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Labdane diterpenoids and highly methoxylated bibenzyls from the liverwort *Frullania inouei*

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ABSTRACT

Four undescribed labdane diterpenoids, 1,2-dehydro-3,7-dioxo-manoyl oxide (**1**), 1,2-dehydro-7β-hydroxy-3-oxo-manoyl oxide (**2**), 3,7-dioxo-manoyl oxide (**3**), and 3β-hydroxy-7-oxo-manoyl oxide (**4**) together with three known diterpenoids (**5**–**7**) and four highly methoxylated bibenzyls (**8**–**11**) were isolated from the liverwort *Frullania inouei*. The absolute structures of **1**–**4** were established by combined analysis of NMR data, CD data coupled with TDDFT CD calculations, and single-crystal X-ray diffraction measurement. Cytotoxicity tests to human tumor KB, KB/VCR, K562 or K562/A02 cells showed bibenzyls **8**–**11** inhibited cell proliferation with ID_{50} values ranging from 11.3 to 49.6 μM and overcame the multidrug resistance (MDR) with the reversal fold (RF) values ranging from 3.19 to 10.91 (5 μM) for vincristine-resistant KB/VCR and RF values from 4.40 to 8.26 (5 μM) for adriamycin-resistant K562/A02 cells, respectively. However, none of the diterpenoids were found to be active (ID_{50} > 50 μM).

1. Introduction

Liverworts (Hepaticae) are known to be rich sources of terpenoids and aromatic compounds with interesting biological activities, including antifungal, anti-HIV, antioxidative, insect antifeedant, neurotrophic, cytotoxic, and multidrug resistance (MDR) reversal activities (Asakawa, 2004, 2007; Shi et al., 2008).

With over 1000 described taxa, *Frullania* is a large, complex genus whose (sub)generic boundaries remain unresolved (Asakawa et al., 2003). Dozens of *Frullania* species have been chemically investigated, and were divided into ten chemo-types according to chemotaxonomy (Asakawa, 2004). This genus contains a variety of sesquiterpene lactones, diterpenoids, and bibenzyl derivatives; which can cause potent allergenic contact dermatitis. The extract also possess piscicidal activity as well as cytotoxicity to tumor cells (Asakawa et al., 2003, 2009).

As part of our ongoing research on bioactive substances from Chinese liverworts (Xie and Lou, 2009), the liverwort *Frullania inouei* Hatt., collected in the mountain area (3000 m) of Yunnan Province was phytochemically investigated. Seven labdane diterpenoids (**1**–**7**) and four highly methoxylated bibenyl (**8**–**11**) derivatives were isolated. The similar labdanes and the same bibenzyls as those found in the present species have been isolated from *F. hamatiloba* (Toyota et al., 1988), *F. serrata* (Asakawa, 1995), and *F. brittoniae* ssp. *truncatifolia* (Asakawa et al., 1976).

In this paper, the absolute structures of four undescribed diterpenoids (**1**–**4**) were determined by combined application of CD measurement, TDDFT CD calculations, as well as X-ray diffraction measurement. Cytotoxicities and MDR reversal activities of the isolated compounds were evaluated in vincristine-resistant KB/VCR, adriamycin-resistant K562/A02 and in their parental cells by MTT assays.

2. Results and discussion

2.1. Structure elucidation

The HRESIMS spectrum of compound **1** showed the $[M+Na]^+$ ion peak at *m*/*z* 339.1936 (calcd. 339.1931) ascribable to the molecular formula $C_{20}H_{28}O_3$, which indicated seven degrees of unsaturation. The IR absorptions at 1732 and 1661 cm^{-1} were ascribable to a carbonyl and a conjungated carbonyl, respectively. The ^1H NMR spectrum of **1** (Table 1) exhibited the presence of a monosubstituted vinyl group (–CH = CH_2) at δ_H 5.92 (H-14, *dd*, *J* = 17.4, 10.8 Hz), 5.23 (H-15a, *dd*, *J* = 17.4, 1.2 Hz) and 4.99 (H-15b, *dd*, *J* = 10.8, 1.2 Hz), and five tertiary methyls at δ_H 1.57 (H_3-17, *s*), 1.36 (H_3-16, *s*), 1.29 (H_3-20, *s*), 1.15 (H_3-18, *s*) and 1.11 (H_3-19, *s*). Addi-

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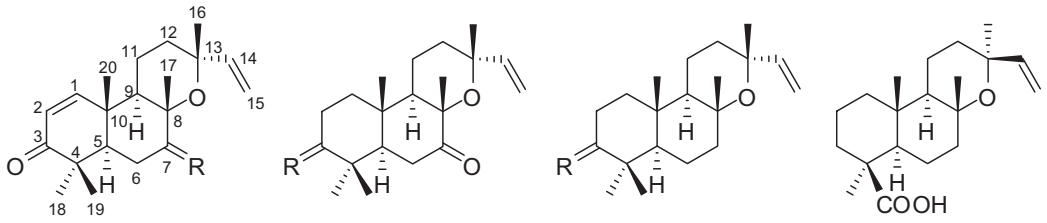
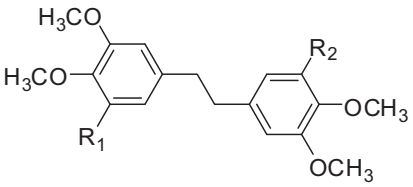


1 R = O
2 R = α-H, β-OH

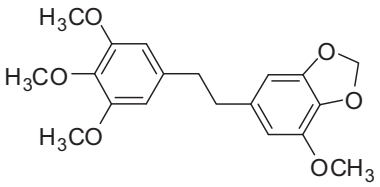
3 R = O
4 R = α-H, β-OH

5 R = O
6 R = α-H, β-OAc

7



8 $R_1 = R_2 = H$
9 $R_1 = OCH_3$, $R_2 = H$
10 $R_1 = R_2 = OCH_3$



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tionally, resonances for a pair of olefinic hydrogens at δ_H 7.06 (H-1, *d*, *J* = 10.8 Hz) and 5.93 (H-2, *d*, *J* = 10.8 Hz) were observed. The ^{13}C NMR (Table 2) and HSQC spectra confirmed the presence of a monosubstituted double bond (–CH = CH_2) and a disubstituted one (–CH = CH–), two carbonyls, five methyls, three methylenes, two methines, as well as four quaternary carbons (including two oxygenated). It is suggested that this compound was a manoyl oxide-type diterpenoid (Toyota et al., 1988) bearing an α,β-unsaturated ketone and another carbonyl. The HMBC correlations (Fig. 1) of H_3-18 and H_3-19 to the carbon signal at δ_C 203.5 indicated that the carbonyl was at C-3. Another carbonyl at C-7 was confirmed by the HMBC correlations of H-5 (δ_H 2.12), H-6 (δ_H 2.80 and 2.44) and H_3-17 to the carbon (C-7) signal at δ_C 207.4. The cross-peaks of H_3-20 with the olefinic carbon (C-1) at δ_C 155.4, and H-1 with C-3 and C-5 (δ_C 52.8) assigned the location of a double bound $\Delta^{1,2}$, which was supported by the correlations of an olefinic hydrogen (H-2) with C-4 (δ_C 44.9) and C-10 (δ_C 39.4), respectively, in the HMBC spectrum.

The NOEs between the protons in NOESY and the single-crystal X-ray diffraction analysis (Fig. 2) determined the relative configuration of **1**. To determine the absolute configuration of **1**, the CD exciton chirality method was applied (Berova and Nakanishi, 2000; Harada et al., 1981). The CD of **1** (Fig. 3) exhibited a positive split between the two chromophores of the α,β-unsaturated ketone (232 nm, $\Delta\varepsilon$ +9.87, $\pi \rightarrow \pi^*$ transition) (Koreeda et al., 1973) and the

Table 1
^1H NMR spectroscopic data for compounds **1**–**4**.[a]

Position	1	2	3	4
1	7.06 *d* (10.8)	7.06 *d* (10.2)	α 1.43 *ddd* (13.2, 10.8, 6.6) β 1.94 *ddd* (13.2, 7.2, 3.6)	α 1.01 *m* β 1.68 *m*
2	5.93 *d* (10.8)	5.86 *d* (10.2)	α 2.44 *ddd* (16.2, 6.6, 3.6) β 2.62 *ddd* (16.2, 10.8, 7.2)	α 1.72 *m* β 1.66 *m*
3				3.24 *dd* (11.4, 4.2)
5	2.12 *dd* (14.4, 2.4)	1.85 *m*	1.85 *dd* (14.4, 3.0)	1.28 *br d* (14.4)
6α	2.44 *dd* (14.4, 2.4)	1.87 *m*	2.35 *dd* (14.4, 3.0)	2.42 *br d* (14.4)
6β	2.80 *t* (14.4)	1.54 *dt* (13.8, 12.0)	2.74 *t* (14.4)	2.63 *t* (14.4)
7		3.69 *dd* (12.0, 4.8)		
9	2.02 *dd* (11.4, 4.8)	1.51 *m*	1.76 *m*	1.65 *m*
11	1.91 *m* (2H)	α 1.83 *m* β 1.77 *m*	α 1.69 *m* β 1.76 *m*	1.64 *m* (2H)
12α	1.84 *m*	1.69 *m*	1.76 *m*	1.72 *m*
12β	1.91 *m*	1.86 *m*	1.83 *m*	1.80 *m*
14	5.92 *dd* (17.4, 10.8)	5.85 *dd* (17.4, 10.8)	5.91 *dd* (16.8, 10.2)	5.91 *dd* (17.4, 10.8)
15a	5.23 *dd* (17.4, 1.2)	5.14 *dd* (17.4, 1.2)	5.21 *br d* (17.4)	5.20 *br d* (17.4)
15b	4.99 *dd* (10.8, 1.2)	4.94 *dd* (10.8, 1.2)	4.96 *br d* (10.8)	4.95 *br d* (10.8)
16	1.36 *s* (3H)	1.30 *s* (3H)	1.33 *s* (3H)	1.31 *s* (3H)
17	1.57 *s* (3H)	1.35 *s* (3H)	1.54 *s* (3H)	1.48 *s* (3H)
18	1.15 *s* (3H)	1.17 *s* (3H)	1.07 *s* (3H)	0.96 *s* (3H)
19	1.11 *s* (3H)	1.10 *s* (3H)	1.05 *s* (3H)	0.81 *s* (3H)
20	1.29 *s* (3H)	1.05 *s* (3H)	1.17 *s* (3H)	1.02 *s* (3H)

[a] Recorded in $CDCl_3$ at 600 MHz. Chemical shifts are given in ppm. Figures in parentheses are coupling constants (*J*) in Hz.

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**4**.[a]

Position	1	2	3	4
1	155.4	157.2	36.9	36.9
2	126.5	126.3	33.6	27.0
3	203.5	204.9	214.9	78.4
4	44.9	44.7	47.5	39.3
5	52.8	51.0	54.8	55.1
6	35.8	27.0	36.1	35.7
7	207.4	80.3	208.3	209.4
8	80.5	78.5	79.9	80.3
9	49.4	48.4	53.6	54.4
10	39.4	39.7	36.7	37.0
11	15.5	15.3	15.6	15.3
12	33.9	35.6	34.1	34.3
13	75.0	73.8	74.7	74.7
14	146.3	147.3	146.4	146.7
15	112.0	110.8	111.6	111.5
16	29.8	28.6	29.3	29.5
17	24.7	20.6	24.1	24.3
18	27.0	27.9	25.7	27.6
19	21.1	21.5	20.9	15.0
20	18.0	19.1	14.3	15.0

[a] Recorded in $CDCl_3$ at 150 MHz. Chemical shifts are given in ppm.