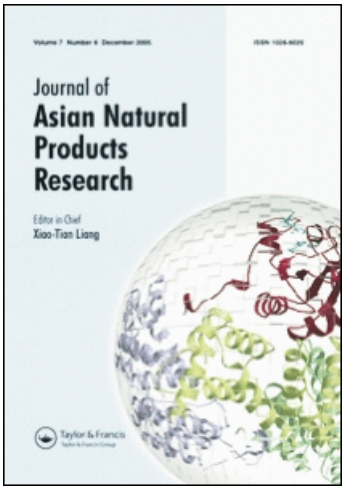

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Two new sesquiterpenoids, vernobockolides A (**1**) and B (**2**), along with five known ones, piptocarphin C (**3**), piptocarphin F (**4**), piptocarphin A (**5**), hirsutolide (**6**), and β-D-glucopyranosyl taraxinic ester (**7**), were isolated from the aerial part of *Vernonia bockiana*. Their structures were determined by spectroscopic analysis, especially 2D NMR. Among them, compounds **2**–**6** showed strong cytotoxic activities against mouse lymphoid tumor cell line P388 with the IC_{50} values of 1.81, 1.32, 0.77, and 0.73 μM, respectively.

Keywords: *Vernonia bockiana*; Compositae; sesquiterpenoid; vernobockolides A and B; cytotoxic activity

1. Introduction

The genus *Vernonia* (Compositae) comprising about 1000 species is mainly distributed in the torrid zones of America, Asia, and Africa, and *ca*. 27 species of this genus grow in the southern part of China, many of which have applications in Chinese folklore medicine.[1] A number of highly oxygenated sesquiterpene lactones have been isolated from this genus,[2] some of which showed significant biological activity, such as vernolide A[3] isolated from *V. cinerea* which exhibited remarkable cytotoxicities against several human tumor cell lines. *Vernonia bockiana* Diels distributed mainly in Sichuan, Yunnan, and Guizhou Provinces of China was not chemically investigated, previously. In the current study, two new sesquiterpenoids, vernobockolides A (**1**) and B (**2**) (Figure 1), along with five known ones, piptocarphins C (**3**),[4] F (**4**),[4] A (**5**),[4] hirsutolide (**6**),[5] and β-D-glucopyranosyl taraxinic ester (**7**),[6] were isolated from the aerial part of *V. bockiana*, and their structures were determined by spectroscopic data, especially 2D NMR. Among them, compounds **2**–**6** showed strong cytotoxic activities against mouse lymphoid tumor cell line P388 with IC_{50} values of 1.81, 1.32, 0.77, and 0.73 μM, respectively.

2. Results and discussion

Vernobockolide A (**1**) was obtained as a gum with an optical rotation of $[\alpha]_D^{20} = +72.3$ (*c* 4.0, $CHCl_3$). The molecular formula of **1**, as determined by the HR-EI-MS (*m/z* 392.1831 $[M - H_2O]^+$), ^1H, and ^{13}C NMR (Table 1), was $C_{21}H_{30}O_8$ with seven degrees of unsaturation. The IR absorptions revealed the presence of hydroxyl (3454 cm^{-1}) and carbonyl (1755 and 1716 cm^{-1}) groups. In the ^1H

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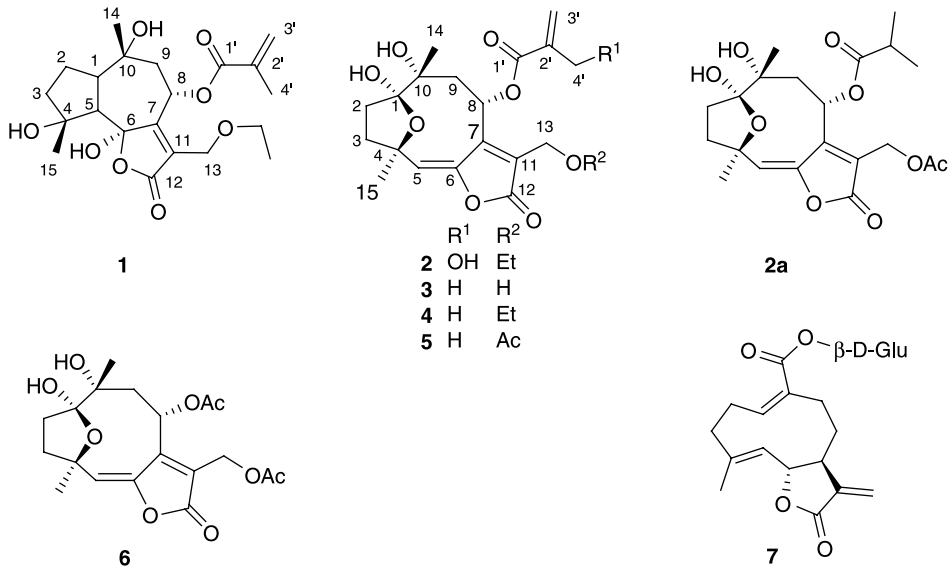

Figure 1. Structures of compounds **1**–**7**.

NMR spectrum, three broad singlets at δ 6.46, 2.50, and 1.86 (each *ca*. 1H), which did not show any correlations in the HSQC spectrum, were assigned to three hydroxyls. In accordance with the above molecular formula, the ^{13}C NMR spectrum of **1** exhibited 21 carbon signals that were classified by DEPT experiments as two carboxyls (δ 169.5 and 166.1), one persubstituted double bond (δ 159.8 and 129.2), one terminal double bond (δ 135.6 and 126.6), one hemiketal group (δ 108.1), two oxygenated quaternary carbons (δ 83.8 and 72.7), three sp^3 methines, five sp^3 methenes, and four sp^3 methyls. The aforementioned data and 1D NMR spectral data of **1** indicated that it is a guaiane-type sesquiterpenoid. The ^{1}H and ^{13}C NMR spectral data also revealed the presence of an ethoxyl and a methacryloxyl in **1**, which was confirmed by the observed HMBC correlations (Figure 2).

The planar structure of **1** was constructed by a comprehensive analysis of the HSQC and HMBC (Figure 2) spectra. An oxygenated quaternary carbon at δ_C 72.7 was attributable to C-10 bearing 10-OH on the basis of its HMBC correlations with H-1, H_2-9, and H_3-14. The oxygenated C-8 was assigned by the mutual correlations of H-8 (δ 6.13)/C-9 and C-10, and H_2-9/C-8 (δ 65.5). The HMBC correlation between H-8 and C-1′ located the methacryloxyl at C-8. The HMBC correlations of H-8 to the olefinic carbons (C-7 and C-11) indicated the occurrence of a $\Delta^{7(11)}$ double bond. The linkage of the ethoxyl via an oxygenated methylene (CH_2-13: δ_C 61.9, δ_H 4.23) to the C-11 was established by the HMBC correlations of H_2-13/C-7, H_2-13/C-11, H_2-13/EtO, and EtO/C-13. The HMBC correlations of H_2-2/C-4, H_2-3/C-4, H-5/C-4, and H_3-15/C-4 allowed the assignment of an oxygenated carbon at δ 83.8 to C-4 bearing a 4-OH. A carbon resonance at δ 169.5 correlating with H_2-13 was assigned to C-12, and a hemiketal carbon at δ 108.1 was then assigned to C-6 on the basis of the HMBC correlations of H-8/C-6, H-5/C-6, H-1/C-6, and 6-OH/C-6. Although no direct HMBC correlation was available to link C-6 and C-12, the remaining one degree of unsaturation (two double bonds, the bicyclic sesquiterpenoid skeleton, and two carboxyls accounted for six out of seven degrees of unsaturation) indicated the linkage of C-6 and C-12 via an oxygen atom to form a 6,12-lactone.

Table 1. The NMR spectral data for compounds **1** and **2** in $CDCl_3$.

	1		**2**		**2a**[a]	**4**
	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_C	δ_C
1	2.93 ddd (11.4, 8.7, 8.7)	50.6		108.8	108.7	108.6
2	2.02–2.06 m	24.0	2.06–2.07 m	32.1	31.8	31.7
	1.55–1.60 m		1.90–1.94 dd (11.9, 5.3)			
3	1.78–1.83 m (2H)	41.9	2.37–2.45 m	37.8[b]	37.5[b]	37.7[b]
			1.85 dd (12.7, 5.3)			
4		83.8		82.4	82.1	82.0
5	1.50 d (11.4)	54.8	5.78 br s	125.3	126.8	125.6
6		108.1		150.4	150.0	150.2
7		159.8		144.1	144.0	144.0
8	6.13 dd (9.6, 3.5)	65.5	6.68 d (10.9)	65.3	66.5	66.1
9	2.50 dd (14.5, 9.6)	44.5	2.59 dd (10.9, 14.9)	38.0[b]	38.1[b]	38.0[b]
	2.05 dd (14.5, 3.5)		2.08 d (14.9)			
10		72.7		77.4	78.1	78.0
11		129.2		133.2	131.2	133.4
12		169.5		167.4	166.5	167.4
13	4.23 s (2H)	61.9	4.52 d (12.2)	66.7	55.8	66.7
			4.33 d (12.2)			
14	1.15 s (3H)	21.7	1.21 s (3H)	26.4	25.4	25.4
15	1.58 s (3H)	29.7	1.59 d (1.6, 3H)	28.9	29.1	29.0
1′		166.1		165.0	175.4	165.7
2′		135.6		138.9	34.1	135.8
3′	6.13 dd (1.4, 1.0)	126.6	6.35 br s	129.4	18.9	127.1
	5.61 dd (1.4, 1.4)		5.83 br s			
4′	1.95 dd (1.4, 1.0, 3H)	18.4	4.40 br d (12.8) 4.18 br d (12.8)	62.3	18.4	18.1
OEt	3.50 q (7.0)	67.0	3.53–3.61 m	61.5		61.6
	1.17 t (7.0, 3H)	14.9	1.22 t (6.9, 3H)	15.1		15.2
OH	6.46 (br s, 6-OH)		NB			
	2.50 (br s)					
	1.86 (br s)					
Ac					170.3	
					20.7	

NB, not observed. [a]Cited data see Kotowicz *et al.*[7] [b]May be exchanged in the same column.

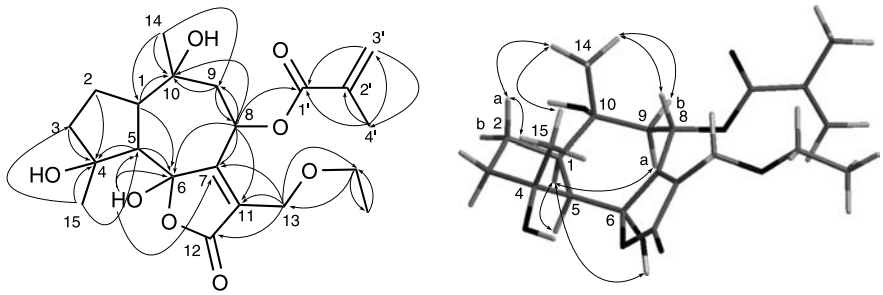

Figure 2. Selected HMBC correlations (H → C) and key ROESY cross-peaks (↔) for vernobockolide A (**1**).

The relative stereochemistry of **1** was established by ROESY experiment. As depicted in Figure 2, the ROESY cross-peaks of H-8/CH_3-14 and CH_3-14/CH_3-15 indicated that H-8, CH_3-14, and CH_3-15 were in β-configuration. Consequently, the ROESY correlations of H-1/H-5 and H-1/6-OH revealed α-orientation for H-1, H-5, and 6-OH.

The NMR spectral data (Table 1) of vernobockolide B (**2**) showed high similarity to those of piptocarphin F (**4**)[4] and (1*S**,4*R**,8*S**,10*R**)-13-acetyloxy-1,4-epoxy-1,10-dihydroxy-8-isobutyryloxygermacra-5*E*, 7(11)-dien-6,12-olide (**2a**)[7] in the sesquiterpenoid core, indicating that they had the same sesquiterpenoid core, and the only structural difference was the C-8 substituent. The ^{1}H and ^{13}C NMR spectra of **2** showed the presence of a 2-hydroxymethyl-acryloxyl (C=O: δ_C 165.0; > C=δ_C 138.9; CH_2=δ_C 129.4, δ_H 6.35, and 5.83; CH_2OH: δ_C 62.3, δ_H 4.40, and 4.18) at C-8. Therefore, the structure of vernobockolide B was established as **2**.

The five known compounds were identified as piptocarphins C (**3**),[4] F (**4**),[4] A (**5**),[4] hirsutolide (**6**),[5] and β-D-glucopyranosyl taraxinic ester (**7**)[6] on the basis of the NMR spectral data. The ^{13}C NMR spectral data of piptocarphin F (**4**) was reported in this study for the first time.

Table 2. The anti-tumor activity for compounds **1**–**7**.

	IC_{50} (μM)	
	P388[a]	A549[b]
1	–	–
2	1.81	–
3	–	–
4	1.32	–
5	0.77	–
6	0.73	–
7	–	–

–, not show activity against tumor cell lines.
[a] Measured by microculture tetrozolium (MTT) method.
[b] Measured by sulforhodamine B (SRB) method.

2.1 *Cytotoxic activities of compounds 1–7*

The cytotoxicities (Table 2) of compounds **1**–**7** were tested against P388 (mouse lymphoid tumor) and A549 (human lung cancer) cell lines by the MTT[8] and the SRB assay,[9] respectively. Four compounds, vernobockolide B (**2**), piptocarphin F (**4**), piptocarphin A (**5**), and hirsutolide (**6**), showed significant activity against P388 with the IC_{50} values of 1.81, 1.32, 0.77, and 0.73 μM, respectively, while compounds **1**–**7** were inactive against A549 tumor cell line (Table 2).

3. Experimental

3.1 *General experimental procedures*

Optical rotations were recorded on a Perkin–Elmer 341 polarimeter. The IR spectra were obtained on a Perkin–Elmer 577 spectrometer with KBr disks. The NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as an internal standard. The EI-MS (70 eV) was carried out on a Finnigan-MAT 95 mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, China). Silica gel (200–300 mesh) was used for column chromatography, and precoated silica gel GF 254 plates (Qingdao Haiyang Chemical Plant, Qingdao, China) were used for TLC. C_{18} reversed-phase silica gel (150–200 mesh, Merck), and MCI gel (CHP20P, 75–150 μ, Mitsubishi Chemical Industry Ltd, Tokyo, Japan) was also used for column chromatography.

3.2 *Plant material*

The aerial part of *Vernonia bockiana* was collected from Chishui County, Guizhou Province, China, in July 2002. The plant was identified by Professor Qian-Hai Chen, Guizhou Biological Research Institute, Guizhou Academy of Sciences. A voucher specimen (accession No.: Ver-2002-2Y) has been deposited at Shanghai Institute of Materia Medica.

3.3 Extraction and isolation

The air-dried powder of the aerial part of *V. bockiana* (2 kg) was percolated with EtOH (20 l) three times at room temperature. The ethanolic extract (103 g) was dissolved in 1.5 l water to form a suspension, and then partitioned with EtOAc 500 ml × 3 to give an EtOAc fraction (62 g). The water phase was extracted subsequently with *n*-BuOH 250 ml × 3 to give the *n*-BuOH fraction (10 g). The EtOAc fraction was subjected to an MCI gel column eluted with a gradient aqueous CH_3OH (0–100%, v/v) to obtain three major fractions 1–3 (as monitored by TLC). Fraction 2 was separated on a column of reversed-phase C_{18} silica gel (aqueous methanol 40–60%), and the major elutes 2a–2f were then subjected to extensive column chromatography over silica gel ($CHCl_3$–MeOH, 50:1) to give compounds **1**–**6** (30, 15, 10, 20, 50, and 10 mg), respectively. The *n*-BuOH-soluble fraction was separated on a silica gel column ($CHCl_3$–MeOH–H_2O, 5:1:0.1) to obtain the major component that was then purified on a reversed-phase C_{18} silica gel column (aqueous methanol 35%) to yield compound **7** (30 mg).

3.3.1 *Vernobockolide A (**1**)*

Obtained as gum; $[\alpha]_D^{20} + 72.3$ (*c* 4.0, $CHCl_3$); IR (KBr) ν_{max}: 3454, 2974, 2931, 2875, 1755, 1716, 1159, 947, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (Table 1); EI-MS *m/z*: (rel. int.): 392 $[M - H_2O]^+$(2), 306 (21), 288 (20), 261 (17), 260 (100), 243 (32), 218 (78), 202 (27), 189 (14), 175 (31), 160 (33), 149 (7); HR-EI-MS *m/z*: 392.1831 $[M - H_2O]^+$ (calcd for $C_{21}H_{28}O_7$, 392.1835).

3.3.2 *Vernobockolide B (**2**)*

Obtained as gum; $[\alpha]_D^{20} + 60.0$ (*c* 11.5, $CHCl_3$); IR (KBr) ν_{max}: 3446, 2978, 2931, 2875, 1759, 1639, 1078, 948, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (Table 1); EI-MS *m/z*: (rel. int.): 424 $[M]^+$(5), 378 (3), 360 (1), 322 (5), 307 (2), 294 (5), 276 (45), 263 (18), 258 (10), 248 (5), 234 (100), 216 (70), 205 (19), 191 (47), 188 (55), 163 (15), 148 (90); HR-EI-MS *m/z*: 424.1735 $[M]^+$ (calcd for $C_{21}H_{28}O_9$, 424.1733).

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