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LABDANOIDS AND BIS(BIBENZYLS) FROM *JUNGERMANNIA* SPECIES

CHIA-LI WU* and HSIANG-RU LIN

Department of Chemistry, Tamkang University, Tamsui, Taiwan 251, R.O.C.

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Key Word Index—*Jungermannia appressifolia*, *J. infusca*; Jungermanniaceae; liverwort; labda-8,14-dien-13-ol; labda-7,14-dien-9,13-diol; 13-hydroxy-7-oxo-labda-8,14-diene; marchiantin C; riccardin C.

Abstract—Two Taiwanese liverworts, *Jungermannia appressifolia* and *J. infusca*, were chemically investigated. *Jungermannia appressifolia* elaborated two new labdane-type diterpenoids, labda-7,14-dien-9,13-diol and 13-hydroxy-7-oxo-labda-8,14-diene, along with the known labda-8,14-dien-13-ol. *Jungermannia infusca* produced two macrocyclic bis(bibenzyls), marchantin C and riccardin C, as its major constituents demonstrating the fourth chemo-type of this complex species. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

The liverwort *Jungermannia appressifolia* was collected in the middle mountainous (1700 m) area of Taiwan. The plants appear blackish in colour, and grow in a pure mat along the roadside slope. The species is reported to be quite common in the Himalayas, but rare in the Indomalayan region [1, 2]. Its chemistry has not been investigated. *Jungermannia infusca*, on the other hand, was earlier thought to be endemic in Japan [3], but was later found in Taiwan, SE China and Korea [2]. Its chemistry had been studied extensively and was found to be as complex as its morphology [4–6]. In the pesent study, three labdane-type diterpenoids (**1–3**) were isolated from *J. appressifolia* and two of them (**2**, **3**) are new. From *J. infusca*, two known macrocyclic bis(bibenzyls) (**4**, **5**) were identified as the major components. Previously, diterpenoids of labdane-, clerodane- and kaurane-types were found in *J. infusca* [6]. The only bis(bibenzyl) compound isolated from *Jungermannia* species was perrottetin E (**6**) from *J. comata* [6].

RESULTS AND DISCUSSION

Column chromatography on silica gel of EtOAc extract of the crushed material of *J. appressifolia* resulted in the isolation of three major diterpenes: labda-8,14-dien-13-ol (**1**), labda-7,14-dien-9,13-diol (**2**), and 13-hydroxy-7-oxo-labda-8,14-diene (**3**).

Compounds **1–3** were closely related, as followed from the ^{1}H NMR spectra. In all cases, the characteristic signals of a terminal vinyl group, a vinyl methyl and four methyls at quaternary carbons were present (Table 1). In the ^{13}C NMR spectrum of the main compound (**1**), a tetrasubstituted double bond and a tertiary hydroxyl group were detected. Since the only position to accommodate the tetrasubstituted double bond in a labdane skeleton is between C-8 and C-9, the structure for compound **1** could be easily dervied. Compound **1** had been isolated from a higher plant [8]. Although the shifts of the reported typical protons were comparable with ours, the identity of compound **1** was only assured after a subsequent HMBC experiment.

The NMR spectra of compound **2** differed from those of **1** due to the presence of a trisubstituted double bond and an additional tertiary hydroxyl group at a relatively deshielded position (δ_C 90.9). Again the structure was unambiguously shown to be that of labda-7,14-dien-9,13-diol (**2**) on the basis of the HMBC data. Although structure **2** has not been reported previously, its exocyclic double bond isomer, jungermanool (**7**), was a component of another liverwort *J. torticalyx* along with manool (**8**) [9]. The axial nature of the hydroxyl group at C-9 was supported by the obvious upfield shifts of C-1 and C-5 on comparison of the ^{13}C NMR data of **1** and **2** (Table 2) as a result of the γ-gauche effects [10]. The EI-mass spectra of compound **2** was quite unique with just one fragment *m/z* 164 ($C_{11}H_{16}O$, HR-mass spectra MS) as the prominent peak, which again clearly demonstrated the positions of double bond at C-7 and the hydroxyl at C-9 as shown in the fragmentation pathway proposed in Scheme 1.

On comparing the spectra of compound **3** (Tables 1 and 2) with those of compound **1**, the conjugated keto group (δ_C 200.3) was assigned at C-7). The structure was again confirmed by HMBC observations. A similar compound (**9**) with a primary hydroxyl group at C-19 had been isolated from a Compositae species [11]. The

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR data of labdane derivatives **1–3**

H	δ *J* (Hz)		
	1*	**2**	**3**
1	1.78 *dt* (13.3, 3.5)	1.56	1.91 (e) *bd* (13.3)
	1.11 *td* (13.3, 4.5)	1.18 *td* (12.7, 4.0)	1.35 (a)
2	1.58	1.76	1.58 (e)
	1.38	1.46	1.65 (a)
3	1.38	1.31	1.48(e) *bd* (13.7)
	1.11 *td* (13.3, 4)	1.20	1.21 (a)
5	1.08 *dd* (12.6, 1.9)	1.75 *dd* (14.0, 4.5)	1.65 (a) *dd* (13.8, 3.8)
6	1.98	1.85	2.48 (e) *dd*(14.2, 3.8)
	1.86	2.03	2.35 (a) *t* (14.2)
7	1.89	5.50 *bs*	
11	1.95	1.90	2.26 2H, *t* (8.9)
	1.6		
12	1.55 2H,	2.09	1.58
		1.89	1.68
14	5.82 *dd* (17.2, 10.7)	6.02 *dd* (17.5, 10.9)	5.94 *dd* (17.3, 10.8)
15	5.20 *dd* (17.2, 1.1)	5.05 *dd* (17.5, 1.3)	5.26 *dd* (17.3, 1.1)
	5.05 *dd* (10.7, 1.1)	4.92 *dd* (10.9, 1.3)	5.13 *dd* (10.8, 1.1)
16	1.27 *s*	1.36 *s*	1.34 *s*
17	1.52 *s*	1.78 *d* (1.5)	1.74 *s*
18	0.85 *s*	0.85 *s*	0.91 *s*
19	0.80 *s*	0.87 *s*	0.88 *s*
20	0.91 *s*	0.78 *s*	1.08 *s*

Assignments were determined by consideration of data from ^{13}C–^{1}H COSY, ^{1}H–^{1}H COSY, and HMBC experiments. Relative configurations of compound **3** were deduced from 2D NOESY (600 MHz spectrum).

All unassigned shifts were either overlapping or obscured.

*Reported data [8] for methyls are δ 1.23, 1.53, 0.87, 0.82, 0.93; δ 4.9–6.1 for olefinic protons.

characteristic IR and UV absorptions of the cyclohexenone moiety of these two compounds agreed well. The absolute configuration was assigned as in the labdane series because the CD sign of compound **3** was positive ($\Delta\epsilon_{342} + 0.26$), the same as compound **9** [11]. Hence, compounds **1** and **2** were assumed to have the same configuration as depicted.

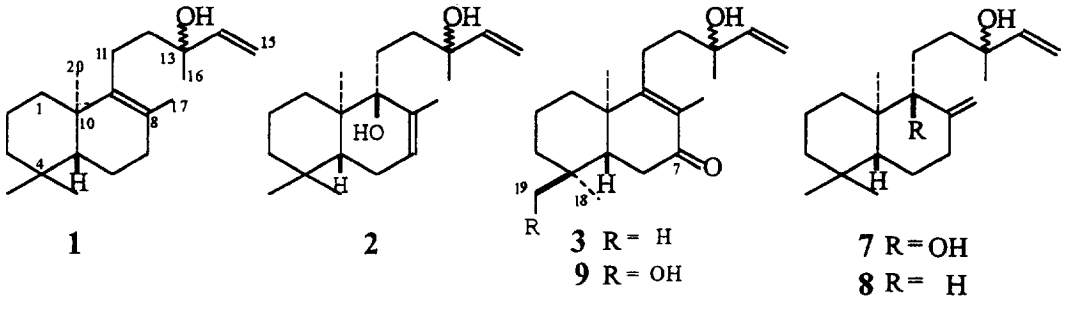
Table 2. ^{13C} NMR data of labdane derivatives **1–3**

C	**1**	**2**	**3**
1	37.1	33.3	36.0
2	19.1	18.7	18.6
3	41.6	42.0	41.2
4	33.2	33.1	33.3
5	51.9	42.4	50.3
6	22.1	24.3	35.2
7	33.7	126.7	200.3
8	125.8	135.1	130.1
9	140.0	90.9	168.2
10	39.2	41.2	41.1
11	19.1	28.4	23.9
12	42.5	37.2	40.5
13	73.6	83.3	73.4
14	144.9	145.3	144.2
15	111.8	110.0	112.5
16	27.7	29.1	28.1
17	19.5	21.0	11.3
18	33.3	32.9	32.5
19	21.7	22.3	21.4
20	20.1	16.9	18.1

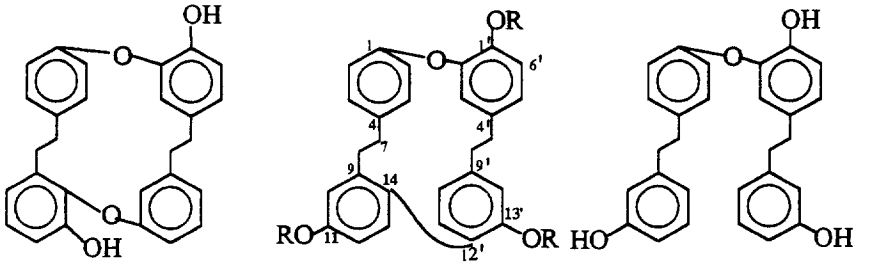
The chemical shift assignments of each atom of compounds **1–3** were supported from ^{1}H–^{1}H and ^{13}C–^{1}H COSY NMR spectra as well as HMBC data, and are compiled in Tables 1 and 2.

Chromatography of the ethanol acetate extract of *J. infusca* on silica gel, Sephadex LH-20 and prep TLC afforded two major macrocyclic aromatic components **4** and **5**. The identity of compound **4** as marchantin C was readily recognized by the typical H-3′ absorption (δ 5.53, *d*, $J = 2.0$ Hz) [12] and the EI-mass spectra ($[M]^+$ $m/z = 424$) [13]. Marchantin C had been isolated by our laboratory from the liverwort *Reboulia hemisphaerica* [14].

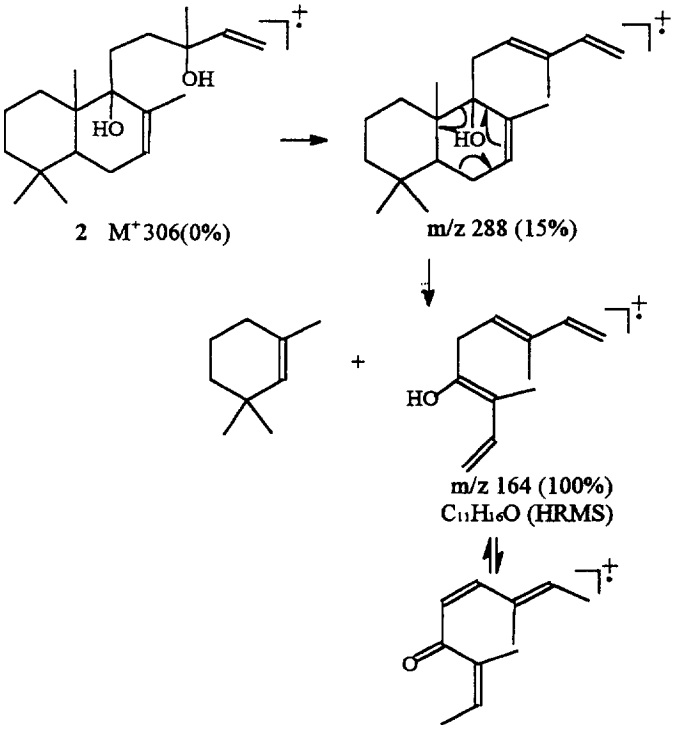
Compound **5** was shown to possess a riccardin skeleton again by the H-3′ absorption at δ 5.53 (*d*, $J = 2.0$ Hz) [15]. Compound **5** showed the same $[M]^+$ ($m/z = 424$) as that of marchantin C (**4**), yet compound **5** was more polar than compound **4**, with a lower R_f value. Therefore, compound **5** should have three hydroxyl groups on the rings. Of the known riccardins, riccardin C (**5**) does have the above-mentioned feature. The published partial ^{1}H NMR data of riccardin C [15] indeed coincided with our data. However, the ^{13}C NMR data of riccardin C was unavailable in the literature, and the consistency of a few proton shifts appeared rather



1 **2** **3** R = H, **9** R = OH **7** R = OH, **8** R = H



4 **5** R = H, **10** R = CH_3 **6**



Scheme 1. Proposed fragmentation pathway of *m/z* 164 from compound **2**.

Table 3. ^1H and ^{13}C NMR data of riccardin C (**5**)

C	C-type	$\delta^{13}C$	δ^1H	J (Hz)
1	4°C	152.5	–	
2	CH	122.1	6.71	*dd*, 8.1, 1.9
3	CH	117.4	6.90	*d*, 8.1
4	4°C	139.8	–	
5	CH	129.8*	7.02	*d*, 8.4
6	CH	117.5	6.78	*dd*, 8.4, 1.9
7	CH_2	34.9	2.70	*m*
8	CH_2	37.6	2.75	*bs*
9	4°C	143.7	–	
10	CH	132.8	6.95	*d*, 2.7
11	4°C	155.9	–	
12	CH	114.3	6.78	*dd*, 8.4, 2.7
13	CH	129.4*	7.02	*d*, 8.4
14	4°C	128.2	–	
1′	4°C	143.3	–	
2′	4°C	146.3	–	
3′	CH	116.1	5.34	*d*, 1.9
4′	4°C	133.1		
5′	CH	122.3	6.71	*dd*, 8.1, 1.9
6′	CH	114.9	6.90	*d*, 8.1
7′	CH_2	36.9	2.63	*m*
8′	CH_2	38.0	2.90	*m*
9′	4°C	141.9	–	
10′	CH	121.7	6.21	*dd*, 7.7, 1.7
11′	CH	131.4	6.76	*d*, 7.7
12′	4°C	124.4	–	
13′	4°C	151.8	–	
14′	CH	116.1	6.37	*d*, 1.7
Ar-OH			4.93	*bs*
			5.71	*bs*
			5.93	*bs*

Assignments were determined by consideration of data from ^{13}C–^1H COSY, ^1H–^1H COSY, HMBC experiments and by comparison of data reported for riccardins D, E and A trimethyl ether [18].

*Assignments may be interchanged.

inconclusive. Therefore, a subsequent methylation of compound **5** from *J. infusca* was performed and successfully yielded a trimethyl ether of which both the ^1H and ^{13}C NMR data were identical to those of riccardin A trimethyl ether (**10**) [16].

The finding of two macrocyclic bis(bibenzyl) compounds from the Taiwanese *J. infusca* suggested a fourth chemo-type for this morphologically complex species. On the other hand, *J. appressifolia* could be grouped with *J. torticalyx* and *J. rosulans* [9, 17] as the simple labdane-type *Jungermannia* species.

EXPERIMENTAL

Methods. Solvents used for spectral measurements were: $CDCl_3$ (^1H and ^{13}C NMR, 400 MHz), 95% EtOH (UV), CHCl3 ($[\alpha]_D$ and IR) and MeCN (CD). GC–MS: 70 eV, column, DBWAX, 30 × 0.25, 100–220° (40 min), 5° min^{-1})

Plant material. Jungermannia appressifolia Mitt. was collected at Shanlin Chi (1700 m), Nantou Hsien in 1991 and *J. infusca* (Mitt.) Steph. at Ali Shan (2000 m.) Chiayi Hsien in 1992. Both species were identified by Dr K. Yamada (Ise-shi, Japan) and specimens are deposited at the Department of Chemistry, Tamkang University.

Extraction and isolation. Air-dried and powered whole plants of *J. appressifolia* (100 g) were extracted successively with *n*-hexane and EtOAc. The combined extracts (1.2 g) were chromatographed on silica gel using an *n*-hexane–EtOAc gradient. The 10%, 15%, and 20% EtOAc–hexane fractions, yielded compounds (**1**) (15 mg), (**2**) (40 mg) and (**3**) (7 mg), respectively. Both compound **1** and compound **3** were further purified on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) as solvent. The powdered material (40 mg) of *J. infusca* was extracted with EtOAc and 1 g of crude oil was obtained. The 40% EtOAc–hexane eluate furnished marchantin C (**4**) (17 mg) after further purification on prep. TLC. Riccardin C (**5**) (50 mg) was obtained from the 60–100% eluate by CC on Sephadex LH-20 and prep. TLC.

Labda-8,14-*dien*-13-*ol* (**1**). EI-MS *m/z* (rel int.): 290 $[M]^+$(5), 191(98), 189(70), 121(90), 119(72), 109(87), 107(63), 95(100), 81(64). [8]

Labda-7,14-*dien*-9,13-*diol* (**2**). $[\alpha]_D$ + 11° (c 0.07); IR ν_{max} cm^{-1}: 3461, 1599; UV λ_{max} nm (log ε): 245(2.94); EI-MS *m/z* (rel. int.): 306 $[M]^+$(0), 288 $([M]^+ - H_2O]^+$ 15), 273(36), 205(27), 203(25), 164(100), 135(28), HR-MS: *m/z* 164.1197, $C_{11}H_{16}O$, requires 164.1202.

13-*Hydroxy*-7-*oxo*-*labda*-8,14-*diene* (**3**), $[\alpha]_D$ + 23° (*c* 0.24); $\Delta\varepsilon_{342}$ + 0.26; IR ν_{max} cm^{-1}: 3425, 1700, 1644; UV λ_{max} nm: 246.5; (EI-MS) *m/z* (rel. int.): 304 $[M]^+$(8), 205(85), 135(100), 123(75), 109(50), 71(80), 69(82), 57(62), 55(95).

Methylation of riccardin C (**5**). To a soln of riccardin C (**5**) (30 mg) in dry Me_2CO (50 ml) was added 15 ml of MeI and 1 g of NaH. The mixture was stirred for 22 hr at 0°. After work-up and purification by prep. TLC, 19 mg of riccardin A trimethyl ether (**10**) was obtained. Both the ^1H and ^{13}C NMR data agreed well with those reported [16].

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