Two Novel Sesquiterpene Lactones, Cytotoxic Vernolide-A and -B, from *Vernonia cinerea*

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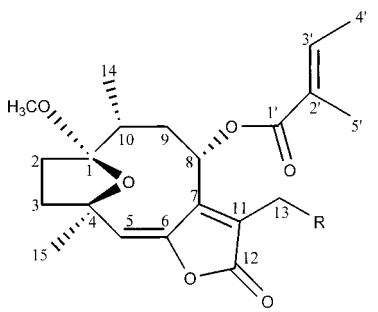
Bioassay-directed fractionation of an ethanolic extract of stems of *Vernonia cinerea* has resulted in the isolation of two novel sesquiterpene lactones, vernolide-A and -B. Their structures were elucidated on the basis of spectroscopic analysis. Biological evaluation showed that vernolide-A demonstrated potent cytotoxicity against human KB, DLD-1, NCI-661, and Hela tumor cell lines (ED_{50}=0.02, 0.05, 0.53, 0.04 μg/ml for KB, DLD-1, NCI-661, and Hela, respectively); vernolide-B had marginal cytoxicity (ED_{50}=3.78, 5.88, 6.42 μg/ml for KB, NCI-661, and Hela, respectively).

Key words *Vernonia cinerea*; vernolide-A; vernonia-B; cytotoxicity

Vernonia cinerea LESS. is a perennial herbaceous plant that is distributed throughout the middle and southern ares of Taiwan. During a search for bioactive agents from the terrestrial plants of Taiwan, we found that a crude extract of *V. cinerea* possessed cytotoxicity. Bioassay-guided fractionation of an EtOH extract led to the isolation of two novel sesquiterpene lactones, vernolide-A (**1**) and-B (**2**). We describe the structural elucidation of **1** and **2** using H–H correlation spectroscopy (COSY), ^{13}C–^{1}H heteronuclear multiple quantum coherence (HMQC), and ^{13}C–^{1}H heteronuclear multiple bond coherence (HMBC) experiments. Biological evaluation of the cytotoxicity of compounds **1** and **2** is also reported.

The molecular formula of **1** ($C_{21}H_{28}O_7$) was indicated by a molecular ion (*m/z* 392 $[M]^+$) in the HR-EI-MS spectra. The IR and ^{13}C-NMR spectra suggested that **1** may contain hydroxyl, ester, and γ-lactone functionalities.

The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra indicated that **1** contained eight quaternary carbons, four tertiary carbons, four secondary carbons, and five primary carbons. In the ^{1}H–^{1}H COSY spectrum, cross peaks between H-9 and H-8, -10, between H-14 and H-10, between H-2 and H-3, and between H-4′ and H-3′ were observed. Inspection of the HMBC spectrum, a singlet olefinic proton (δ_H 5.82, s), a doublet methyl (δ_H 0.86 ppm, 3H, d, J=6.5), a singlet methyl (δ_H 1.44 ppm, 3H, s), and a methoxyl (3.25 ppm, 3H, s) were assigned at H-5, -10, -4, and -1, respectively. Three oxygenated quaternary carbons could be assigned at C-1, -4, and -6, respectively, not only from ^{13}C-NMR but also from the HMBC spectrum. In addition, a correlation between an oxygenated methylene (H-13) and the carbonyl carbon (C-12) was found, together with the above corroborations and referenced data,[1—4] suggesting that **1** is a sesquiterpene with a lactone skeleton which was usually isolated from *Vernonia* spp. and named as a hirsutinolide type sesquiterpene. Besides the major skeleton of **1** based on the above deduction, an additional functional group was also predicted by the HMBC spectrum. Thus, H-3′ was correlated with a carbonyl carbon (C-1′), doublet methyl (C-4′), and singlet methyl (C-5′) signals, indicating the presence of a tiglate.[2] Moreover, an oxygenated proton, (H-8), has a long-range correlation with a carbonyl carbon (C-1′), suggesting this functional group was at C-8. These findings together with the molecular ion at *m/z* 392 confirmed compound **1** as 1α-methoxyl-8α-tigloyloxy hirsutinolide, which is tentatively named vernolide-A.



R
1 OH
2 OAc

Vernolide-B (**2**) gave *m/z* 434 $[M^+]$, for $C_{23}H_{30}O_8$ by MS. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**, and indicated the characteristic signals for a substituted hirsutinolide type sesquiterpene. However, additional signals (δ_H 2.01, δ_C 20.6, 170.1) for an acetate group were observed in the NMR spectra, and the oxygenated methylene (H-13a, b) shifted to low field (from δ_H 4.61, 4.51 to 5.05, 4.97), revealing that compound **2** possesses an acetate group at C-13. Moreover, the correlation between the carbonyl carbon and H-13 in the HMBC spectrum, and a corresponding fragment ion at *m/z* 375 $[M^+-CH_3COO]$ in the mass spectrum, are in good agreement with the acetic acid ester at C-13. In addition to the above corroboration, on the basis of 2D ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectral analyses, the complete assignments for the chemical shifts of ^{1}H- and ^{13}C-NMR of **2** were definitively undoubtedly.

As to the stereochemistry of compounds **1** and **2**, correlations between the methoxyl and CH_3-15, and CH_3-14 in the nuclear Overhauser effect spectroscopy (NOESY) and NOE spectra were observed. This evidence possibly supported that **1** and **2** possessed a 1,4β ether ring and 8α-tiglate acid ester configurations as does the known 8α-tigloyloxy hirsutinolide,[1] and then their structures were established unambigu-

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ously.

After cytotoxicity assay in human KB (oral epidermoid carcinoma), DLD-1 (colon adenocarcinoma), NCI-661 (lung large cell carcinoma), and Hela (cervix epithelioid carcinoma) tumor cell lines, compound **1** exhibited potent cytotoxicity against the above tumor cells (ED_{50}=0.02, 0.05, 0.53, 0.04 μg/ml for KB, DLD-1, NCI-661, Hela, respectively). Compound **2** showed only marginal cytotoxic effects (ED_{50}=3.78, 5.88, 6.42 μg/ml for KB, NCI-661, and Hela, respectively). These results indicate revealed that replacement of a hydroxy by an acetate group in C-13 would decrease the activity. It seems that the C-13 position in the hirsutinolide type sesquiterpenes may play an important role in their activity. Detailed structure–activity relationships of the substituted hirsutinolide need to be investigated.

Table 1. ^{1}H-NMR Data[a] of Compounds **1** and **2** ($CDCl_3$)

Position	**1**	**2**
2a	2.12 br d (2.8)	2.13 br d (3.1)
2b	1.85 m	1.86 m
3a	2.07 br d (3.5)	2.06 br d (2.4)
3b	1.91 s	1.92 s
5	5.82 s	5.82 s
8	6.25 d (8.0)	6.17 d (7.8)
9a	1.71 m	1.76 m
9b	2.33 dd (16, 11)	2.38 dd (16,11)
10	1.94 m	1.95 m
13a	4.61 d (13.2)	5.05 (13)
13b	4.51 d (13.2)	4.97 (13)
14	0.86 d (6.5)	0.84 d (6.9)
15	1.44 s	1.43 s
1-OCH_3	3.25 s	3.21 s
13-OAc		2.01 s
3′	7.03 q (7.5)	6.99 q (7)
4′	1.78 br d (7.5)	1.74 d (7)
5′	1.80 br s	1.77 br s

a) Chemical shift values are given in ppm, and *J* values in parentheses are given in Hz. Assignments were confirmed by ^{1}H–^{1}H COSY, HMQC, and HMBC experiments.

Table 2. ^{13}C-NMR Data[a] of Compounds **1** and **2** ($CDCl_3$)

Carbon	**1**	**2**	HMBC (^{13}C→^{1}H)
1	111.3 (s)	111.1 (s)	1-OCH_3, H-2, 3, 9,10, 14
2	32.8 (t)	32.6 (t)	H-3
3	39.9 (t)	39.8 (t)	H-5, 15
4	80.6 (s)	80.4 (s)	H-2, 3, 5, 15
5	126.3 (d)	126.6 (d)	H-3, 15
6	146.7 (s)	146.4 (s)	H-5, 8
7	147.6 (s)	150.3 (s)	H-5, 8, 9, 13
8	68.4 (d)	68.2 (d)	H-9, 10
9	36.1 (t)	35.8 (t)	H-8, 10, 14
10	42.6 (d)	42.5 (d)	H-8, 9, 14
11	133.7 (s)	129.6 (s)	H-8, 13
12	168.1 (s)	167.6 (s)	H-13
13	54.4 (t)	55.4 (t)	
14	16.8 (q)	16.7 (q)	H-9, 10
15	27.6 (q)	27.1 (q)	H-3, 5
1-OCH_3	48.9 (q)	48.6 (q)	
$\underline{C}H_3COO$		20.6 (q)	
$CH_3\underline{C}OO$		170.1 (s)	H-13
1′	168.4 (s)	167.6 (s)	H-8, 3′
2′	128.4 (s)	128.3 (s)	H-4′, 5′
3′	139.4 (d)	138.4 (d)	H-4′, 5′
4′	14.6 (q)	14.3 (q)	H-3′
5′	11.9 (q)	11.8 (q)	H-3′

a) Assignments were confirmed by HMQC and HMBC experiments.

Experimental

General Experimental Procedures ^{1}H- and ^{13}C-NMR spectra were recorded at 300.13 and 75.46 MHz, respectively, on a Bruker 300 AC spectrometer. The spectra of heteronuclear correlation, HMBC was established by the coupling of 8 Hz. Electron impact (EI)-MS and FAB-MS were performed on a JEOL SX-102A instrument. Silica gel (Merck 70—230 mesh) was used for column chromatography, and precoated Silica gel (Merck 60F-254) plates were used for TLC. HPLC was accomplished on an SPD-6AV liquid chromatograph using a preparative C_{18} column. Melting points were determined on a Fisher–Johns apparatus and are uncorrected.

Plant Material The stems of *Vernonia cinerea* Less. were collected in June 1999 at Kaohsiung, southern Taiwan. A voucher specimen is deposited at the National Research Institute of Chinese Medicine, Shih-Pai, Taipei, Taiwan, R.O.C.

Extraction and Isolation The dried stems of *V. cinerea* (5.3 kg) were extracted exhaustively with ethanol. An EtOH extract (102 g) of dried stems of *V. cinerea* was extracted successively with *n*-hexane and $CHCl_3$. The $CHCl_3$ extract was chromatographed by column chromatography over Si gel and eluted with *n*-hexane–EtOAc and EtOAc to give 8 fractions. The bioactive fr. 5 (*n*-hexane : EtOAc=2 : 1) was further separated by HPLC ($5C_{18}$, 250×10 mm) with MeOH–H_2O (9 : 1) to furnish **1** (11 mg) and **2** (8 mg).

Vernolide-A (**1**): Red brown amorphous powder; IR ν_{max} (KBr) 3450 (OH), 1760 (γ-lactone), 1720 (C=CCO_2R) cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 1; HR-EI-MS *m/z* 392.1834 $[M]^+$ (Calcd for $C_{21}H_{28}O_7$: 392.1835).

Vernolide-B (**2**): Red brown amorphous powder; IR ν_{max} (KBr) 3450 (OH), 1760, (γ-lactone), 1725 (OAc, C=CCO_2R) cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 1; HR-EI-MS *m/z* 434.1949 $[M]^+$ (Calcd for $C_{23}H_{30}O_8$: 434.1941).

Cytotoxicity Assay An *in vitro* cytotoxicity assay was performed as previously described.[5]

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References

1) Bohlmann F., Brindöpke G., Rastogi R. C., *Phytochemistry*, **17**, 475—482 (1978).
2) Bohlmann F., Zdero C., King R. M., Robinson H., *Phytochemistry*, **21**, 1045—1048 (1982).
3) Bohlmann F., Mahanta P. K., Dutta L. N., *Phytochemistry*, **18**, 289—291 (1979).
4) Borkosky S., Bardon A., Catalan C. A. N., Diaz J. G., Herz W., *Phytochemistry*, **44**, 465—470 (1997).
5) Kuo Y. H., Huang H. C., Kuo Yang L. M., Chen C. F., *J. Org. Chem.*, **64**, 7023—7027 (1999).