-ol (**6**) (23 mg), respectively. Fractions 4 and 7 were rechromatographed on Sephadex LH-20 using the same solvent system described above to furnish cyclomyltaylane-1β,5α-diol (**2**) (18 mg) and marchantin C (718 mg),[15] respectively. Fraction 6 was further chromatographed on Sepahdex LH-20 and then silica gel (*n*-hexane/ether 1 : 1) to give colorless crystals and was recrystallized from *n*-hexane and ether to give 12-acetoxylcyclomyltaylane-1β,5α-diol (**3**) (67 mg).

Compound **1**: $[\alpha]_D$ −12.3° (c=0.4); IR (KBr) cm^{-1}: 3454 (OH), 2926, 1461; EI-MS: *m/z* (%) 220 (M^+, 40), 205 (39), 161 (47), 135 (59), 119 (80), 91 (77), 69 (89), 43 (100). HR-EI-MS: *m/z* 220.1804 (M^+), $C_{15}H_{24}O$ requires 220.1827. ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2.

Compound **2**: $[\alpha]_D$ +29.7° (c=0.8); IR (KBr) cm^{-1}: 3479 (OH), 3400 (OH); EI-MS: *m/z* (%) 236 (M^+, 49), 218 (39), 203 (64), 175 (82), 147 (35), 119 (100), 105 (39); HR-EI-MS: *m/z* 236.1780 (M^+), $C_{15}H_{24}O_2$ requires 236.1776. ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2. X-ray data: Orthorhombic, space group $P2_12_12_1$, a=13.5800(4) Å, b=14.01(5) Å, c=14.4340(5) Å, V=2746.15(15) Å^3, α=90.00°, β=90.00°, γ=90.00°, R=0.0584.

Compound **3**: $[\alpha]_D$ +29.7° (c=0.8); IR (KBr) cm^{-1}: 3422 (OH), 2933, 1699 (C=O), 1259 (OAc); EI-MS: *m/z* (%): 294 (M^+, 3), 276 (48), 234 (8), 216 (29), 203 (100), 175 (53), 145 (38), 119 (39), 105 (24), 91 (22), 43 (51); HR-EI-MS: *m/z* 296.1834 (M^+), $C_{17}H_{26}O_4$ requires 294.1831. ^{1}H- and ^{13}C-NMR spectral data: see Tables 1 and 2. X-ray data: Triclinic, space group $P1$, a=7.2530(4) Å, b=6.9490(5) Å, c=8.7310(7) Å, V=400.41(5) Å^3, α=82.680(2)°, β=70.628(3)°, γ=74.908(3)°, R=0.0356.

Compound **4a**: $[\alpha]_D$ +33.7° (c=1.0); IR (KBr) cm^{-1}: 3623, 3486, 1460, 1218; EI-MS: *m/z* (%) 220 (M^+, 80), 205 (94), 187 (70), 162 (77), 147 (100), 121 (15), 91 (82), 81 (53), 41 (64); HR-EI-MS: *m/z* 220.1827 (M^+),

$C_{15}H_{24}O$ requires 220.1827. ^{1}H- and ^{13}C-NMR spectral data (see Tables 1, 2) were identical to those reported in the literature.[13)]

Compound **5a**: $[\alpha]_D$ +23.7° (c=1.0); IR (KBr) cm^{-1}: 3479 (OH), 2965, 2911, 2864, 1630 (C=C), 1044, 1029, 892; EI-MS: *m/z* (%) 220 (M^+, 39), 205 (87), 187 (70), 176 (86), 161 (72), 109 (94), 105 (100), 81 (73), 41 (79); HR-EI-MS: *m/z* 220.1835 (M^+), $C_{15}H_{24}O$ 220.1827; ^{1}H- and ^{13}C-NMR spectral data: see Table 3.

Compound **6**: $[\alpha]_D$ −64.4° (c=1.0); IR (KBr) cm^{-1}: 3392 (OH), 2928, 1632 (C=C), 1445; EI-MS: *m/z* (%) 220 (M^+, 49), 205 (39), 187 (54), 164 (64), 131 (72), 93 (95), 91 (100), 79 (83), 41 (65); HR-EI-MS: *m/z* 220.1823 (M^+), $C_{15}H_{24}O$ requires 220.1827: ^{1}H- and ^{13}C-NMR spectral data: see Table 3.

Marchantin C: IR (KBr) cm^{-1}: 3521 (OH), 2923, 1601, 1505, 1467, 1224, 758; EI-MS: *m/z* (%): 424 (M^+, 100); HR-EI-MS: *m/z* 424.1666 (M^+), $C_{28}H_{24}O_4$ requires 4424.1675. These spectral data and ^{1}H- and ^{13}C-NMR spectra were identical to those of marchantin C.[15)]

Benzoylation of 4a To compound **4a** (15.5 mg) in pyridine (3 ml) was added 3,5-dinitrobenzoylchloride (165 mg) and dimethylaminopyridine (DMAP) (10 mg) and stirred for 4 d. Work-up as usual gave a reaction mixture that was chromatographed on silica gel (*n*-hexane–ether gradient) to give cyclomyltaylan-5α-yl 3,5-dinitrobenzoate (**4b**) (17.1 mg, 66%): mp 162—164 °C; $[\alpha]_D$ +116.1° (c=1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3105, 2948, 1729, 1548, 1344, 1273, 1167; UV λ_{max} nm (log ε): 234.6 (4.40); ^{1}H-NMR ($CDCl_3$): δ 1.56 (1H, d, J=11.0 Hz, H-1α), 1.61 (1H, ddd, J=11.0, 1.4 Hz, H-1β), 1.12 (d, J=3.6 Hz, H-2α,β), 1.10 (1H, d, J=5.6 Hz, H-3), 5.29 (1H, s, H-5β), 2.27 (1H, m, H-8α), 1.63 (1H, m, H-8β), 1.75 (1H, m, H-9α,β), 1.48 (1H, m, H-10α), 1.21 (1H, m, H-10β), 0.86 (3H, s, H-12), 1.13 (3H, s, H-14), 1.07 (3H, s, H-15), 9.19, 9.20 (each 1H, H-2′, 6′), 9.25 (1H, t, J=2.2 Hz, H-4′); ^{13}C-NMR ($CDCl_3$): δ 28.5 (C-1), 18.3 (C-2), 34.4 (C-3), 22.8 (C-4), 88.9 (C-5), 52.9 (C-6), 45.7 (C-7), 31.8 (C-8), 18.7 (C-9), 37.2 (C-10), 31.7 (C-11), 29.4 (C-12), 25.5 (C-13), 22.7 (C-14), 13.0, C-15), 162.1 (C=O), 134.2 (C-1′), 129.4 (C-2′, 6′), 122.3 (C-4′), 148.8 (C-3′, 5′); EI-MS: *m/z* 414 (M^+), HR-EI-MS: *m/z* 414.1817 (M^+), $C_{15}H_{26}O_6N_2$ requires 414.1791. X-ray data: Trigonal, space group $P3_2$, a=15.4570(12) Å, b=15.46(3) Å, c=7.4760(3) Å, V=1546.86(14) Å^3, α=90.00°, β=90.00°, γ=120.00°, R=0.0481.

Benzoylation of 5a Compound **5a** (24.7 mg) was treated in the same manner as described above to give *ent*-β-chamigrene-1α-yl 3,5-dinitorbenzoate (**5b**) (33.6 mg, 72%): mp 129—131 °C ; $[\alpha]_D$ −93.6° (c=1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3103, 2966, 1729, 1630, 1548, 1461, 1344, 1273, 1167; UV λ_{max} nm (log ε): 244.4 (4.01); ^{1}H-NMR ($CDCl_3$): δ 5.45 (1H, dd, J=10.4, 4.9 Hz, H-1α), 2.06 (1H, m, H-2α), 2.22 (1H, m, H-2α), 5.35 (1H, m, H-4), 2.18 (1H, m, H-5α), 2.27 (1H, m, H-5β), 1.22 (1H, m, H-8α), 1.83 (1H, m, H-8β), 1.70 (1H, m, H-9α), 1.96 (1H, m, H-9β), 2.24 (1H, m, H-10α), 2.80 (1H, m, H-10β), 0.92 (3H, s, H-12), 0.89 (3H, s, H-13), 4.81, 5.11 (each 1H, s, H-11); 1.65 (3H, s, H-15), 9.20, 9.21 (each 1H, H-2′, 6′), 9.25 (1H, t, J=2.2 Hz, H-4′); ^{13}C-NMR ($CDCl_3$): δ 78.2 (C-1), 34.4 (C-2), 130.8 (C-3), 120.2 (C-4), 32.3 (C-5), 48.3 (C-6), 37.6(C-7), 39.4 (C-8), 23.0 (C-9), 34.8 (C-10), 146.3 (C-11), 24.9 (H-12), 24.7 (C-13), 113.1 (C-14), 22.3 (C-15), 161.7 (C=O), 134.3 (C-1′), 129.5 (C-2′,6′), 122.4 (C-4′), 148.8 (C-3′, 5′); EI-MS: *m/z* 414 (M^+), HR-EI-MS: *m/z* 414.1805 (M^+), $C_{22}H_{26}O_6N_2$ requires 414.1794. X-ray crystallographic data: Orthorhombic, space group $P2_12_12_1$, a=7.29490(3) Å, b=8.4180(5) Å, c=34.556(3) Å, V=2108.7(2) Å^3, α=90.00°, β=90.00°, γ=90.00°, R=0.0596.

Biotransformation of Compound 4a *A. niger* was inoculated in Czapek peptone medium and cultivated in a rotary (100 rpm) at 30 °C for 3 d. Compound **4a** (100 mg×3) was added and further cultivated under the same conditions as described above for 5 d. The cultured liquid was filtered using centrifugation to give the culture broth, which was extracted with ethyl acetate to give the crude extract (332 mg) and was then chromatographed on silica gel using *n*-hexane–ethyl acetate as the gradient solvent to give three fractions. From fractions 1 and 2, compounds **7a** (88 mg) and **8** (33 mg) were obtained as a pure state. Fraction 3 was further chromatographed on silica gel using $CHCl_3$ and MeOH gradients to yield compounds **10** (6 mg) and **9** (43 mg).

Compund **7a**: $[\alpha]_D$ +50.9° (c=1.0); IR (KBr) cm^{-1}: 3407 (OH), 2950, 2894, 1081, 989; EI-MS: *m/z* (%) 236 (M^+, 57), 203 (100), 178 (56), 161 (66), 119 (65), 107 (59), 91 (49), 41 (30); HR-EI-MS: *m/z* 236.1775 (M^+), $C_{15}H_{24}O_2$ requires 236.1776. ^{1}H- and ^{13}C-NMR spectral data: see Tables 4 and 5.

Compound **8**: $[\alpha]_D$ +27.9° (c=1.0); IR (KBr) cm^{-1}: 3429 (OH), 3045, 2946, 1083, 987, 758; EI-MS: *m/z* (%) 236 (M^+, 8), 203 (74), 161 (44), 150 (79), 119 (59), 105 (48), 91 (44), 43 (43); HR-EI-MS: *m/z* 236.1778 (M^+), $C_{15}H_{24}O_2$ requires 236.1776. ^{1}H- and ^{13}C-NMR spectral data: see Tables 4 and 5.

Compound **9**: mp 108—110 °C; $[\alpha]_D$ +56.0° (c=1.0, MeOH); IR (KBr) cm^{-1}: 3390 (OH), 2952, 2905, 1083, 1016, 757; EI-MS: *m/z* (%) 252 (M^+, 10), 234 (86), 219 (100), 216 (44), 201 (48), 163 (821), 145 (33), 105 (33), 105 (31), 91 (32), 79 (18), 43 (20); HR-EI-MS: *m/z* 252.1740 (M^+), $C_{15}H_{24}O_3$ requires 252.1725. ^{1}H- and ^{13}C-NMR spectral data: see Tables 4 and 5. X-ray data of **9**: Monoclinic, space group $P2_1$, a=6.8010(3) Å, b=12.5510(5) Å, c=9.1060(8) Å, V=777.26(8) Å^3, β=90.469(2)°, R=0.0381.

Compound **10**: Colorless oil; $[\alpha]_D$ −2.0° (c=1.0); IR (KBr) cm^{-1}: 3372 (OH), 2932, 2881, 1732 (C=O), 1018, 756; EI-MS: *m/z* (%) 250 (M^+, 63), 235 (45), 217 (100), 199 (66), 163 (52), 145 (55), 105 (68), 91 (69), 77 (41), 41 (32); HR-EI-MS: *m/z* 250.1572 (M^+), $C_{15}H_{22}O_3$ requires 250.1569. ^{1}H- and ^{13}C-NMR spectral data: see Tables 4 and 5.

Preparation of (+)-MTPA Esters of 7a To compound **7a** (10 mg) in pyridine (0.25 ml) was added DMAP (18.3 mg) and (+)-MTPA chloride (0.05 ml) and stirred for 3 h at room temperature. The reaction mixture was partitioned between $CHCl_3$ and H_2O and the lower layer was washed with 1 N HCl, saturated NaCl, 5% $NaHCO_3$, and saturated NaCl again. The organic layer was dried over $MgSO_4$, then the solvent was evaporated *in vacuo* to give a residue which was chromatographed on silica gel (*n*-hexane–EtOAc gradient) to afford (+)-MTPA ester (18.6 mg) (**7b**). Colorless oil; $[\alpha]_D$ +56.5° (c=1.0, $CHCl_3$); IR(KBr) cm^{-1}: 3568 (COO), 1739 (COO); ^{1}H-NMR ($CDCl_3$): δ 1.38 (1H, dd, J=10.7, 1.4 Hz, H-1α), 1.24 (1H, d, J=10.4, H-1β), 0.93 (1H, d, J=5.5 Hz, H-2), 0.95 (1H, d, J=5.5 Hz, H-3), 3.67 (2H, s, H-5), 2.57 (1H, dd, J=14.8, 4.7 Hz, H-8α),1.67 (1H, br d, J=14.8 Hz, H-8β), 5.53 (1H, m, H-9), 2.21 (dd, J=15.1, 4.7 Hz, H-10α), 1.41 (1H, m, H-10β), 0.57 (3H, s, H-12), 0.90 (3H, s, H-13), 1.11 (3H, s, H-14), 1.02 (3H, s, H-15); EI-MS; *m/z* 452 (M^+), 452 (3), 218 (100), 189 (53), 145 (52), 105 (49), 91 (38), 77 (30), 41 (19); HR-EI-MS: *m/z* 452.2192 (M^+), $C_{25}H_{21}O_4F_3$ requires 452.2175.

Preparation of (−)-MTPA Ester of 7a Compound **7a** (9.9 mg) was treated in the same manner as describe above to give (−)-MTPA ester **7c** (16.1 mg). Colorless oil; $[\alpha]_D$ +0.7° (c=1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3558 (OH), 1739 (COO); ^{1}H-NMR ($CDCl_3$): δ 1.38 (1H, dd, J=10.7, 0.8 Hz, H-1α), 1.25 (1H, d, J=10.7 Hz, H-1β), 0.90 (1H, d, J=5.8 Hz, H-2), 0.91 (1H, d, J=5.8 Hz, H-3), 3.68 (2H, s, H-5), 2.47 (1H, dd, J=14.8, 4.1 Hz, H-8α), 1.56 (1H, br d, J=14.8 Hz, H-8β), 5.52 (1H, m, H-9), 2.21 (dd, J=16.2, 4.7 Hz, H-10α), 1.53 (1H, m, H-10β), 0.68 (3H, s, H-12), 0.94 (3H, s, H-13), 1.09 (3H, s, H-14), 0.98 (3H, s, H-15); EI-MS; *m/z* 452 (M^+), 452 (3), 218 (100), 189 (78), 145 (75), 105 (66), 91 (45), 77 (39), 41 (22); HR-EI-MS: *m/z* 452.2175 (M^+), $C_{25}H_{21}O_4F_3$ requires 452.2175.

Biotransformation of Compound 5a Compound **5a** (192 mg) was treated in the same manner as described above to give the culture broth that was extracted with ethyl acetate to give the crude extract (168 mg). The extract was chromatographed on silica gel using the same solvent as described above to afford three fractions from which compounds **11** (10 mg), **12a** (52 mg), and **13** (95 mg) were obtained, respectively.

Compound **11**: $[\alpha]_D$ −12.9° (c=1.0, MeOH); IR (KBr) cm^{-1}: 3422 (OH), 2909, 2894, 1633 (C=C); EI-MS: *m/z* (%) 236 (M^+, 28), 218 (73), 203 (64), 159 (82), 133 (85), 119 (100), 91 (96), 79 (56), 41 (94); HR-EI-MS: *m/z* 236.1771 (M^+), $C_{15}H_{24}O_2$ requires 236.1776. ^{1}H- and ^{13}C-NMR spectral data: see Table 6.

Compound **12a**: $[\alpha]_D$ +2.0° (c=1.0); IR (KBr) cm^{-1}: 3409 (OH), 1633 (C=C); EI-MS: *m/z* (%) 236 (M^+, 57), 203 (100), 178 (56), 161 (66), 119 (65), 107 (59), 91 (49), 41 (30); HR-EI-MS: *m/z* 236.1775 (M^+), $C_{15}H_{24}O_2$ requires 236.1776. ^{1}H- and ^{13}C-NMR spectral data: see Table 6.

Compound **13**: $[\alpha]_D$ −42.6° (c=1.0, MeOH); IR (KBr) cm^{-1}: 3368 (OH),1632 (C=C); CI-MS: *m/z* (%) 251 (M^++1, 30), 217 (100), 199 (56), 189 (21), 173 (20); HR-CI-MS: *m/z* 251.1663 (M^++1), $C_{15}H_{23}O_3$ requires 251.1647. ^{1}H- and ^{13}C-NMR spectral data: see Table 6.

Preparation of (+)-MTPA Esters of 12a To compound **12a** (5.4 mg) in CH_2Cl_2 (4 ml) was added DMAP (33.3 mg), (+)-MTPA chloride (23.1 mg), and DCC (59.1 mg) and stirred for 1 d at room temperature. One day, there after, (+)-MTPA (60 mg), DCC (69.3 mg), and DMAP (64.0 mg) were added to the reaction mixture and stirred for 5 d. The reaction mixture was treated in the same manner as described above to afford the crude extract, which was purified on silica gel chromatography (*n*-hexane–EtOAc gradient) to give (+)-MTPA ester (**12b**) (7.6 mg). $[\alpha]_D$ −12.3° (c=1.0, $CHCl_3$); IR (KBr) cm^{-1}: 3552 (OH), 1732; ^{1}H-NMR ($CDCl_3$): δ 4.07 (1H, dd, J=6.3, 6.3 Hz, H-1α), 1.95 (2H, m, H-2), 5.26 (1H, m, H-4), 2.05 (2H, m, H-5), 6.08 (1H, dd, J=4.9, 4.9 Hz, H-8β), 1.51 (1H, m, H-9α), 1.97 (1H, m, H-9β), 2.23 (1H, m, H-10α), 2.79 (1H, ddd, J=13.7, 4.9, 4.9 Hz, H-10β), 1.08 (3H, s, H-12), 0.78 (3H, s, H-13), 4.69 (1H, s, H-14), 4.97 (1H, t, J=1.6 Hz,

H-14), 1.59 (3H, s, H-15); HR-EI-MS: *m/z* 452.2167 (M^+), $C_{25}H_{31}O_4F_3$ requires 452.2174.

Preparation of (−)-MTPA Esters of 12a Compound **12a** ((5.6 mg) was treated in the same manner as decribe above to afford (−)-MTPA ester (**12c**) (8.6 mg): $[\alpha]_D$ −69.8° (c=1.0, $CHCl_3$), IR (KBr) cm^{-1}; 3549 (OH), 1732; ^{1}H-NMR ($CDCl_3$): δ 4.06 (1H, dd, J=6.9, 6.9 Hz, H-1α), 1.94 (2H, d, J=7.4 Hz, H-2), 5.26 (1H, m, H-4), 2.04 (2H, m, H-5), 6.12 (1H, dd, J=4.9, 4.9 Hz, H-8β), 1.04 (1H, m, H-9α), 2.01 (1H, m, H-9β), 2.26 (1H, m, H-10α), 2.81 (1H, ddd, J=13.7, 5.2, 5.2 Hz, H-10β), 0.97 (3H, s, H-12), 0.77 (3H, s, H-13), 4.70 (1H, s, H-14), 4.99 (1H, t, J=1.6 Hz, H-14), 1.59 (3H, s, H-15); HR-EI-MS: *m/z* 452.2177 (M^+), $C_{25}H_{31}O_4F_3$ requires 452.2174.

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