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ent-Isopimarane-type diterpenoids from the New Zealand liverwort *Trichocolea mollissima*

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Abstract

Two *ent*-isopimarane-type diterpenoids were isolated from the New Zealand liverwort *Trichocolea mollissima* (Hook. f. and Tayl.) Gott., together with a known 1α-hydroxy-*ent*-sandaracopimara-8(14),15-diene. Their absolute structures have been established by modified Mosher's method, X-ray crystallography and by analyses of their CD spectra.

Keywords: *Trichocolea mollissima*; Liverwort; *Hepaticae*; *ent*-Isopimarane-type; Diterpenoid

1. Introduction

Liverworts are rich sources of terpenoids and aromatic compounds, many of them possessing novel carbon skeletons (Asakawa, 1982, 1995). Their constituents are valuable as chemosystematic and genetic markers (Asakawa, 1982, 1995). Additionally, it is known that geographical differences in the main components are occasionally observed in the same species (Asakawa, 1982, 1995; Nagashima and Asakawa, 1998). Some compounds often showed interesting biological properties such as cytotoxic, antifungal, 5-lipoxygenase inhibitory, and antimicrobial activities (Asakawa, 1999). As part of a search for novel compounds and biologically active substances in the Hepaticae, we are studying the chemical constituents of the Southern Hemispheric liverworts. A number of endemic liverwort species have been found in New Zealand, which are not related to those found in Japan (Allison and Child, 1975). Therefore, we have been interested in their chemical constituents (Asakawa, 2001).

Recently, we obtained large amounts of *Trichocolea mollissima*, which enabled us to continue our studies on the chemical constituents of its ether extract. The Japanese, Taiwanese, East Malaysian, New Zealand and European *Trichocolea* species contain diterpenoids and methyl benzoate with prenyl ether group (Asakawa, et al., 1978, 1981, 1991; Chang and Wu, 1987; Wu and Chang, 1988; Perry et al., 1996; Baek et al., 1998). Previously, prenyl ethers and 1α-hydroxy-*ent*-sandaracopimara-8(14),15-diene (**1**) have been isolated from the New Zealand *T. mollissima* (Perry et al., 1996; Lorimer et al., 1997). We wish to report here on the isolation and characterization of two new *ent*-isopimarane-type diterpenoids **2** and **3** and the determination of the absolute configuration of **1**, using X-ray crystallographic analysis.

2. Results and discussion

A combination of column chromatography on silica gel, Sephadex LH-20 and preparative HPLC of the ether extract of *T. mollissima* gave two new *ent*-isopimarane-type diterpenoids **2** and **3**, along with a known 1α-hydroxy-*ent*-sandaracopimara-8(14),15-diene (**1**) (Lorimer et al., 1997).

The IR spectrum of **2** showed the presence of a hydroxyl group (3364 cm^{-1}). The high resolution-EIMS (HR-EIMS) of **2** (*m*/*z* 304 $[M]^+$) confirmed the molecular formula as $C_{20}H_{32}O_2$. Acetylation of **2** gave a diacetate **4** (*m*/*z* 388 $[M]^+$) which showed two acetyl groups (δ 2.00, 2.03 each *s*) on the ^1H NMR spectrum. The ^1H NMR spectrum (Table 1) of **2** displayed four

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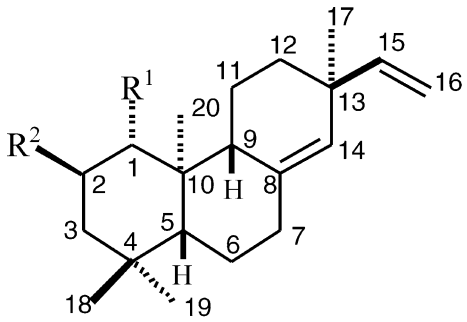
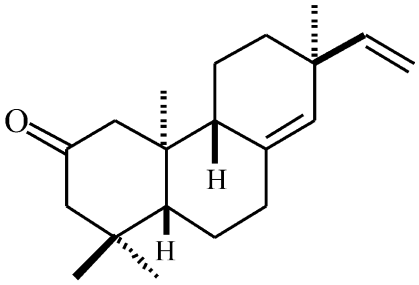


1 R^1=OH, R^2=H
2 R^1=R^2=OH
3 R^1=H, R^2=OH
4 R^1=R^2=OAc



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tertiary methyls (δ 0.88, 0.91, 0.93 1.06), an olefinic (δ 5.29 *br s*), vinyl (δ 4.88, 4.92, 5.78 each *dd*) and two methine protons (δ 3.23 *d*, 3.63 *ddd*) bearing hydroxyl groups. The ^{13}C NMR spectrum (Table 2) and its distortionless enhancement by polarization transfer (DEPT) spectra of **2** confirmed the presence of trisubstituted olefinic carbons (δ 130.4 *d*, 136.2 *s*), vinyl carbons (δ 109.9 *t*, 149.2 *s*) and two oxygenated methines (δ 69.5, 83.8), as well as four methyls, five methylenes, three methines and two quaternary carbons, respectively. The above spectral data and the similarity to those of **1** suggested that compound **2** is an isopimarane (=sandaracopimarane) -type diterpenoid.

The ^{1}H–^{1}H COSY (Fig. 1) of **2** confirmed the presence of three partial structures, (i) –CH(OH)–CH(OH)–CH_2–, (ii) –CH–CH_2–CH_2–, and (iii) –CH–CH_2–CH_2–. The connectivity of each partial structure was clarified by the HMBC spectrum as shown in Fig. 1. Thus, **2** was determined as 1,2-dihydroxyisopimara-8(14),15-diene. The phase sensitive NOESY spectrum of **2** showed NOEs between (i) H-18 and H-3α, H-3β, H-5, H-6β, (ii) H-5 and H-1β, H-3β, H-7β, H-9β, (iii) H-9β

Table 1
^{1}H NMR spectral assignments of **2** and **3** (600 MHz, $CDCl_3$)

H	**2**	**3**
1	3.23 *d* (9.3)	2.02–2.08 *m*, α
		0.99 *t* (11.8), β
2	3.63 *ddd* (12.4, 9.3, 4.4)	3.84 *dddd* (11.5, 11.5, 4.1, 4.1)
3	1.75 *dd* (12.6, 4.4), α	1.75–1.79 *m*, α
	1.31 *t* (12.4), β	1.16 *t* (12.1), β
5	1.11 *dd* (12.4, 2.5)	1.02 *dd* (12.4, 2.5)
6	1.41 *dddd* (12.9, 12.9, 12.9, 4.7), α	1.28 *dddd* (12.9, 12.9, 12.9, 4.7), α
	1.59 *d quint.* (12.9, 2.2), β	1.60 *m*, β
7	2.27 *ddd* (14.3, 4.4, 1.9), α	2.28 *ddd* (14.3, 4.4, 2.2), α
	2.03 *ddd* (14.3, 14.3, 5.8), β	2.02–2.08 *m*, β
9	1.98 *br t* (8.5)	1.75–1.79 *m*
11	1.83–1.94 2H, *m*	1.54 *m*, α
		1.63 *m*, β
12	1.50 *d t like* (12.6, 3.3), α	1.48 *m*, α
	1.35 *ddd* (12.6, 12.6, 4.1), β	1.37 *ddd* (12.4, 12.4, 3.6), β
14	5.29 *br* s	5.25 *s*
15	5.78 *dd* (17.6, 10.7)	5.78 *dd* (17.3, 10.7)
16	4.88 *dd* (10.7, 1.4)	4.89 *dd* (10.7, 1.4)
	4.92 *dd* (17.6, 1.4)	4.92 *dd* (17.3, 1.4)
17	1.06 3H, *s*	1.04 3H, *s*
18	0.93 3H, *s*	0.95 3H, *s*
19	0.91 3H, *s*	0.90 3H, *s*
20	0.88 3H, *s*	0.84 3H, *s*

J values (Hz) in parentheses.

Table 2
^{13}C NMR spectra assignments of **2–5** (100 MHz, $CDCl_3$)

C	2	3	4	5
1	83.8	48.6	80.5	54.0
2	69.5	65.4	70.9	211.7
3	47.4	51.1	44.5	56.4
4	34.3	35.0	34.2	39.5
5	54.3	54.1	54.1	54.5
6	22.28[a]	22.3	22.1	22.7
7	36.2	35.8	36.1	35.5
8	136.2	136.4	135.3	135.8
9	51.8	50.6	50.7	50.4
10	44.1	39.9	44.0	44.4
11	22.25[a]	18.9	20.1	18.8
12	34.7	34.5	34.6	34.3
13	37.1	37.4	36.8	37.4
14	130.4	129.3	131.0	129.9
15	149.2	149.0	149.1	148.5
16	109.9	110.1	110.0	110.5
17	25.5	26.0	25.3	26.2
18	33.4	33.8	33.2	33.5
19	22.8	23.1	22.5	23.4
20	9.9	15.9	10.7	15.4
OAc			21.1	
			21.7	
			170.5	
			170.8	

[a]May be interchanged.

and H-12β, (iv) H-20 and H-2α, H-6α, (v) H-2α and H-3α, H-19, H-20, and (vi) H-17 and H-12α, H-20, respectively. Accordingly, the stereostructure of **2** was determined to be 1α,2β-dihydroxyisopimara-8(14),15-diene. The absolute configuration of the hydroxyl group at C-2 was established to be *R* by modified Mosher's method (Kusumi et al., 1992) as shown in Fig. 2. Thus, the absolute structure of **2** was established to be (1*R*,2*R*)-*ent*-1,2-dihydroxyisopimara-8(14),15-diene.

Since the ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **3** resembled those of **1** and **2**, the structure of **3** was assumed to be an isopimarane-type diterpenoid. The IR spectrum of **3** (*m*/*z* 288, M^+) showed the presence of a hydroxyl group (3269 cm^{-1}), and its HR-EIMS confirmed the molecular formula as $C_{20}H_{32}O$. The ^1H and ^{13}C NMR spectra showed the presence of a methine (δ_C 65.4) bearing secondary hydroxyl group, a trisubstituted olefin (δ_C 129.3 *d*, 136.4 *s*), a vinyl group (δ_C 110.1 *t*, 149.0 *d*) and four tertiary methyls (δ_H 0.84, 0.90, 0.95, 1.04). The analysis of the ^1H–^1H COSY, HMQC and HMBC spectra of **3** as indicated in Fig. 3 clarified that the structure of **3** was 2-hydroxyisopimara-8(14),15-diene. The orientation of the hydroxyl group at C-2 was shown to be β by the NOESY spectrum (Fig. 3). The oxidation of **3** gave a ketone **5** (*m*/*z* 286 M^+) which displayed a negative Cotton effect (294 nm) in the CD spectrum. Accordingly, the absolute configuration of **5** was clarified to be *ent*-8(14),15-isopimaradien-2-one by the application of the back octant rule for the CD spectrum as shown in Fig. 4. Thus, the absolute structure of **3** was characterized to be (2*R*)-*ent*-2-hydroxyisopimara-8(14),15-diene.

While the ^1H and ^{13}C NMR spectral data of compound **1** were identical with those of 1α-hydroxy-*ent*-sandaracopimara-8(14),15-diene (Lorimer et al., 1997), its absolute configuration remained to be clarified. Consequently, the absolute configuration of **1** was confirmed by X-ray crystallographic analysis of *m*-bromobenzoate derivative **6** displayed from **1** as shown in Fig. 5.

Occurrence of pimarane- and isopimarane-type diterpenoids in liverworts is rare. At present, *ent*-pimara-8(14),15-dien-19-ol and *ent*-8-hydroxypimara-15-ene have been isolated from *Jungermannia thermarum* (Matsuo et al., 1976) and *ent*-pimara-8(14),15-dien-19-oic acid and (−)-sandaracopimaric acid from *Mastigophora diclados* (Wu et al., 1992).

T. tomentella (Asakawa, 1995; Perry et al., 1996) and *T. hatcheri* (Baek et al., 1998) produce various methyl benzoates with a prenyl ether which are the most significant chemical markers of *Trichocolea* genus. On the other hand, *T. pluma* (Asakawa et al., 1991; Chang and Wu, 1987) and *T. mollissima* (Perry et al., 1996; Lorimer et al., 1997) elaborate not only such benzoates but also

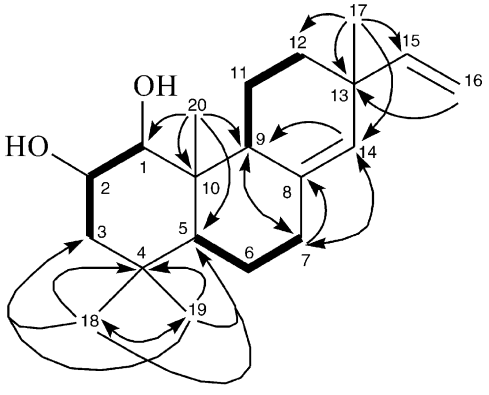

Fig. 1. Long-range ^1H–^{13}C (arrows) and ^1H–^1H (bold lines) correlations of **2**.

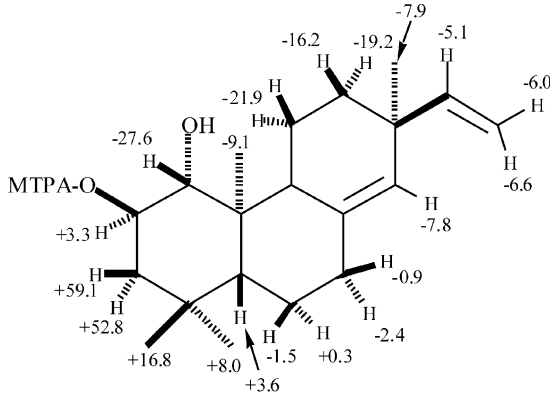

Fig. 2. Δδ values (Hz) for (*R*)- and (*S*)- MTPA esters of **2**.

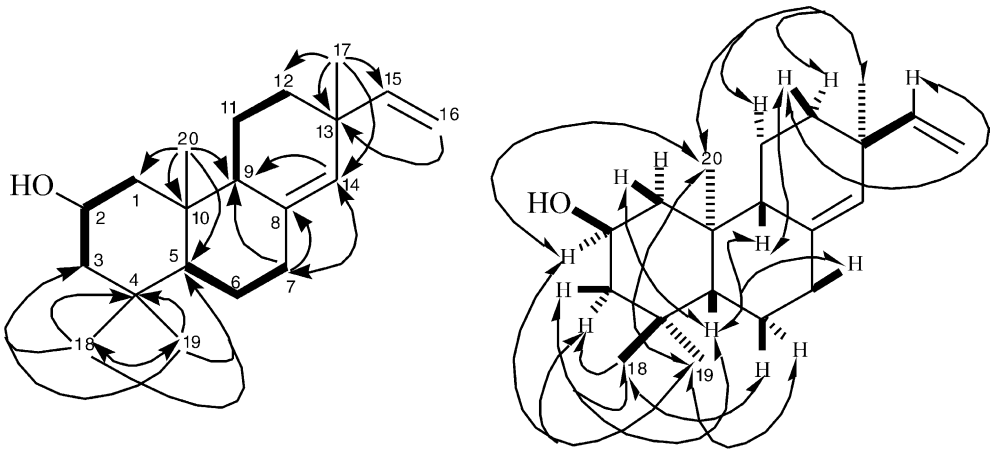

Fig. 3. Long-range ^{1}H-^{13}C (arrows) and ^{1}H-^{1}H (bold lines) and NOE (half arrows) correlations of **3**.

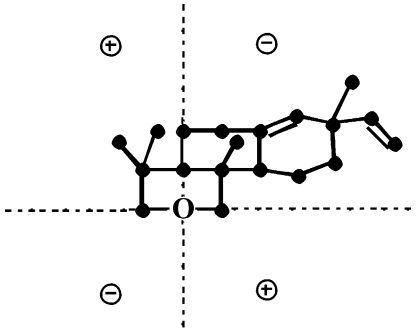

Fig. 4. The octant diagram of **5**.

labdane- or isopimarane-type diterpenoids. The former species also contain mono- and sesquiterpenoids. Thus, *T. mollissima*, from which we could not isolate methyl benzoates, and *T. pluma* are chemically different from the above two *Trichocolea* species except for the presence of the common methyl benzoate derivatives.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR: 400 and 600 MHz (^{1}H NMR), and 100 and 150 MHz (^{13}C NMR). Chemical shift values were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard (^{1}H NMR), and δ 77.03 (ppm) from $CHCl_3$ as a standard (^{13}C NMR). TLC: visualized under UV (254 nm) light and by spraying with 10% H_2SO_4 or Godin reagent (Godin, 1954), followed by heating. MeOH–CH_2Cl_2 (1:1) was used for Sephadex LH-20. $CHCl_3$ for the optical rotations.

3.2. Plant material

Trichocolea mollissima (Hook. f. and Tayl.) Gott. (NZ124) was collected in Cascade, New Zealand, 2000, and identified by Dr. J.E. Braggins (University of Auckland), and a voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

3.3. Extraction and isolation

The ether extract (615 mg) of *T. mollissima* (26 g) was divided into seven fractions by column chromatography (CC) on silica gel using *n*-hexane–EtOAc gradient solvent system. Fr. 2 was subjected to Sephadex LH-20 and silica gel CC (*n*-hexane–Et_2O 17:3) to give 1α-hydroxy-*ent*-isopimara(=sandaracopimara)-8(14),15-diene (**1**) (70.8 mg). (2*R*)-*ent*-2-hydroxyisopimara-8(14),15-diene (**3**) (15.0 mg) was isolated by CC on silica gel (*n*-hexane–EtOAc 7:3), Sephadex LH-20 and finally preparative HPLC (Nucleosil, *n*-hexane–EtOAc 1:1) of Fr. 4. Fr. 5 was applied to a Sephadex LH-20 and silica gel (CH_2Cl_2–EtOAc 19:1) CC to give three subfractions. A mixture of diterpenoids of subfraction 3 was subjected to reversed phase silica gel (Cosmosil 75C_{18}, CH_3CN) to yield (1*R*,2*R*)-*ent*-1,2-dihydroxyisopimara-8(14),15-diene (**2**) (38.0 mg).

3.4. (1R,2R)-ent-1,2-Dihydroxyisopimara-8(14),15-diene (2)

Crystal, mp 132–133 °C; $[\alpha]_D^{20}$ −16.5° (*c* 2.51); HR-EIMS: found 304.2404, $C_{20}H_{32}O_2$ requires 304.2403; FTIR ν_{max} cm^{-1}: 3364 (OH); For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2; EIMS *m*/*z* (rel. int.): 304[M]$^{+}$(100), 289(86), 276(22), 253(14), 241(10),

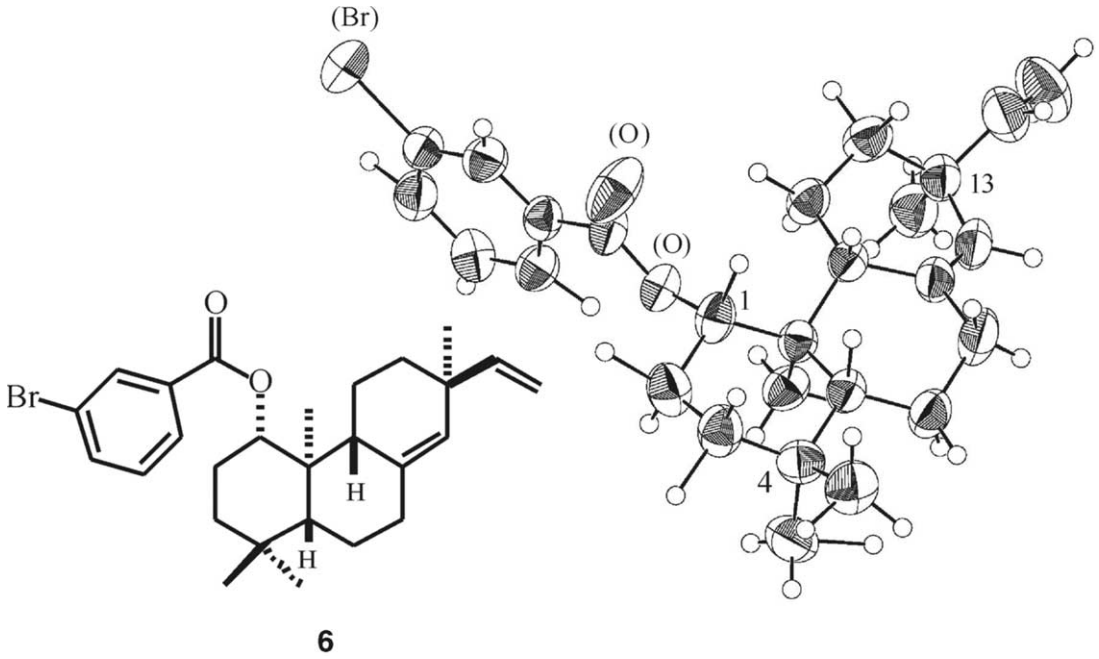

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Fig. 5. The ORTEP depiction of **6**. Anisotropic ellipsoids are represented by a 50% probability level.

219(8), 203(22), 187(27), 168(37), 147(23), 135(54), 121(74), 107 (47), 91(53), 71(11), 67(24), 55(35), 41(35).

3.5. (2R)-ent-2-Hydroxyisopimara-8(14),15-diene (3)

Crystal, mp 104–105 °C; $[\alpha]_D$ −9.6° (*c* 0.81); HR-EIMS: found 288.2454, $C_{20}H_{32}O$ requires 288.2454; FTIR ν_{max} cm^{-1}: 3269 (OH); For ^1H and ^{13}C NMR spectra, see Tables 1 and 2; EIMS *m*/*z* (rel. int.): 288[M]$^+$(39), 273(40), 255(40), 227(4), 201(11), 187(46), 175(8), 159(8), 135(100), 121(22), 107(20), 93(24), 91(19), 79(13), 69(11), 55(10).

3.6. Acetylation of 2

Compound **2** (9 mg) was added to pyridine (1 ml) and Ac_2O (1 ml), and kept at room temperature overnight, then worked up as usual to give (1*R*, 2*R*)-*ent*-1,2-diacetoxyisopimara-8(14),15-diene (**4**) (10 mg). Oil; $[\alpha]_D^{20}$ −5.6° (*c* 0.86); HR-EIMS: found 388.2616, $C_{24}H_{36}O_4$ requires 388.2614; FTIR ν_{max} cm^{-1}: 1745, 1246; ^1H NMR: δ 4.93 (1H, *d*, *J* = 9.9 Hz, H-1), 5.02 (1H, *ddd*, *J* = 12.1, 9.9, 4.1 Hz, H-2), 1.78 (1H, *dd*, *J* = 12.6, 4.4 Hz, H-3α), 1.46 (1H, *t*, *J* = 12.6 Hz, H-3β), 1.20 (1H, *dd*, *J* = 12.4, 2.5 Hz, H-5), 1.43–1.48 (2H, *m*, H-6α, H-11β), 1.62 (1H, *m*, H-6β), 2.26 (1H, *ddd*, *J* = 14.3, 4.4, 1.9 Hz, H-7α), 2.02 (1H, *m*, H-7β), 2.05 (1H, *br t*, *J* = 9.3 Hz, H-9), 1.65 (1H, *m*, H-11α), 1.41 (1H, *br d*, *J* = 12.4 Hz, H-12α), 1.30 (1H, *ddd*, *J* = 12.4, 12.4, 3.6 Hz, H-12β), 5.32 (1H, *br s*, H-14), 5.76 (1H, *dd*, *J* = 17.6, 10.7 Hz, H-15), 4.88 (1H, *dd*, *J* = 10.7, 1.4 Hz, H-16), 4.91 (1H, *dd*, *J* = 17.6, 1.4 Hz, H-16), 1.04 (3H, *s*, H-17), 0.94 (3H, *s*, H-18), 0.98 (3H, *s*, H-19), 1.01 (3H, *s*, H-20), 2.00 (3H, *s*, OCO$\underline{CH_3}$), 2.03 (3H, *s*, OCO$\underline{CH_3}$). For ^{13}C NMR spectrum, see Table 2; EIMS *m*/*z* (rel. int.): 388[M]$^+$(5), 328(11), 313(4), 286(11), 268(100), 253(41), 240(4), 225(5), 211(5), 187(38), 151(24), 135(11), 119(12), 105(13), 91(12), 81(7), 55(8), 43(22).

3.7. Preparation of (R)- and (S)-MTPA esters of 2

To compound **2** (6.8 mg) in CH_2Cl_2 (1 ml) was added (*R*)-α-methoxy-α-trifluorophenylacetic acid (MTPA, 2 mg), dicyclohexylcarbodiimide (DCC, 1.9 mg) and 4(N,N-dimethylamino)pyridine (DMAP, 5.1 mg), and the mixture allowed to stand at room temp. for 5 h. The reaction mixture was purified by preparative TLC (*n*-Hexane–EtOAc 4:1) to give (*R*)-MTPA ester (3.0 mg). Compound **2** (4.9 mg) was treated with (*S*)-MTPA (2.3 mg) in the same manner as described above to afford (*S*)-MTPA ester (5.2 mg); *(R)-MTPA ester*: ^1H NMR (600 MHz): δ 3.53 (1H, *dd*, *J* = 9.9, 5.2 Hz, H-1), 5.09 (1H, *ddd*, *J* = 12.4, 9.9, 4.4 Hz, H-2), 1.84 (1H, *dd*, *J* = 12.4, 4.4 Hz, H-3α), 1.35 (1H, *t*, *J* = 12.4 Hz, H-3β), 1.11 (1H, *dd*, *J* = 12.4, 2.5 Hz, H-5), 1.43 (1H, *ddd*, *J* = 12.9, 12.9, 4.7 Hz, H-6α), 1.60 (1H, *d like*, *J* = 12.9 Hz, H-6β), 2.28 (1H, *ddd*, *J* = 14.3, 4.4, 1.9 Hz, H-7α), 2.03 (1H, *ddd*, *J* = 13.5, 13.5, 4.4 Hz, H-7β), 1.98 (1H, *br t*, *J* = 8.0 Hz, H-9), 1.89 (2H, *m*, H-11), 1.48 (1H, *br dt*, *J* = 11.5, 3.6 Hz, H-12α), 1.33 (1H, *m*, H-12β), 5.31 (1H, *s*, H-14), 5.77 (1H, *dd*, *J* = 17.5, 10.7 Hz, H-15), 4.88 (1H, *dd*, *J* = 10.7, 1.4 Hz, H-16), 4.92 (1H, *dd*, *J* = 17.5, 1.4 Hz, H-16), 1.05 (3H, *s*, H-17), 0.93 (3H, *s*, H-18), 1.00 (3H, *s*, H-19), 0.95 (3H, *s*, H-20); *(S)-MTPA ester*: ^1H NMR (600 MHz): δ 3.48 (1H, *dd*, *J* = 9.9, 3.8 Hz, H-1), 5.10 (1H, *ddd*, *J* = 12.4, 9.9, 4.4 Hz,

H-2), 1.93 (1H, *dd*, $J=12.4$, 4.4 Hz, H-3α), 1.44 (1H, *t*, $J=12.4$ Hz, H-3β), 1.12 (1H, *dd*, $J=12.4$, 2.5 Hz, H-5), 1.43 (1H, *m*, H-6α), 1.60 (1H, *d sex.*, $J=13.2$, 2.2 Hz, H-6β), 2.28 (1H, *ddd*, $J=14.0$, 4.4, 1.9 Hz, H-7α), 2.02 (1H, *ddd*, $J=14.0$, 14.0, 6.0 Hz, H-7β), 1.94 (1H, *m*, H-9), 1.85 (2H, *m*, H-11), 1.45 (1H, *m*, H-12α), 1.30 (1H, *m*, H-12β), 5.29 (1H, *s*, H-14), 5.76 (1H, *dd*, $J=17.6$, 10.7 Hz, H-15), 4.87 (1H, *dd*, $J=10.7$, 1.4 Hz, H-16), 4.91 (1H, *dd*, $J=17.6$, 1.4 Hz, H-16), 1.04 (3H, *s*, H-17), 0.96 (3H, *s*, H-18), 1.01 (3H, *s*, H-19), 0.94 (3H, *s*, H-20).

3.8. Oxidation of 3

To compound **3** (4 mg) in CH_2Cl_2 (2 ml) was added *N*-methylmorpholine *N*-oxide (NMO, 10 mg), 4 Å molecular sieves (12 mg), and then tetrapropylammonium perruthenate (TPAP, 3 mg), and stirred at room temp. for 2 h. The reaction mixture was filtered through a short pad column to give *ent*-8(14),15-isopimaradien-2-one (**5**) (2.5 mg). Oil; $[\alpha]_D^{22}$ −4.9° (*c* 1.26); HR-EIMS: found 286.2298, $C_{20}H_{30}O$ requires 286.2297; FTIR ν_{max} cm^{-1}: 1701 (C=O); CD: $\Delta\varepsilon_{294}$ −0.60, $\Delta\varepsilon_{211}$ +3.54 (c 8.74×10^{-4}); ^1H NMR: δ 2.37 (1H, *d*, $J=12.4$ Hz, H-1α), 2.23 (1H, *d*, $J=12.4$ Hz, H-1β), 2.14 (1H, *dd*, $J=12.9$, 2.5 Hz, H-3α), 2.36 (1H, *d*, $J=12.9$ Hz, H-3β), 1.61 (1H, *dd*, $J=12.4$, 2.7 Hz, H-5), 1.41 (1H, *m*, H-6α), 1.73 (1H, *d quint.*, $J=13.2$, 2.7 Hz, H-6β), 2.34 (1H, *ddd*, $J=14.0$, 4.4, 2.2 Hz, H-7α), 2.13 (1H, *m*, H-7β), 1.98 (1H, *t*, $J=7.7$ Hz, H-9), 1.48 (1H, *m*, H-11), 1.55 (1H, *m*, H-11), 1.50 (1H, *m*, H-12α), 1.39 (1H, *m*, H-12β), 5.30 (1H, *br s*, H-14), 5.77 (1H, *dd*, $J=17.3$, 10.7 Hz, H-15), 4.90 (1H, *dd*, $J=10.7$, 1.4 Hz, H-16), 4.92 (1H, *dd*, $J=17.3$, 1.4 Hz, H-16), 1.04 (3H, *s*, H-17), 1.08 (3H, *s*, H-18), 0.89 (3H, *s*, H-19), 0.83 (3H, *s*, H-20). For ^{13}C NMR spectrum, see Table 2; EIMS *m*/*z* (rel. int.): 286$[M]^+$(73), 271(100), 258(7), 243(4), 201(8), 187(44), 173(15), 159(15), 151(21), 135(47), 119(45), 105(42), 91(61), 79(30), 67(19), 55(24), 41(27).

3.9. m-Bromobenzoylation of 1

To compound **1** (7 mg) in pyridine (2 ml) was added *m*-bromobenzoic acid (6 mg), DCC (6 mg) and DMAP (2 mg) and stirred at room temp. overnight. The reaction mixture was filtered and subjected to silica gel CC to yield *m*-bromobenzoate **6** (11.4 mg). ^1H NMR: δ 0.91 (3H, *s*), 0.92 (3H, *s*), 1.04 (3H, *s*), 1.10 (3H, *s*), 1.19 (1H, *ddd*, $J=13.2$, 13.2, 3.3 Hz), 2.28 (1H, *ddd*, $J=14.3$, 4.4, 1.8 Hz), 4.85 (1H, *dd*, $J=11.0$, 1.5 Hz), 4.87 (1H, *dd*, $J=17.6$, 1.5 Hz), 4.94 (1H, *dd*, $J=10.6$, 4.8 Hz), 5.29 (1H, *br s*), 5.73 (1H, *dd*, $J=17.6$, 11.0 Hz), 7.34 (1H, *t*, $J=7.7$ Hz), 7.69 (1H, *dq*, $J=7.7$, 1.1 Hz), 7.96 (1H, *dt*, $J=7.7$, 1.1 Hz), 8.16 (1H, *t*, $J=1.8$ Hz); Crystal data: $C_{27}H_{35}BrO_2$, Mr = 471.484, orthorhombic, $P2_12_12_1$, $a=6.1910$ (3), $b=17.41000$ (11), $c=22.561$ (2), $\alpha=90.00$ Å, $\beta=90.00$ Å, $\gamma=90.00$ Å, Mo Kα radiation, $\lambda=0.71073$, DIP Image plate, Refinement on F^2, full-matrix least squares refinement, $R(gt)=0.046$, $wR(gt)=0.085$, $S(gt)=0.715$, 2688 reflections, 273 parameters, cell refinement: Scalepack (HKL), data reduction: maXus, program used to refine structure: maXus. The supplementary material has been deposited at the Cambridge Data Centre.

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