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SESQUI- AND DI-TERPENOIDS FROM THE LIVERWORT *JUNGERMANNIA VULCANICOLA*

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Key Word Index—*Jungermannia vulcanicola*; Jungermanniaceae; Hepaticae; chiloscypholone; dihydrochiloscypholone; *ent*-13-epi-manool; chiloscyphane-type; labdane-type; sesquiterpenoid; diterpenoid; chemo-type.

Abstract—A new chiloscyphane-type sesquiterpenoid, dihydrochiloscypholone, was isolated from the liverwort *Jungermannia vulcanicola*, together with the previously known chiloscypholone. Another collection of *J. vulcanicola* gave a new labdane-type diterpenoid along with the previously known *ent*-13-epi-manool. Their structures were determined by extensive NMR techniques and chemical transformation. There are at least three chemo-types of *J. vulcanicola*.

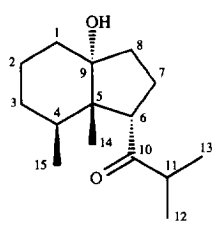
INTRODUCTION

We are continuing to study the chemical constituents of liverworts in our search for biologically active substances [1, 2]. Most of the liverworts are morphologically small and the identification of each species is quite difficult. The *Jungermannia* species contain mainly diterpenoids such as those of the *ent*-kaurane-, clerodane-, trachylobane- and labdane-type [1, 2]. We reported the isolation of *ent*-kaurane-type diterpenoids from *J. vulcanicola* collected in Nagano, Japan [3]. Reinvestigation of the chemical constituents of *J. vulcanicola* collected in Okayama and in Shiga, Japan, resulted in the isolation of a new chiloscyphane-type sesquiterpenoid (**1**) and a previously known chiloscyphane-type sesquiterpenoid, chiloscypholone (**2**), from the material collected in Okayama and a new labdane-type diterpenoid (**3**) and a known labdane-type diterpenoid, *ent*-13-epi-manool (**4**), from the collection from Shiga. In this paper, we report on the isolation and characterization of the new compounds.

RESULTS AND DISCUSSION

A new chiloscyphane-type sesquiterpenoid, dihydrochiloscypholone (**1**), was isolated from the ether extract of *J. vulcanicola* collected in Okayama, along with the known sesquiterpenoid, chiloscypholone (**2**), which was isolated from the liverwort *Chiloscyphus pallescens* [4] and its absolute structure determined by X-ray

The IR spectrum of **1**, $C_{15}H_{26}O_2$ (*m/z* 238.1938), indicated the presence of a hydroxyl (3400 cm^{-1}) and a carbonyl group (1700 cm^{-1}). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) closely resembled those of **2** [4, 5], except for the presence of four secondary methyls (δ_H 0.69, 0.89, 1.08 and 1.15, each *d*, J = 6.8 Hz), indicating that **1** was a dihydro derivative of **2** [4, 5], i.e. a chiloscyphane-type sesquiterpenoid. This assumption was confirmed by the hydrogenation of **2** on 10% Pd–C to give a dihydro derivative, whose spectral data were completely identical with those for **1**.



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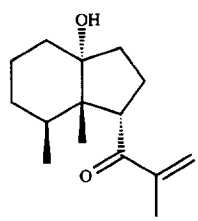

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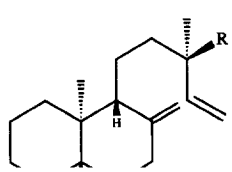
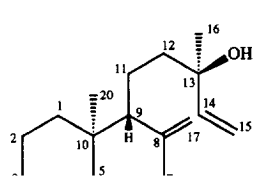




Thus, the absolute structure of dihydrochiloscypholone was established to be **1**.

The ether extract of *J. vulcanicola* collected in Shiga gave a new labdane-type diterpenoid (**3**) and the known *ent*-13-epi-manool (**4**) [6].

The EI-mass spectrum of **3** showed *m/z* 306 $[M]^+$ and its molecular formula was found to be $C_{20}H_{34}O_2$ by high-resolution mass spectrometry. The IR and ^{13}C NMR spectra of **3** indicated the presence of two tertiary hydroxyl groups (3400 cm^{-1}, δ_C 73.6 and 77.4, each *s*). The ^1H NMR spectrum (Table 3) showed the presence of four tertiary methyls, an *exo*-methylene group (δ 4.53 *d* and 4.83 *dd*) and a terminal vinyl group (δ 5.05, 5.20 and 5.91, each *dd*). The ^{13}C NMR spectrum (Table 2) displayed 20 carbons; four methyls, seven methylenes, a methine and two quaternary carbons, along with an *exo*-methylenic carbon, a terminal vinyl carbon and two quaternary carbons each with a tertiary hydroxyl group. These spectral data were similar to those for **4** [6] isolated from the present species, indicating that **3** was a labdane-type diterpenoid with two tertiary hydroxyl groups. The ^1H–^1H and ^{13}C–^1H COSY spectra of **3** showed the presence of three partial structures: (i) –CH(14)=CH_2(15), (ii) –CH(9)–CH_2(11)–CH_2(12)– and (iii) –CH_2(6)–CH_2(7)–. The long-range ^{13}C–^1H correlations observed by HMBC are shown in Fig. 1. Thus, the above spectral evidence established that **3** was a labdane-type diterpenoid with two tertiary hydroxyl groups at C-5 and C-13. In the NOESY spectrum of **3** in pyridine-d_5, NOEs were observed between (i) H-20 and H-18, (ii) H-2α and H-18 and 20, and (iii) H-9 and H-1β and 7β. On comparison of the ^{13}C NMR spectra of **3** and **4** [6], the signals assigned to C-1, C-3, C-7 and C-9 were observed in the most upfield regions as a result of the γ-effects [7] of the tertiary hydroxyl group. Furthermore, of the pyridine-induced solvent shifts [8] observed in ^1H NMR spectrum of **3**, the shifts of H-1 (Δ 0.52), H-3 (Δ 0.48), H-7 (Δ 0.45) and H-9 (Δ 0.61) also supported the stereochemistry of the hydroxyl group at C-5 as β. The stereochemistry of the hydroxyl group at C-13 was clarified by the circular dichroic (CD) spectrum of the *p*-bromobenzoate (**5**) of **3**. The CD spectrum of **5** showed a negative Cotton effect ($\Delta\varepsilon_{258}$ − 0.80), indicating that the configuration of C-13 was *R* [9, 10]. Therefore, the structure of **3** was elucidated as *ent*-8(17), 14-labdadiene-5α, 13*R*-diol.

We have already reported the isolation of *ent*-kaurane-type diterpenoids from the Japanese *J. vulcanicola* collected in Nagano [3]. From one of the

Table 2. ^{13}C NMR data for compounds **1**–**4** (100 MHz, $CDCl_3$)

C	1*	2	3	4†
1	29.9	29.9	31.9	39.1
2	20.6	20.6	18.8	19.4
3	29.4	29.4	36.3	42.2
4	29.5	29.6	38.6	33.5
5	53.6	53.8	77.4	55.6
6	57.2	52.0	28.9	24.5
7	222.4	209.0	32.7	38.4
8	27.6	27.8	148.4	148.7
9	38.2	38.4	48.6	57.4
10	81.4	81.4	43.7	39.9
11	43.1	145.6	17.7	17.7
12	17.3‡	18.0	41.3	41.5
13	18.7‡	125.8	73.6	73.6
14	20.5	20.7	145.3	145.3
15	17.7	17.4	111.5	111.5
16			27.7	27.7
17			106.6	106.5
18			24.4	33.5
19			28.0	21.7
20			17.8	14.5

*Measured at 50 MHz.
†Measured at 150 MHz.
‡May be interchanged.

Table 1. ^1H NMR data for compounds **1** and **2** ($CDCl_3$, 400 MHz)

H	1	2
1	1.37–1.50 *m*	1.50 *m*
	1.63–1.93 *m*	1.66–1.84 *m*
2	1.37–1.50 *m*	1.46 *m*
	1.63–1.93 *m*	1.85–1.97 *m*
3	1.37–1.50 *m*	1.16 *dddd* (*J* = 11.7, 11.7, 11.7, 8.3 Hz)
	1.17 *m*	1.41 *m*
4	2.07–2.20 *m*	2.16 *m*
6	3.04 *dd* (*J* = 10.3, 4.9 Hz)	3.55 *dd* (*J* = 10.3, 4.9 Hz)
8	2.07–2.20 2H, *m*	1.66–1.84 *m*
		2.10 *m*
9	1.63–1.90 2H, *m*	1.66–1.84 *m*
		1.85–1.97 *m*
11	2.78 *sept.* (*J* = 6.8 Hz)	
12	1.08* 3H, *d* (*J* = 6.8 Hz)	1.91 3H, *d* (*J* = 1.0 Hz)
13	1.15* 3H, *d* (*J* = 6.8 Hz))	5.84 *d* (*J* = 1.5 Hz)
		6.07 *s*

Table 3. ^{1}H NMR data for compound **3** (600 MHz)

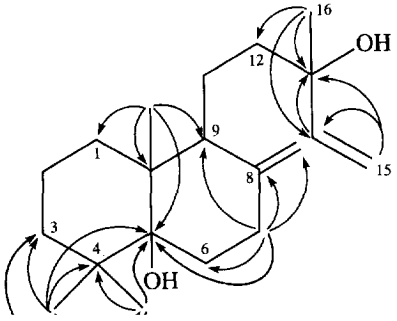
H	$CDCl_3$	C_6D_6	Pyridine-d_5
1	1.44 2H, *m*	1.36–1.40 *m*, α	1.52–1.58 *m*, α
		1.44–1.57 *m*, β	1.96 *m*, β
2	1.63–1.68 *m*, α	1.44–1.57 *m*, α	1.70 *m*, α
	1.50 *m*, β	1.36–1.40 *m*, β	1.52–1.58 *m*, β
3	1.15 *dd* (*J* = 9.0, 2.9 Hz), α	1.00 *br d*, α	1.14 *m*, α
	1.63–1.68 *m*, β	1.58–1.65 *m*, β	2.13 *m*, β
6	1.76 *m*, α	1.58–1.65 *m*, α	1.85 2H, *m*
	1.62 *m*, β	1.36–1.40 *m*, β	
7	2.18 *ddd* (*J* = 13.7, 5.1, 2.2 Hz), α	2.12 like *dd*, α	2.28 like *d*, α
	2.39 *ddd* (*J* = 13.7, 13.7, 5.1 Hz), β	2.46 *ddd* (*J* = 13.2, 13.2, 4.6 Hz), β	2.84 *ddd* (*J* = 12.5, 12.5, 7.1 Hz), β
9	2.52 *d* (*J* = 9.3 Hz)	2.68 *br d*	3.13 *br s*
11	1.36 *m*	1.44–1.57 2H, *m*	1.21 2H, *m*
	1.41–1.47 *m*		
12	1.33 *m*	1.27 *m*	1.52–1.58 *m*
	1.78 *m*	1.84 *m*	2.11 *m*
14	5.91 *dd* (*J* = 17.3, 10.7 Hz)	5.78 *dd* (*J* = 17.3, 10.7 Hz)	6.20 *dd* (*J* = 17.3, 10.7 Hz)
15	5.05 *dd* (*J* = 10.7, 1.5 Hz)	4.95 *dd* (*J* = 10.7, 1.5 Hz)	5.16 *dd* (*J* = 10.7, 2.2 Hz)
	5.20 *dd* (*J* = 17.3, 1.5 Hz)	5.19 *dd* (*J* = 17.3, 1.5 Hz)	5.58 *dd* (*J* = 17.3, 2.2 Hz)
16	1.27 3H, *s*	1.13 3H, *s*	1.49 3H, *s*
17	4.53 *d* (*J* = 1.7 Hz)	4.78 *d* (*J* = 1.7 Hz)	5.00 *br s*
	4.83 *dd* (*J* = 3.4, 1.7 Hz)	4.98 *d* (*J* = 1.7 Hz)	5.88 *br s*
18	1.01 3H, *s*	0.88 3H, *s*	1.12 3H, *s*
19	0.93 3H, *s*	0.83 3H, *s*	1.05 3H, *s*
20	0.85 3H, *s*	0.80 3H, *s*	0.96 3H, *s*

above two *J. vulcanicola* collections, chiloscyphane-type sesquiterpenoids were isolated as the main components while from the other collection a labdane-type diterpenoid was the main component. Thus, it seems that there are at least three chemo-types of *J. vulcanicola* in Japan.

EXPERIMENTAL

Mps: uncorr. The solvents used for spectral measurements were TMS–$CDCl_3$ (^{1}H and ^{13}C NMR); $CHCl_3$ ($[\alpha]_D$), MeOH (CD and UV). TLC was carried out as previously reported [11].

Plant material. Jungermannia vulcanicola (Schiffn.) Steph. was collected in Okayama, Japan, in 1994, and in Shiga, Japan, in 1994, and identified by Dr M. Mizutani (Hattoir Botanical Laboratory, Japan). The voucher specimens were deposited at the Institute of Pharmacognosy, Tokushima Bunri University.



Extraction and isolation. The ground material (14 g) of *J. vulcanicola* from Okayama was extracted with Et_2O. The crude extract (420 mg) was divided into two frs by CC on Sephadex LH-20 (CH_2Cl_2–MeOH; 1:1). Fr. 2 was rechromatographed on silica gel (*n*-hexane–EtOAc, 4:1) and finally purified by prep. HPLC (Nucleosil 50-5, *n*-hexane–EtOAc, 5:1) to give **2** [4, 5] (34 mg) and **1** (5 mg): crystals; mp 80–82°; $[\alpha_D]$ + 147.0° (*c* 0.46); HREIMS: Found $[M]^+$ 238.1938; $C_{15}H_{26}O_2$ requires 238.1933; FTIR ν_{max} cm^{-1}: 3400 (OH), 1700 (C=O); ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 238$[M]^+$(7), 220(9), 210(13), 195(12), 177(22), 167(11), 159(3), 145(100), 139(32), 122(17), 107(16), 93(19), 81(11), 69(13), 55(13), 43(22).

The ground material (12 g) of *J. vulcanicola* from Shiga was extracted with Et_2O. The crude extract (350 mg) was divided into two by CC on Sephadex LH-20 (CH_2Cl_2–MeOH, 1:1). Fr. 2 was rechromatographed on silica gel (*n*-hexane–EtOAc; 9:1) to give **4** [5] (6 mg) and **3** (39 mg): oil; $[\alpha]_D$ −51.4° (*c* 3.87); HREIMS: Found $[M]^+$ 306.2562; $C_{20}H_{34}O_2$ requires 306.2559; FTIR ν_{max} cm^{-1}: 3500 (OH); ^{13}C and ^{1}H NMR: Tables 2 and 3; EIMS *m/z* (rel. int.): 306$[M]^+$(1), 288(10), 270(26), 260(49), 242(19), 227(11), 201(35), 189(31), 177(12), 159(27), 147(23), 134(35), 119(29), 111(100), 95(35), 81(38), 69(67), 55(35), 41(28).

Catalytic hydrogenation of **2**. Compound **2** (20 mg) in EtOH (5 ml) was hydrogenated in the presence of

benzoylchloride (20 mg) and 4-dimethylaminopyridine (3 mg) in pyridine (2 ml) was stirred at room temp. for 3 days. The resulting mixt. was diluted with H_2O and then extracted with Et_2O. The Et_2O layer was washed with 1N HCl, 5% $NaHCO_3$ and satd NaCl, successively, and dried over $MgSO_4$. The residue was purified by prep. TLC to give the benzoate **5** (0.5 mg); UV λ_{max}nm (log ε): 243 (1.57), 206 (1.43) (c 5.4×10^{-5}); ^{1}H NMR: δ 0.83, 0.93, 1.00, 1.68 (each 3H *s*), 2.58 (1H, *d*, $J = 9.8$ Hz), 4.53 (1H, *d*, $J = 1.5$ Hz), 4.85 (1H, *d*, $J = 2.0$ Hz), 5.21 (1H, *dd*, $J = 18.1$, 1.0 Hz), 6.07 (1H, *dd*, $J = 17.6$, 10.7 Hz), 7.56 (2H, *d*, $J = 8.8$ Hz), 7.86 (2H, *d*, $J = 8.8$ Hz); CD: $\Delta\varepsilon_{258} - 0.80$ (c 3.2×10^{-4}).

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