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SESQUI- AND DITERPENOIDS FROM THE JAPANESE LIVERWORT *JUNGERMANNIA HATTORIANA*†

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Key Word Index—*Jungermannia hattoriana*; Jungermanniales; Hepaticae; acoradiepoxide; neocuprenenol; cuparadiepoxide; *epi*-cuparadiepoxide; (+)-cuparane; (+)-cuprenenol; rosulantol; (+)-labda-8(17), 14-diene-9*R**, 13*S**-diol; labda-8, 14-dien-13β-ol; 13-*epi*-sclareol; (−)-pimara-8(14), 15-dien-19-oic acid; β-cyclocitral; cuparane-type; acorane-type; labdane-type; monoterpenoid; sesquiterpenoid; diterpenoid; X-ray analysis.

Abstract—A labdane-type diterpenoid, an acorane-type and three cuparane-type sesquiterpenoids have been isolated from the Japanese liverwort *Jungermannia hattoriana* (Amak.) Amak. as new natural products, together with a known monoterpenoid, three cuparane-type sesquiterpenoids and three labdane- and a pimarane-type diterpenoids. Their structures were determined by spectral analysis, chemical degradation and X-ray crystallographic analysis. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

As a part of a chemosystematic study and search for biologically active substances, we are continuing to study the chemical constituents of liverworts. The species belonging to the genus *Jungermannia* are morphologically very small, therefore, their classification is rather difficult. Furthermore, this genus contains a large number of taxonomically complex species which are polymorphic. It is known that the *Jungermannia* species generally contain a large amount of diterpenoids such as *ent*-kaurane-, clerodane-, trachylobane-, pimarane- and labdane-type [1, 2], whereas a few species contain sesquiterpenoids such as chilocyphane- and cuparane-type [5, 6] as the main components. Exceptionally, *J. comata* elaborates a bisbibenzyl-type aromatic compound, perrottetin E, as the main component [7, 8]. In order to clarify the chemical relationships in the *Jungermannia*, the chemical constituents of *J. hattoriana* were analyzed. In this paper, we report the isolation and characterization of a new labdane-type diterpenoid, (+)-labda-8(17), 14-diene-9*R**, 13*S**-diol (**1**), a new acorane-type sesquiterpene, acoradiepoxide (**2**), three new cuparane-type sesquiterpenoids, neocuprenenol (**3**), cuparadiepoxide (**4**) and *epi*-cuparadiepoxide (**5**), from the ether extract of the Japanese *J. hattoriana*, along with a known monoterpenoid (**6**), three cuparane-type sesquiterpenoids (**7**–**9**), two labdane-type (**10**, **11**) and a pimarane-type diterpenoids (**12**).

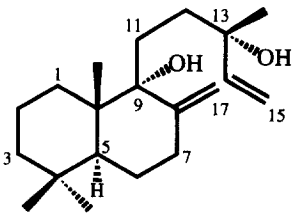
RESULTS AND DISCUSSION

A combination of column chromatography on silica gel, Sephadex LH-20 and preparative HPLC of the ether extract of *J. hattoriana* gave five new terpenoids: (+)-labda-8(17), 14-diene-9*R**, 13*S**-diol (**1**), acoradiepoxide (**2**), neocuprenenol (**3**), cuparadiepoxide (**4**), *epi*-cuparadiepoxide (**5**), along with seven known terpenoids, β-cyclocitral (**6**) [3], (+)-cuparene (**7**) [4], (+)-cuprenenol (**8**) [5], rosulantol (**9**) [5], labda-8,14-dien-13β-ol (**10**) [6, 7], 13-*epi*-sclareol (**11**) [8] and (−)-pimara-8(14),15-dien-19-oic acid (**12**) [9–11]. The structures of the known compounds were determined by comparison with the reference data of authentic samples. Furthermore, the stereostructures of **9** and **11** were confirmed by the X-ray crystallographic analysis as shown in Fig. 1.

The molecular formula of **1** was determined as $C_{20}H_{34}O_2$ ([M]$^+$ at *m/z* 306.2566) by high-resolution mass spectrometry. The IR and ^{13}C NMR spectra indicated the presence of two tertiary hydroxyl groups (3450 cm^{-1}, δ 73.4, 79.7 each *s*). The ^1H NMR spectrum (Table 1) exhibited the presence of four tertiary methyls (δ 0.83, 0.83, 0.90, 1.92 each *s*), an *exo*-methylene group (δ 4.64, 4.91 each *s*) and a vinyl proton (δ 5.05, 5.21, 5.92 each *dd*). The ^{13}C NMR spectrum (Table 1) also displayed 20 carbon signals and its

†This paper is dedicated with best wishes to Professor Guy Ourisson on the occasion of his 70th birthday.

‡Author to whom correspondence should be addressed.



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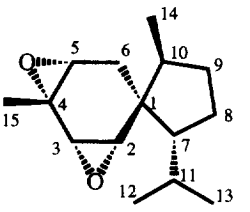

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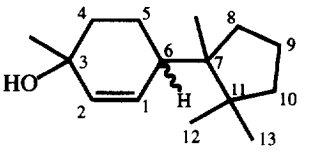

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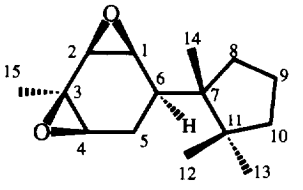

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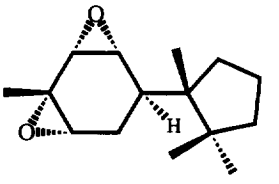

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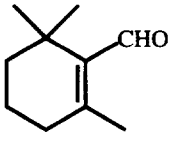

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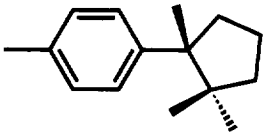

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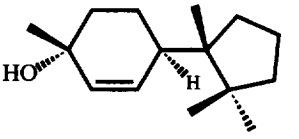

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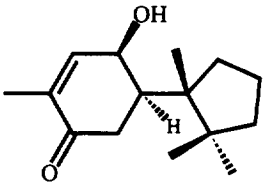

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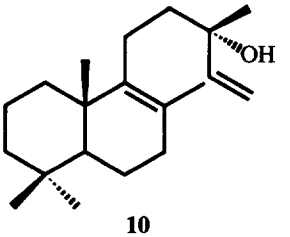

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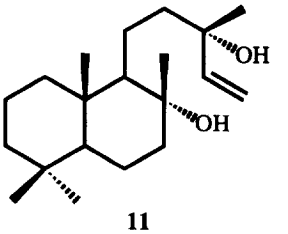

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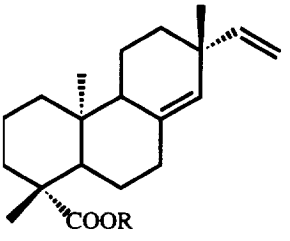
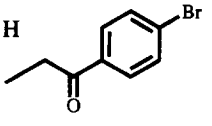
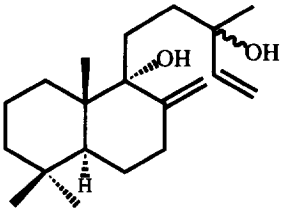


12 R: H
13 R:





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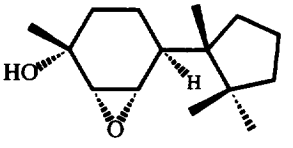

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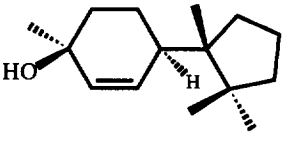

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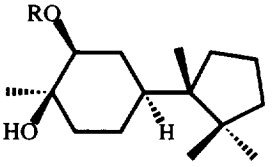
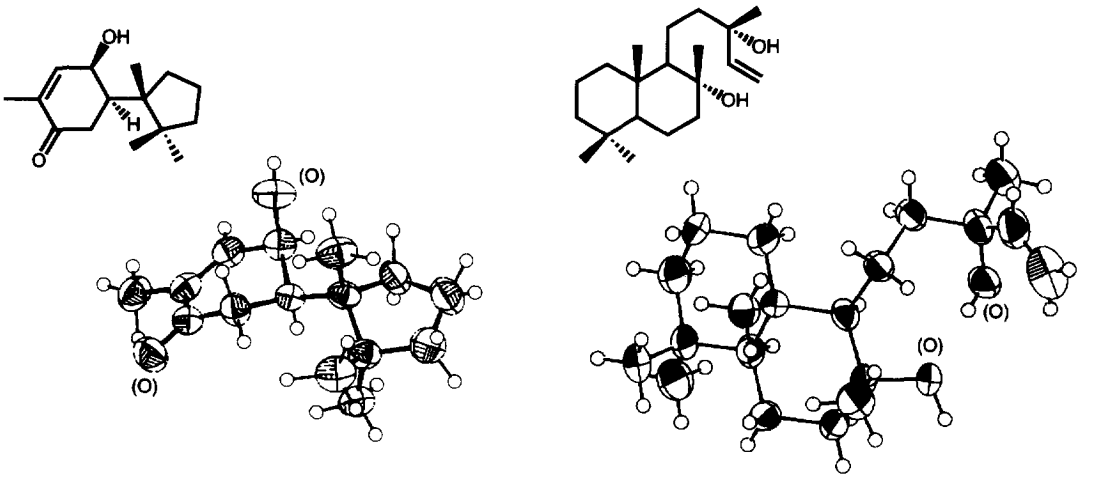


17 R: (*R*)-MTPA
18 R: (*S*)-MTPA



9 **11**

Fig. 1. The ORTEP drawing of compounds **9** and **11**.

Table 1. ^{1}H and ^{13}C NMR data of compound **1** ($CDCl_3$) and ^{13}C NMR data of compound **14**

	1		**14** [12]
	H*	C†	C
1	1.48–1.58 2H, *m*	32.1	31.9
2	1.48–1.58 *m*	19.2	18.8
	1.59–1.74 *m*		
3	1.20 *m*	41.7	32.7
	1.27 *m*		
4		33.5	38.6
5	1.75–1.86 *m*	45.9	48.6
6	1.32 *m*	24.0	27.5
	1.59–1.74 *m*		
7	2.14(1H *dq*, *J* = 13.2, 4.4, 2.4Hz)	33.3	36.2
	2.42(1H *ddd*, *J* = 13.2, 13.2, 5.4Hz)		
8		149.1	148.2
9		79.7	77.3
10		43.6	43.6
11	1.59–1.74 *m*	23.3	28.9
	1.75–1.86 *m*		
12	1.59–1.74 2H, *m*	36.0	41.2
13		73.4	73.4
14	5.92(1H *dd*, *J* = 17.6, 10.8Hz)	145.5	145.4
15	5.05(1H *dd*, *J* = 10.8, 1.0Hz)	111.7	111.4
	5.21(1H *dd*, *J* = 17.6, 1.5Hz)		
16	1.92(3H *s*)	27.7	28.0
17	4.64(1H *s*)	109.9	106.5
	4.91(1H *s*)		
18	0.83(3H *s*)	22.0	24.4
19	0.90(3H *s*)	33.9	17.8
20	0.83(3H *s*)	16.4	17.8

*400MHz.
†50MHz.

DEPT spectrum contained four methyls, nine methylenes, two methines and five quaternary carbons. The comparison of the above spectral data with those of **10** and **11** suggested that **1** was a labdane-type diterpenoid with two tertiary hydroxyl groups. In order to confirm the above assumption, the ^{1}H-^{1}H and ^{13}C-^{1}H COSY and HMBC spectra of **1** were measured. In the HMBC spectrum, the methine proton (H-14) at the vinyl group were both correlated with a tertiary methyl (C-16), a methylene carbon (C-15) at the vinyl group and a quaternary carbon (C-13) with a tertiary hydroxyl group. The *exo*-methylenic protons (H-17)

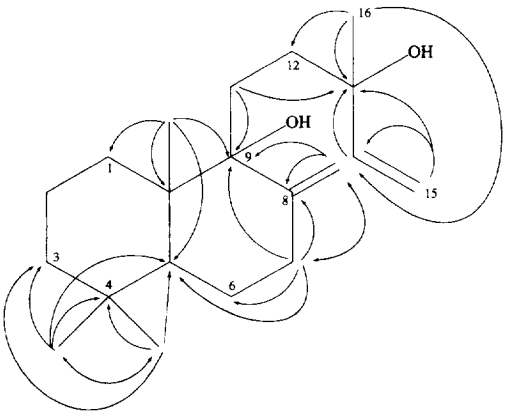


Fig. 2. Long-range H-C correlations observed by the HMBC spectrum of compound **1**.

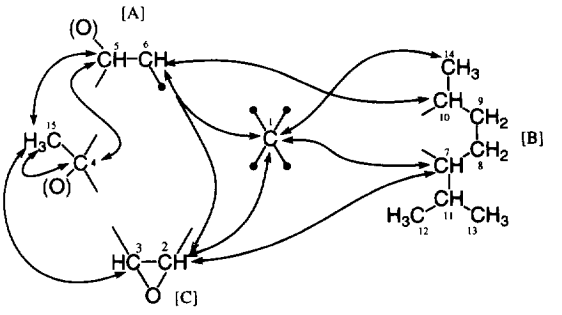


Fig. 4. The partial structures [A]–[C] and the long-range C-H correlations of compound **2**.

were correlated with a methylene (C-7), a quaternary carbon (C-8) and a quaternary carbon (C-9) with a tertiary hydroxyl group. Moreover, the analysis of the other long-range ^{1}H-^{13}C correlation, as shown in Fig. 2, led to the conclusion that compound **1** was labda-8(17), 14-dien-9, 13-diol as the same plane structure as jungermanool (**14**) isolated from *J. torticalyx* [12], although the ^{13}C NMR data of **1** were not in accordance with those of **14** (Table 1). Since the stereochemistry of **1** could not be clarified by the difference NOE spectrum, its X-ray crystallographic analysis was carried out and gave the ORTEP drawing as shown in Fig. 3. Consequently, the stereostructure of **1** was established to be (+)-labda-8(17), 14-diene-9*R**, 13*S**-diol.

The EI-mass spectrum of **2** showed *m/z* 236 and its molecular formula was determined to be $C_{15}H_{24}O_2$ ($[M]^+$ at *m/z* 236.1768) by the HR mass spectrum. The two oxygen atoms in the molecule of **2** were suggested to be epoxides, since its IR spectrum contained neither hydroxyl nor carbonyl absorption bands. The ^{1}H NMR spectrum (C_6D_6) (Table 2) indicated the presence of a tertiary methyl (δ 1.11 *s*), three secondary methyls (δ 0.71, 0.82 and 1.14 each *d*) and three protons (δ 2.45, 2.49 and 2.80 each *dd*) on carbon bearing the oxygen atom. The ^{13}C NMR spectrum (C_6D_6) (Table 3) displayed 15 carbons and its DEPT spectrum showed the presence of four methyls, three methylenes, two methines, two quaternary carbons and three methines (δ 52.8, 53.7 and 57.7), and a quaternary carbon (δ 53.6) bearing an epoxy group.

The above spectral data established that compound **2** was a bicyclic sesquiterpene with two epoxide rings. The ^{1}H-^{1}H COSY and TOCSY spectra of **2** indicated the presence of the three partial structures [A]–[C] (Fig. 4). The connectivity of each partial structure was confirmed by the HMBC spectrum as shown in Fig. 4. Namely, the quaternary carbon (δ 46.9) was correlated with a methylene proton (δ 1.15) in the partial structure A, a secondary methyl (δ 0.71) and methine proton (δ 1.54) in partial structure B and a methine proton (δ 2.49) in the partial structure C. The tertiary methyl (δ 1.11) was also correlated with a quaternary carbon (δ 53.6), a methine carbon (δ 53.7) in the partial structure A and a methine carbon (δ 52.8) in the partial structure C. Thus, the structure of **2** was established to be an acorane-type sesquiterpenoid with C-2/C-3 and C-4 C-5 diepoxides. The NOEs by the NOESY spectrum of **2** were observed between (i) H-2 and H-3; (ii) H-2 and H-7; (iii) H-2 and H-14; (iv) H-3 and H-15; (v) H-5 and H-15; and (vi) H-7 and H-14, as shown in Fig. 5. From the above spectral evidence, the stereostructure of acoradiepoxide was established to be **2**, although the absolute configuration remains to be determined.

Compounds **3** (HRMS: $[M]^+$ at *m/z* 222.1986; $C_{15}H_{26}O$) and **8** (HRMS: $[M]^+$ at *m/z* 222.1989; $C_{15}H_{26}O$) [5] were obtained by preparative HPLC, respectively. The relative stereostructure of the latter

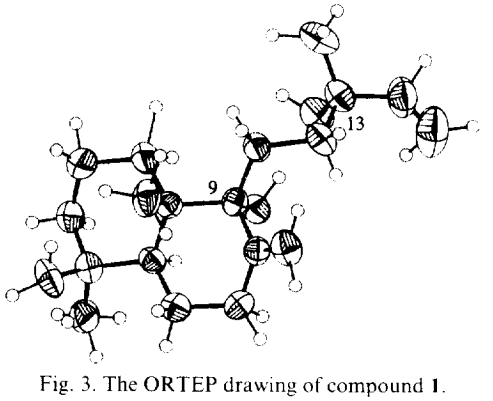


Fig. 3. The ORTEP drawing of compound **1**.

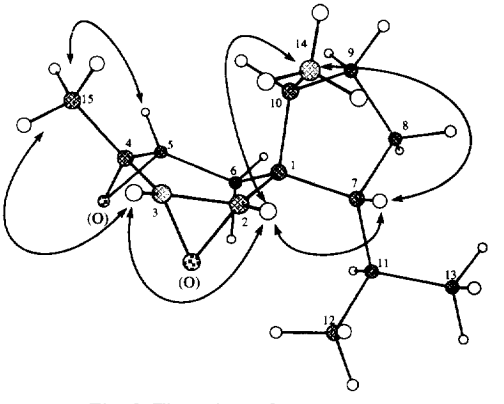


Fig. 5. The NOEs of compound **3**.

Table 2. ^{1}H-NMR data of compounds **2**–**5** and **8** (TMS, $CDCl_3$)

H	**2*** (in C_6D_6)	**3***	**4**†	**5**†	**8**† ([5])
1		5.90 *ddd*, J = 10.3, 1.7, 1.7 Hz	3.09 *d*, J = 4.2 Hz	2.90 *dd*, J = 4.4, 2.4 Hz	5.76 *d*, J = 10.3 Hz (5.83 *d*, J = 10 Hz)
2	2.49 *dd*, J = 3.9, 1.0 Hz	5.65 *ddd*, J = 10.3, 2.7, 2.0 Hz	3.04 *dd*, J = 4.2, 0.7 Hz	3.12 *dd*, J = 4.4, 2.4 Hz	5.59 *d t*, J = 10.3, 2.0 Hz (5.60 *d*, J = 10 Hz)
3	2.80 *dd*, J = 3.9, 0.7 Hz				
4		1.51–1.61 *m* 1.84 *d* like	2.86 *dd*, J = 7.3, 2.7 Hz	2.96 *t*, J = 2.9 Hz	1.60–1.77 *m* 1.90 *m*
5	2.45 *dd*, J = 6.8, 2.9 Hz	1.51–1.61 2H *m*	1.53 *t* like 1.95–2.01 *m*	1.86–1.99 2H, *m*	1.48–1.58 *m* 1.60–1.77 *m*
6	1.09–1.17 *m* 1.64–1.74 *m*	2.19 *br d*	1.95–2.01 *m*	1.86–1.99 *m*	2.29 *m*
7	1.54 *q* like				
8	1.09–1.17 *m* 1.64–1.72 *m*	1.48 *m* 1.71 *m*	1.72–1.82 *m*	1.58–1.80 2H, *m*	1.36–1.47 *m* 1.60–1.77 *m*
9	0.97 *m* 1.64–1.72 *m*	1.51–1.61 2H, *m*	1.63 2H, *m*	1.58–1.80 2H, *m*	1.48–1.58 *m*
10	1.64–1.72 *m*	1.40 *m* 1.74 *m*	1.38 *quit.* like 1.72–1.82 *m*	1.41 *m* 1.58–1.80 *m*	1.36–1.47 *m* 1.60–1.77 *m*
11	1.37 *m*				
12	0.82 3H, *d*, J = 6.6 Hz‡	1.07 3H, *s*	0.98 3H, *s*	1.02 3H, *s*	1.05 3H, *s* (1.07 3H, *s*)
13	1.14 3H, *d*, J = 6.6 Hz‡	0.95 3H, *s*	0.95 3H, *s*	0.93 3H, *s*	0.95 3H, *s* (0.97 3H, *s*)
14	0.71 3H, *d*, J = 7.3 Hz	0.80 3H, *s*	0.92 3H, *s*	0.89 3H, *s*	0.77 3H, *s* (0.78 3H, *s*)
15	1.11 3H, *s*	1.26 3H, *s*	1.47 3H, *s*	1.54 3H, *s*	1.28 3H, *s* (1.28 3H, *s*)

*Measured by 600MHz.
†Measured by 400MHz.
‡May be interchanged.

Table 3. ^{13}C NMR data of compounds **2–5**, **8** and **9**

	2*	3†	4‡	5‡	8*	9‡
1	46.9	132.7	54.1	51.7	130.3	67.2
2	57.7	133.7	50.2	52.5	135.0	143.9
3	52.8	66.9	53.4	52.7	69.7	135.8
4	53.6	37.8	54.5	57.5	39.2	201.5
5	53.7	21.9	21.5	23.4	24.5	36.0
6	26.1	42.4	40.6	37.6	42.10	43.0
7	53.1	47.1	43.8	46.6	47.4	47.2
8	28.4	38.6	40.3	38.9	38.5	36.8
9	31.2	19.0	19.2	19.3	19.0	18.9
10	41.6	42.1	41.9	42.0	42.06	40.8
11	28.6	43.9	47.3	44.0	44.0	44.8
12	22.8§	25.9	25.1	25.4	25.9	25.9
13	22.9§	25.6	24.4	24.8	25.6	25.5
14	17.6	17.6	18.3	18.6	17.6	18.2
15	20.4	29.7	20.6	21.1	28.3	15.5

*In $CDCl_3$(150MHz).
†In C_6D_6(150MHz).
‡In $CDCl_3$(50MHz).
§May be interchanged.

compound was established by the X-ray crystallographic analysis of the monoepoxide **15** derived from **8** [5], as shown in Fig. 6. The IR and ^{13}C NMR spectra of **3** showed the presence of a tertiary hydroxyl group (3340 cm^{-1}, δ_C 66.9 *s*) and the ^1H (Table 2) and ^{13}C NMR (Table 3) spectra resembled those of **8** [5], indicating that **3** might be the same cuparene-type sesquiterpene as **8** [5] with a tertiary hydroxyl group at C-3. The above assumption was also confirmed by the ^1H-^1H, ^{13}C-^1H COSYs and HMBC spectral data of **3** as shown in Table 4. In order to clarify the relative stereochemistry of **3**, a NOESY spectrum was measured. However, no useful information was obtained. From the above evidence, and the slight difference of the chemical shifts of cyclohexene ring in ^{13}C NMR (Table 3) between **3** and **8** [5], we suggested that **3** might be the C-3 epimer of **8** [5]. Matsuo *et al.* [5] reported that **8** epimerize to give **16** during the oxidation reaction and only ^1H NMR chemical shifts of four methyl and a disubstituted olefinic protons has been demonstrated [5]. However, the chemical shifts of four tertiary methyl groups (**3**, δ 0.80, 0.95, 1.07, 1.26; **16** [5], δ 0.82, 0.87, 1.10, 1.20) and two olefinic protons (**3**, δ 5.65, 5.90; **16** [5], δ 5.55, 5.83) were slightly different. Thus, the plane structure of neocuparenenol was depicted as **3**. However, its structure might be the same as **16** from consideration of the co-occurence of the cuparene-type sesquiterpenoids **7–9** [4, 5] obtained from the present species.

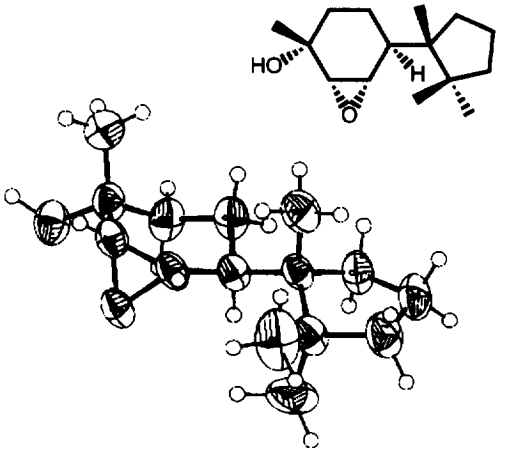


Fig. 6. The ORTEP drawing of compound **15**.

The HR-mass spectra of **4** and **5** showed the same molecular formula $C_{15}H_{24}O_2$ (**4**, $[M]^+$ at *m/z* 236.1782; **5**, $[M]^+$ at *m/z* 236.1781). The IR spectra of both compounds showed no hydroxyl or carbonyl absorption bands, indicating that two oxygen functions are present as ethers. The ^1H NMR (Table 2) and ^{13}C NMR (Table 3) spectra of **4** and **5** closely resembled each other and indicated the presence of three tertiary methyls and three protons (**4**, δ 2.86 *dd*, 3.04 *dd*, 3.09 *d*; **5**, δ 2.90 *dd*, 2.96 *t*, 3.12 *dd*) bearing the oxygen atom. The three methines (**4**, δ 50.2, 54.1, 54.5; **5**,

Table 4. The long-range C/H correlations of compound **3**

H	C
1	3, 5, 6, 7
2	4, 6
4	3, 6
5	3
6	1, 2, 5, 7
12	7, 10, 11, 13
13	7, 10, 11, 12
14	6, 7, 8, 11
15	2, 3, 4

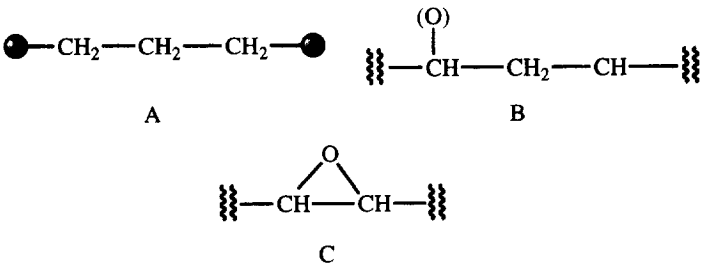


Fig. 7. Partial structures of compound **4**.

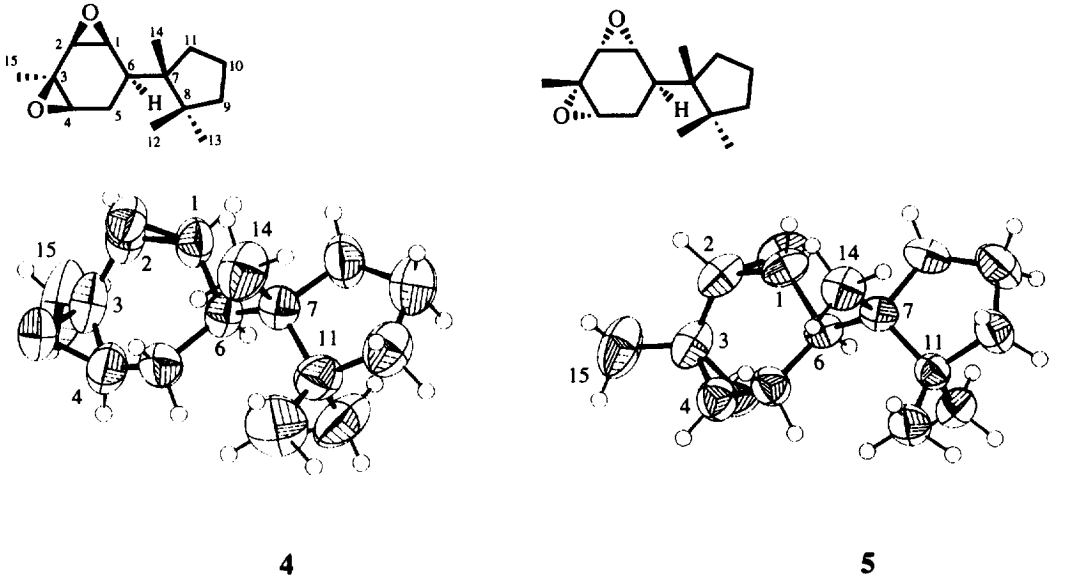


Fig. 8. The ORTEP drawing of compound **4** and **5**.

δ 51.7, 52.5, 57.5) bearing the oxygen atom and a quaternary carbon (**4**, δ 53.4; **5**, δ 52.7) bearing the oxygen atom were confirmed by the ^{13}C NMR spectra of **4** and **5**. Furthermore, the ^{13}C NMR spectra of **4** and **5** contained signals representing four methyls, four methylenes, a methine and two quaternary carbons, respectively. The above spectral data supported the view that compounds **4** and **5** possessed two epoxides in the molecule. The ^1H-^1H and ^{13}C-^1H COSYs of **4** indicated the presence of three partial structures (Fig. 7). The connectivity of the partial structure of **4** was clarified by the HMBC spectrum, as summarized in Table 5. The above evidence indicated that **4** was a cuparane-type sesquiterpenoid with C-1/C-2 and C-3/C-4 diepoxides. The analysis of ^{13}C-^1H COSY and HMBC spectra (Table 5) of **5** supported the suggestion that compound **5** was the diastereoisomer of **4**. Since the stereochemistry of **4** and **5** could not be clarified by the difference NOE spectra, X-ray crystallographic analyses of both compounds were carried out. The ORTEP drawings of **4** and **5** are shown in Fig. 8. The absolute structure of **4** was clarified by MTPA esters **17** and **18** derived from **4**. The modified Moscher's method [13] was applied to the ^1H NMR spectra of **17** and **18** (Table 6 and Fig. 9). Thus, the absolute configuration of C-2, C-6 and C-7 is *S* and that of C-3 is *R*. Therefore, the absolute structure of cuparadiepoxide (**4**) was established to be 1*S*, 2*S*; 3*R*, 4*R*-diepoxy-6*R*, 7*S*-cuparane. The absolute structure of *epi*-cuparadiepoxide (**5**) is depicted as

Table 5. Long range H-C correlations of compound **4** and **5**.

H	C	
	4	**5**
1	2, 4, 5, 6	2, 5, 6, 7
2	1, 3, 15	1, 4, 15
4	3, 5	5, 6
6	4, 5, 8, 11	1, 4, 5, 14
12	7, 10, 11, 13	7, 10, 11, 13
13	7, 10, 11, 12	7, 10, 11, 12
14	8, 11	6, 7, 8, 11
15	2, 3, 4	2, 3, 4

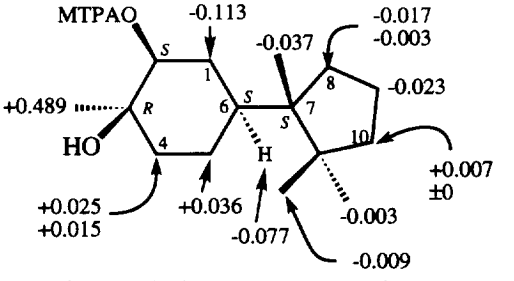


Fig. 9. $\Delta\delta$ values (δS-δR) for MTPA ester of compound **4**.

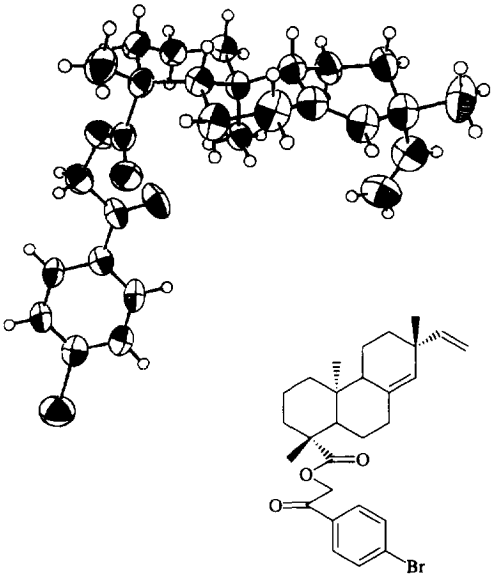


Fig. 10. The ORTEP drawing of compound **13**.

Table 6. ^{1}H NMR data of compounds **17** and **18** ($CDCl_3$, 600 MHz)

H	**18**	**17**	$\Delta\delta$
1	1.622	1.735	−0.113
2	4.844	4.879	−0.034
4	1.355	1.330	+0.025
	1.827	1.812	+0.015
5	1.544	1.508	+0.036
6	1.460	1.537	−0.077
8	1.487	1.504	−0.017
	1.645	1.648	−0.003
9	1.553	1.576	−0.023
10	1.370	1.377	−0.007
	1.720	1.720	±0.000
12	0.986	0.995	−0.009
13	0.916	0.919	−0.003
14	0.796	0.833	−0.037
15	1.516	1.027	+0.489

1*R*, 2*R*; 3*S*, 4*S*-diepoxy-6*R*, 7*S*-cuparane, the epimer of **4**.

(−)-Pimara-8(14),15-dien-19-oic acid (**12**) has already been isolated from two liverworts, *J. thermarum* (= *J. vulcanicola*) [9] and *Mastigophora diclados* (Ptilidiaceae) [10] and the higher plant, *Aralia cordata* (Araliaceae) [11]. The X-ray crystallographic analysis of the *p*-bromophenacyl derivative **13** from **12** was carried out. The R-factor of **13** was 0.056 and Eta value was + 1.15 (alternative chirality; R-factor 0.056, Eta value −1.181) and its absolute structure is depicted in Fig. 10.

In the biosynthesis of the labdane- and pimarane-type diterpenoids, the compounds possessing at 10α-Me-type and 10β-Me-type are known to be biosynthesized by an enzymatically controlled process (Fig. 11) [14], although the presence of both stereo-type compounds is rare in the same plant. Both stereo-type compounds, labda-8, 14-dien-13β-ol (**10**) [6, 7] and 13-*epi*-sclareol (**11**) [8] with a 10β-Me, and (−)-pimara-8(14), 15-dien-19-oic acid (**12**) [9–11] with a 10α-Me were isolated from the present species. This is the first example of the isolation of diterpenoids with both the 10α-Me and 10β-Me groups in the same liverwort species. The isolation of acorane- (**2**) and cuparane-type sesquiterpene diepoxides (**4**, **5**) is the first instance from the Hepaticae. In addition, β-cyclocitral (**6**) is the first isolation of a monoterpenoid from the liverwort as a natural product. Only one example of the isolation of a monoterpene, α-terpineol has been known in *J. vulcanicola* [2]. The present species is chemically close to *J. rosulans* [5] since both species biosynthesize the same cuparane-type sesquiterpenes (**3**–**5**, **7**–**9**), although neither labdane- nor pimarane-type diterpenoids has been found in *J. rosulans* so far examined.

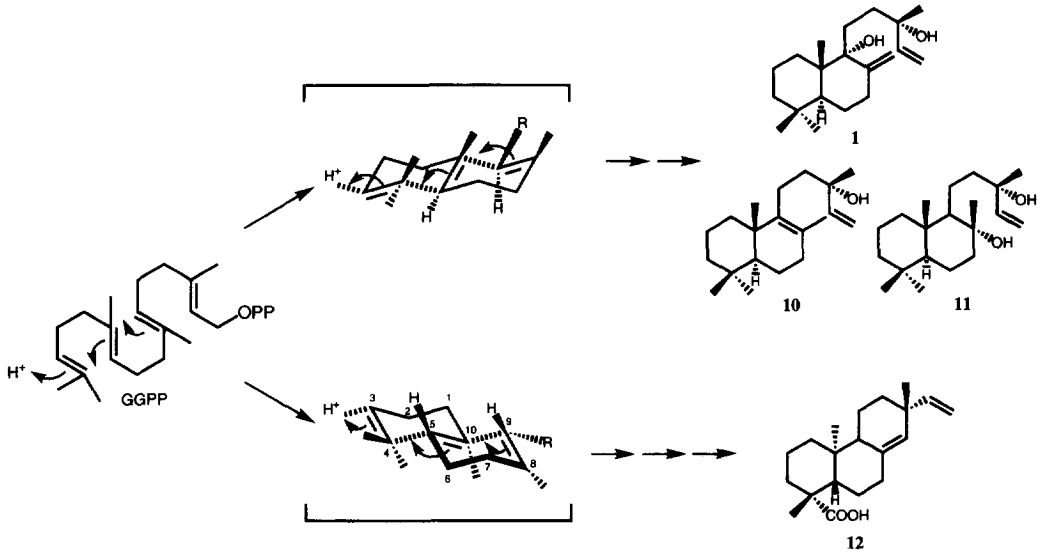


Fig. 11. Formation of the diterpenoids possessing 10α-Me (**12**) and 10β-Me (**1**, **10**, **11**) [14].

EXPERIMENTAL

Mps uncorr. The solvents used for spectra were TMS–$CDCl_3$ [^1H- and ^{13}C NMR] unless otherwise stated; $CHCl_3$ ($[\alpha]_D$); EtOH (UV). MeOH–CH_2Cl_2 (1 : 1) was used for Sephadex LH-20 CC. TLC was carried out using silica gel and detection was with 30% H_2SO_4 or Godin reagent [15].

Plant materials. Jungermannia hattoriana (Amak.) Amak. was collected in Niigata, Japan, 1994 and identified by Dr M. Mizutani (Hattori Bot. Lab.). The voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation. The ground material of *J. hattoriana* (1.14 kg) was extracted with Et_2O for 5 weeks. The crude extract (8.2 g) was divided into 6 fractions by CC on silica gel using an *n*-hexane–EtOAc gradient. Fr. 1 was rechromatographed on silica gel (pentane) impregnated 10% $AgNO_3$ to afford (+)-cuparene (**7**) (684.2 mg) ($[\alpha]_D$ + 58.0 *c* 4.02 $CHCl_3$; lit. [16] $[\alpha]_D$ −27.6). Fr. 2 was rechromatographed on Sephadex LH-20 and silica gel eluted with *n*-hexane–EtOAc (19 : 1) and CH_2Cl_2–Et_2O (1 : 1) and finally purified by the prep. TLC (*n*-hexane–EtOAc, 9 : 1) to give β-cyclocitral (**6**) (3.5 mg) [3]. Fr. 3 was chromatographed on Sephadex LH-20 to divide it into 4 frs. The diterpenoid containing fr. was rechromatographed on silica gel (*n*-hexane–EtOAc, 9 : 1) and finally purified by reverse phase MPLC (Lobar^R column, RP-18, MeOH) to give labda-8,14-dien-13β-ol (**10**) (101.2 mg) [6, 7]. Fr. 4 was chromatographed on Sephadex LH-20 and silica gel (*n*-hexane–EtOAc, 9 : 1) to divide it into Fr. A–D. Fr. B was purified by the prep. HPLC (NUCLEOSIL 50-5, *n*-hexane–EtOAc, 9 : 1) and prep. reverse phase TLC (RP-8, MeOH) to afford acoradiepoxide (**2**) (3.6 mg). Cuparadiepoxide (**4**) (22.6 mg) and *epi*-cuparadiepoxide (**5**) (18.5 mg) were purified by the CC on silica gel (CH_2Cl_2–Et_2O, 49 : 1), MPLC (Lobar^R column, CH_2Cl_2–Et_2O, 49 : 1) and finally prep. HPLC (NUCLEOSIL 50-5, *n*-hexane–EtOAc, 9 : 1) of the Fr. C. Fr. D was rechromatographed on MPLC (Lobar^R column, *n*-hexane–EtOAc, 9 : 1) to give (−)-pimara-8(14), 15-dien-19-oic acid (**12**) (10.0 mg) [9–11]. Fr. 5 was divided into Fr. 5A–5E by CC on Sephadex LH-20 and silica gel (*n*-hexane–EtOAc, 9 : 1). Cuparadiepoxide (**4**) (6.0 mg) and *epi*-cuparadiepoxide (**5**) (9.5 mg) was purified by the CC on Sephadex LH-20, silica gel (*n*-hexane–EtOAc, 4 : 1) and MPLC (Lobar^R column, *n*-hexane–EtOAc 4 : 1) of Fr. 5B. Fr. 5C was chromatographed on silica gel (CH_2Cl_2–Et_2O, 19 : 1) to divide it into two fractions. The first fraction was rechromatographed on MPLC (Lobar^R column, *n*-hexane–Et_2O, 1 : 1) and prep. HPLC (NUCLEOSIL 50-5, *n*-hexane–Et_2O, 1 : 1) to afford (+)-labda-8(17), 14-diene-9*R**, 13*S**-diol (**1**) (12.8 mg) and rosulantol (**9**) (32.0 mg) [5]. The second fraction was also rechromatographed on MPLC (Lobar^R column, *n*-hexane–Et_2O, 7 : 3) and prep. HPLC (NUCLEOSIL 50-5, CH_2Cl_2–Et_2O, 9 : 1) to give neocuprenenol (**3**) (5.6 mg) and (+)-cuprenenol (**8**) (36.0 mg) [5]. Fr. 6 was chromatographed on Sephadex LH-20 and silica gel (CH_2Cl_2–Et_2O, 7 : 3) and finally purified by the recrystallization from *n*-hexane to afford 13-*epi*-sclareol (**11**) (35.8 mg) [8].

(+)-*Labda*-8(17), 14-*diene*-9*R**, 13*S**-*diol* (**1**). mp 124–125 °; $[\alpha]_D$ + 34.2 (*c* 3.60); HREIMS: found 306.2566 $C_{20}H_{34}O_2$ requires 306.2559. FTIR ν_{max} cm^{-1}: 3450 (OH); ^1H and ^{13}C NMR: Table 1. EIMS *m/z* (rel. int.): 306[M]$^+$(1), 288(74), 273(8), 255(7), 243(1), 220(28), 205(29), 189(9), 177(35), 163(38), 151(100), 135(23), 123(44), 109(38), 95(33), 81(41), 69(33), 55(23), 41(22), 32(63).

Acoradiepoxide (**2**): $[\alpha]_D$ − 76.8 (*c* 1.44); HREIMS: found 236.1768 $C_{15}H_{24}O_2$ requires 236.1776. FTIR ν_{max} cm^{-1}: 3000, 1480, 1390. ^1H NMR and ^{13}C NMR (C_6D_6): Tables 2 and 3. ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88(3H, *d*, *J* = 6.4 Hz), 1.00(3H, *d*, *J* = 7.3 Hz), 1.02(3H, *d*, *J* = 7.3 Hz), 1.23(1H, *m*), 1.37(1H, *m*), 1.48(1H, *m*), 1.49(3H, *s*), 1.59(1H, *dd*, *J* = 5.4, 3.4 Hz), 1.76(1H, like *q*), 1.88–2.05(4H, *m*), 2.84(1H, *d*, *J* = 3.9 Hz), 2.86(1H, *d*, *J* = 3.9 Hz), 3.22(1H, *d*, *J* = 3.9 Hz). ^{13}C-NMR (100 MHz): δ 46.7 (C-1), 57.7 (C-2), 52.8 (C-3), 54.4 (C-4), 54.5 (C-5), 25.8 (C-6), 52.9 (C-7), 28.0 (C-8), 31.0 (C-9), 41.7 (C-10), 28.3 (C-11), 22.6 (×2, C-12, 13), 17.6 (C-14), 20.4 (C-15); EIMS *m/z* (rel. int.): 236[M]$^+$(1), 221(3), 203(3), 193(15), 175(13), 165(6), 149(38), 137(31), 123(55), 107(46), 95(48), 85(46), 81(100), 69(44), 55(43), 43(72), 32(10).

Neocuprenenol (**3**). mp 96–98°; $[\alpha]_D$ + 18.0 (*c* 1.00); HREIMS: found 222.1986 $C_{15}H_{26}O$ requires 222.1985. FTIR ν_{max} cm^{-1}: 3340 (OH). ^1H NMR and ^{13}C NMR: Tables 2 and 3. EIMS *m/z* (rel. int.): 222[M]$^+$(1), 207(8), 189(3), 151(4), 137(10), 111(100), 94(96), 79(11), 69(75), 55(28), 43(14).

Cuparadiepoxide (**4**). mp 72–74 °; $[\alpha]_D$ + 12.9 (*c* 0.87); HREIMS: found 236.1782 $C_{15}H_{24}O_2$ requires 236.1776. FTIR ν_{max} cm^{-1}: 3000, 2900, 1480, 1390. ^1H and ^{13}C-NMR: Tables 2 and 3. EIMS *m/z* (rel. nit): 236[M]$^+$(1), 221(1), 207(1), 193(3), 165(5), 151(11), 135(11), 119(13), 111(48), 93(47), 81(36), 69(100), 55(62), 43(53).

epi-*Cuparadiepoxide* (**5**). mp 88–90 °; $[\alpha]_D$ + 1.46 (*c* 5.72); HREIMS: found 236.1781 $C_{15}H_{24}O_2$ requires 236.1776. FTIR ν_{max} cm^{-1}: 3000, 2900, 1480, 1390. ^1H NMR and ^{13}C-NMR: Tables 2 and 3. EIMS *m/z* (rel. int.): 236[M]$^+$(1), 221(2), 207(12), 193(4), 175(6), 165(11), 151(14), 135(17), 123(22), 111(42), 95(45), 82(46), 69(100), 55(73), 43(73), 32(3).

(R)- *and* (S)-*MTPA esterfication of* **4**. To a suspension of $LiAlH_4$ (12 mg) in dry Et_2O (4 ml) was added **4** (12 mg) in Et_2O and the mixture stirred at room temp. for 5 min. Work-up as usual gave a dialcohol (14 mg). To the dialcohol (6 mg) in CH_2Cl_2 (1 ml) was added (*R*)-α-methoxy-α-trifluorophenylacetic acid (MTPA, 2 mg), dicyclohexylcarbodiimide (2 mg) and DMAP (5 mg), and the mixture allowed to stand at room temp. for 5 hr. The residue was purified by

prep. TLC (*n*-hexane–EtOAc, 9 : 1) to give (*R*)-MTPA ester **17** (7.5 mg). The esterification of a remaining dialcohol (8 mg) with (*S*)-MTPA in the same manner as described above afforded (*S*)-MTPA ester **18** (7.3 mg). ^{1}H NMR spectra of **17** and **18**—see Table 6.

Esterification of **12**. A suspension of KF (5 mg) and *p*-bromophenacylbromide (6 mg) in DMF (0.5 ml) was stirred for 1 min and then **12** (5 mg) in DMF (0.5 ml) was added and the mixture stirred at room temp. for 30 min. The reaction mixture was extracted with Et_2O and washed with satd. NaCl, dried $MgSO_4$, filtered, evapd and finally purified by prep. TLC (*n*-hexane–EtOAc, 4 : 1) to give **13** (6.5 mg); ^{1}H NMR (200 MHz): δ 0.63 (3H, *s*), 1.00 (3H, *s*), 1.33 (3H, *s*), 4.91 (1H, *dd* like), 4.94 (1H, *dd* like), 5.14 (1H, *d*, J = 16.2 Hz), 5.15 (1H, *br s*), 5.33 (1H, *d*, J = 16.2 Hz), 5.71 (1H, *dd*, J = 16.9, 10.8 Hz), 7.62 (2H, *d*, J = 9.5 Hz), 7.77 (2H, *d*, J = 9.5 Hz).

Epoxidation of **8**. To **8** (23 mg) in dry CH_2Cl_2 (2 ml) was added *m*-chloroperbenzoic acid (38 mg) and the mixture stirred at room temp. for 90 min. The reaction mixture was extracted with CH_2Cl_2 and the extract was washed successively with 10% $Na_2S_2O_3$, 5% $NaHCO_3$ and satd NaCl, dried with $MgSO_4$ and finally purified by recrystallyzation from pentane to afford **15** (21.8 mg): mp. 113–114°; $[\alpha]_D$ + 19.0 (*c* 1.39); HREIMS: found 238.1933 $C_{15}H_{26}O_2$ requires 238.1933: FTIR ν_{max} cm^{-1}: 3450(OH), 3000, 2900, 1470, 1390. ^{1}H NMR (400 MHz): δ 3.37 (1H, *dd*, J = 2.4, 1.5 Hz), 2.93 (1H, *d*, J = 3.9 Hz), 1.94 (1H, *dd*, J = 11.7, 5.9 Hz), 1.32, 1.11, 1.05, 0.91 (each 3H, *s*). ^{13}C-NMR (100 MHz): δ 17.6, 23.0, 25.9, 26.0 (each *q*), 18.8, 23.9, 34.5, 38.6, 41.7 (each *t*), 40.0, 58.1, 60.2 (each *d*), 44.2, 47.0, 70.2 (each *s*). EIMS *m/z* (rel. int.): 238[M]$^+$(1), 220(7), 205(10), 191(9), 177(22), 164(21), 149(21), 137(47), 123(42), 109(100), 95(66), 82(68), 69(81), 55(56), 43(67).

X-ray crystallographic analysis. All X-ray crystallographic analysis were carried out on a Mac Science MXC 18 diffractometer with Cu Kα radiation. The structures of compounds **1**, **4**, **9** and **15** were solved by direct methods using *SHELXS*-86 and refined by full-matrix least-squares. The structure of compound **5** was solved by direct methods using *Montecarlo Multane* and refined by full-matrix least-squares. The structures of compounds **11** and **13** were solved by direct methods using *CRYSTAN SIR*92 and refined by full-matrix least-squares using *CRYSTAN*.

The crystal data of **1**. Compound **1** was recrystallized from *n*-hexane. $M_f = C_{20}H_{34}O_2$, M_r = 306.00, Orthorhombic, $P2_12_12_1$ (#19), a = 14.859 (4), b = 16.193 (5), c = 7.755 (3) Å, V = 1866 (1) Å^3, Z = 4, D_x = 1.09 g cm^{-3}, μ = 4.53 cm^{-1} (Cu Kα), λ (Cu Kα) = 1.54178, F (000) = 680, Maximum $\sin(\theta)/\lambda$ = 0.580, Total reflections measured 1609, Unique reflections 1563, Rint = 0.00, Reflections used 1366, no. of Variables 302, R = 0.066, Rw = 0.058, S = 1.37.

The crystal data of **4**. Compound **4** was recrystallized from *n*-pentane. $M_f = C_{15}H_{24}O_2$, M_r = 236.00, Orthorhombic, $P2_12_12_1$ (#19), a = 8.848 (2), b = 23.956 (5), c = 6.725 (2) Å, V = 1425.5 (5) Å^3, Z = 4, D_x = 1.10 g cm^{-3}, μ = 4.84 cm^{-1} (Cu Kα), λ (Cu Kα) = 1.54178, F (000) = 520, Maximum $\sin(\theta)/\lambda$ = 0.580, Total reflections measured 1431, Unique reflections 1384, Rint = 0.00, Reflections used 1224, No. of Variables 236, R = 0.055, Rw = 0.055, S = 1.51.

The crystal data of **5**. Compound **5** was recrystallized from *n*-pentane. $M_f = C_{15}H_{24}O_2$, M_r = 236.00, Orthorhombic, $P2_12_12_1$ (#19), a = 8.013 (2), b = 22.515 (5), c = 7.506 (2) Å, V = 1354.3 (6) Å^3, Z = 4, D_x = 1.16 g cm^{-3}, μ = 5.09 cm^{-1} (Cu Kα), λ (Cu Kα) = 1.54178, F (000) = 520, Maximum $\sin(\theta)/\lambda$ = 0.580, Total reflections measured 1359, Unique reflections 1316, Rint = 0.00, Reflections used 1271, No. of Variables 236, R = 0.037, Rw = 0.047, S = 1.69.

The crystal data of **9**. Compound **9** was recrystallized from *n*-hexane–Et_2O. $M_f = C_{15}H_{24}O_2$, M_r = 236.00, Trigonal, $P3_2$ (#145), a = 6.851 (1), b = 6.851 (1), c = 25.282 (4) Å, V = 1027.7 (4) Å^3, Z = 3, D_x = 1.14 g cm^{-3}, μ = 5.03 cm^{-1} (Cu Kα), λ (Cu Kα) = 1.54178, F(000) = 390, Maximum $\sin(\theta)/\lambda$ = 0.579, Total reflections measured 3682, Unique reflections 1106, Rint = 0.03, Reflections used 1065, No. of Variables 235, R = 0.048, Rw = 0.055, S = 1.88.

The crystal data of **11**. Compound **11** was recrystallized from Et_2O. $M_f = C_{20}H_{36}O_2$, M_r = 208.00, Orthorhombic, $P2_12_12_1$, a = 11.298 (3), b = 25.669 (6), c = 6.596 (3) Å, V = 1913.0 (1) Å^3, Z = 4, D_x = 0.868 Mg m^{-3}, μ = 4.794 cm^{-1} (Cu Kα), λ (Cu Kα) = 1.54178, Measured reflections 1455, Independent reflections 927, Rint = 0.0, Refinement on *F*, Reflections 927, Parameters 206, R = 0.058, Rw = 0.065, S = 2.027.

The crystal data of **13**. Compound **13** was recrystallized from *n*-hexane. R-factor of **13** is 0.056 and an Eta value is +1.15 (alternative chirality: R-factor 0.056, Eta value −1.181). $M_f = C_{28}H_{45}O_3Br$, Mr = 507.00, Orthorhombic, $P2_12_12_1$, a = 9.473 (4), b = 38.36 (1), c = 6.841 (2) Å, V = 2485.969971 (1) Å^3, Z = 4, D_x = 1.317 Mg m^{-3}, μ = 24.528 mm^{-1} (Cu Kα), λ (Cu Kα) = 1.54178, Measured Reflections 2489, Independent Reflections 2126, Observed Reflections 2114 [$I > 2.00\ \sigma(I)$], Rint = 0.0, Refinement on *F*, Reflections 2114, Parameters 396, R = 0.056, Rw = 0.073, S = 1.093 (alternative chirality: R = 0.056, Rw = 0.074, S = 1.108).

The crystal data of **15**. Compound **15** was recrystallized from *n*-pentane. $M_f = C_{15}H_{26}O_2$, M_r = 238.00, Monoclinic, $P2_1$ (#4), a = 11.377 (3), b = 6.586 (2), c = 9.699 (3) Å, β = 105.07 (2)°, V = 701.8 (4) Å^3, Z = 2, D_x = 1.13 g cm^{-3}, μ = 4.91 cm^{-1} (Cu Kα), λ (Cu Kα) = 1.54178, F (000) = 264, Maximum $\sin(\theta)/\lambda$ = 0.580, Total reflections measured 1104, Unique reflections 1001, Rint = 0.02, Reflections used 967, No. of Variables 241, R = 0.043, Rw = 0.050, S = 1.76.

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