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Chemical constituents from *Vernonia bockiana*

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[ABSTRACT] A new sesquiterpenoid and two pregnane steroids, named vernobockolide C (**1**) and vernobockones A and B (**2** and **3**), respevtively, along with a known sesquiterpenoid, 7, 10-epoxy-11-hydroxy-bisabol-2-en-15-al, were isolated from the aerial part of *Vernonia bockiana*. Their structures were elucidated on the basis of extensive spectroscopic data analysis, especially 2D NMR (HSQC, HMBC, and ROESY). This study further expands the chemical space of this underexplored species.

[KEY WORDS] *Vernonia bockiana*; Sesquiterpenoid; Steroid

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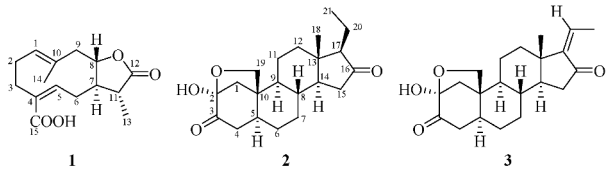
Introduction

Vernonia bockiana, a species of genus *Vernonia* (Compositae), is native to China, and mainly distributed in Sichuan, Yunnan, and Guizhou Provinces [1]. There are limited studies on the chemical constituents of *V. bockiana*, which have led to the discovery of 11 sesquiterpenoids, including vernobockolides A and B [2], piptocarphins A, C, and F [3], hirsutolide [4], *β*-D-glucopyranosyl taraxinic ester [5], 8*α*-(4-hydroxymethacryloyloxy)-10*α*-hydroxy-13-methoxyhirsutinolide [6-7], 8*α*-methacryloyloxy-10*α*-hydroxy-13-*O*-methylhirsutinolide [6, 8], 8*α*-[4-hydroxymethacryloyloxy]-10*α*-hydroxyhisutinolide-13-*O*-acetate [6, 9], and 8*α*-acetoxy-10*α*-hydroxy-13-*O*-methylhirsutinolide [6, 8], together with one known inone glycoside, saussureosides B [6, 10]. Some of those sesquiterpenoids were shown to possess strong cytotoxicity against several tumor cell lines [2, 6]. During the efforts to discover more structurally distinct natural products from this underexplored species, a new sesquiterpenoid, vernobockolide C (**1**), and two new pregnane steroids, vernobockones A and B (**2** and **3**) (Fig. 1), along with a known sesquiterpenoid, 7, 10-epoxy-11-hydroxy-bisabol-2-en-15-al [11], were isolated from the aerial part of *Vernonia bockiana*. Their chemical structures were elucidated by detailed spectroscopic data analysis. Herein, we present the isolation and structural elucidation of these compounds.



1 **2** **3**

Fig. 1 Chemical structures of compounds 1−3

Results and Discussion

Vernobockolide C (**1**) was obtained as a gum, and its molecular formula was determined as $C_{15}H_{20}O_4$ by the HRESI-MS, requiring six degrees of unsaturation. The IR spectrum of **1** revealed the presence of an ester carbonyl (1739 cm^{-1}) and an *α*, *β*-unsaturated carboxyl group (1707 cm^{-1}) [12-13]. The ^{13}C NMR data (Table 1) with 2D NMR experiments (HSQC and HMBC) revealed the presence of two methyl, four methylene, and five methine (two olefinic and one oxygenated) groups, as well as four quaternary carbons (two carbonyls and two olefinic). The two trisubstituted double bonds (δ_C 124.2, 128.9, 134.1, and 150.7) and two

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Dedicated to Professor SUN Han-Dong on the Occasion of His 80th Birthday

carbonyl groups (δ_C 172.8 and 177.5) accounte for four of the six degrees of unsaturation, and the remaining two thus require the structure of **1** being bicyclic. Two structural fragments, **a** (C-1 to C-3) and **b** (C-5 to C-9, C-7 to C-11, and C-11 to C-13), were deduced as drawn in bold lines (Fig. 2A) by combining the 1D and 2D NMR data (^{1}H–^{1}H COSY and HSQC). The linkages of the two structural fragments (**a**/**b**) and quaternary carbons were mainly achieved by examination of the HMBC spectrum (Fig. 2A), in which the $\Delta^{1(10)}$ double bond and the linkages of C-9/C-10 and Me-14/C-10 were fixed by the correlations of Me-14 to C-1, C-10, and C-9, H–C-1 to C-9, and CH_2-9 to C-1 and C-10. The C-15 carboxyl group at δ_C 172.8, conjugated with the Δ^4 double bond, and the linkage of C-3/C-4 were assigned by the HMBC correlations from H–C-5 to C-3 and C-15 and from CH_2-3 to C-4 and C15 (Fig. 2A). According to the molecular formula, a lactone is necessary to satisfy the unsaturation. The presence of the *γ*-lactone between C-12 (δ_C 177.5) and C-8 (δ_C 82.7) were confirmed by the HMBC correlations from H–C-11 and Me-13 to C-12 and from CH_2-6, H–C-7, CH_2-9, and H–C-11 to C-8 (Fig. 2A). As a result, the planar structure of **1** was determined.

Table 1 ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compounds 1–3 (in $CDCl_3$; δ in ppm, *J* in Hz)

Position	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1*α*	5.07 (dd, *J* = 12.5, 4.6)	128.9	1.49 (d, *J* = 12.5)	42.6	1.46 (d, *J* = 12.5)	42.6
1*β*			2.62 (d, *J* = 12.5)		2.61 (d, *J* = 12.5)	
2*α*	2.19 (m)	26.7		102.0		102.0
2*β*	2.29 (m)					
3*α*	1.88 (m)	34.6		205.4		205.4
3*β*	2.92 (m)					
4*α*		124.2	2.57 (m)	40.7	2.56 (m)	40.7
4*β*			2.44 (m)		2.41 (m)	
5	5.50 (dd, *J* = 11.3, 2.3)	150.7	1.86 (m)	44.3	1.84 (m)	44.4
6*α*	2.60 (d, *J* = 16.3)	31.5	1.77 (m)	29.2	1.74 (m)	29.3
6*β*	3.24 (m)		1.48 (m)		1.45 (m)	
7*α*	1.81 (m)	53.4	1.04 (m)	31.3	1.01 (m)	31.0
7*β*			1.69 (m)		1.72 (m)	
8	4.20 (dt, *J* = 11.5, 2.3)	82.7	1.15 (m)	35.9	1.14 (m)	35.8
9*α*	2.34 (m)	45.9	1.37 (m)	45.9	1.31 (m)	45.8
9*β*	2.90 (m)					
10		134.1		47.8		47.9
11*α*	2.38 (m)	41.9	1.83 (m)	20.8	1.90 (m)	21.0
11*β*			1.60 (m)		1.73 (m)	
12*α*		177.5	1.99 (dt, *J* = 12.5, 3.2)	37.6	1.89 (m)	35.2
12*β*			1.40 (m)		1.39 (m)	
13	1.28 (d, *J* = 7.0)	13.0		41.8		42.9
14	1.36 (s)	16.7	1.42 (m)	50.2	1.38 (m)	49.3
15*α*		172.8	1.72 (m)	38.3	1.99 (m)	39.2
15*β*			2.25 (dd, *J* = 18.3, 7.7)		2.20 (dd, *J* = 17.4, 7.0)	
16				218.8		207.8
17			1.70 (m)	65.1		147.7
18			0.69 (s)	13.4	0.89 (s)	19.4
19a			4.22 (d, *J* = 8.5)	67.2	4.22 (d, *J* = 8.6)	67.2
19b			4.03 (d, *J* = 8.5)		4.02 (d, *J* = 8.6)	
20a			1.59 (m)	17.6	5.73 (q, *J* = 7.3)	130.8
20b			1.28 (m)			
21			1.03 (t, *J* = 7.3)	13.3	2.08 (d, *J* = 7.3)	14.1

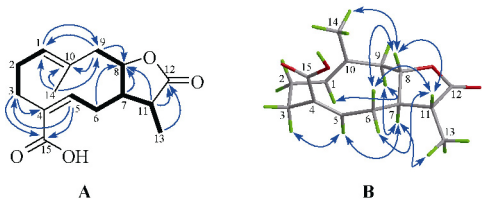

Fig. 2 A) Key ^{1}H–^{1}H COSY (bold lines), HMBC (H→C arrows), and B) ROESY (H↔H double arrows) correlations of 1

The relative configuration of **1** was defined by the analysis of its ROESY spectrum (Fig. 2B). The ROESY cross-peaks of H–C-8/Hβ–C-6, H–C-8/H–C-11, and H–C-11/CH_2-6 indicated that C-6, H–C-8, and H–C-11 were cofacial and randomly assigned in a β-orientation. In consequence, the ROESY correlations of H–C-7/Hα–C-9 and H–C-7/Me-13 suggested that they were α-oriented. The *E*-geometry of $\Delta^{1(10)}$ double bond was fixed by the ROESY correlations of H–C-1/Hα–C-9 and Me-14/H–C-8. By the similar manner, Δ^{4} double bond was assigned as *Z*-geometry based on the ROESY correlations of H–C-5/Hα–C-3 and H–C-5/H–C-7. Thus, the structure of **1** was depicted as shown in Fig. 1.

Vernobockone A (**2**), a white, amorphous powder, gave a molecular formula of $C_{21}H_{30}O_4$ as established on the basis of the HRESI-MS spectrum, indicating seven degrees of unsaturation. The IR spectrum of **2** revealed the presence of OH (3435 cm^{-1}) and carbonyl (1736 cm^{-1}) groups. The 1D NMR data of **2** (Table 1) revealed the presence of two methyl (a singlet and a triplet), nine methylene (an oxygenated), and five methine groups, as well as five quaternary carbons (two keto carbonyls and a hemiketal). The aforementioned data suggested that **2** was likely a C3 oxygenated derivative of 2α, 3β-dihydroxypregnan-16-one 2β, 19-hemiketal [14], which belongs to the pregnane steroid family. The presence of C3 keto carbonyl at δ_C 205.4 was confirmed by the HMBC correlations from CH_2-1, CH_2-4, and H–C-5 to C-3 (Fig. 3A). The relative configuration of **2** was established by analysis of the ROESY spectrum (Fig. 3B) as being identical with that of 2α, 3β-dihydroxypregnan-16-one 2β, 19-hemiketal [14]. Thus, the structure of **2** was elucidated as 2α-hydroxypregnan-3, 16-dione 2β, 19-hemiketal.

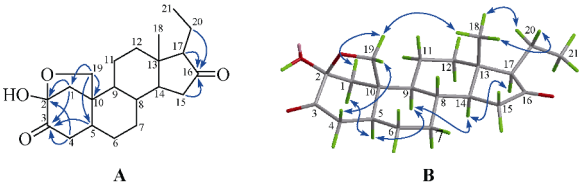

Fig. 3 A) Key HMBC (H→C arrows) and B) ROESY (H↔H double arrows) correlations of 2

Vernobockone B (**3**) was obtained as a white, amorphous powder, and its molecular formula was determined as $C_{21}H_{28}O_4$ by its HRESI-MS. The IR spectrum of **3** indicated the presence of OH (3437 cm^{-1}) and carbonyl (1728 and 1705 cm^{-1}) groups. The 1D NMR data of **3** (Table 1) closely resembled those of **2**, except for the presence of a trisubstituted double bond (δ_H 5.73 (*q*, *J* = 7.3, 1H), δ_C 130.8 and 147.7) in **3**. The $\Delta^{17(20)}$ trisubstituted double bond was located by the HMBC correlations of H–C-14/C-17, Me-18/C-17, and Me-21/C-17 and C-20. As a result of α, β-unsaturation, the keto carbonyl at C-16 shifted upfield by 11.0 ppm as compared to that in **2**. The *Z*-geometry of $\Delta^{17(20)}$ double bond was fixed by the ROESY correlations of H–C-20/CH_2-12. The relative configurations of other stereocenters in **3** were identical to those in **2** as assigned by its ROESY spectrum, as well as by comparing their NMR data. Thus, the structure of **3** was elucidated as 2α-hydroxypregnan-17(20)-ene-3, 16-dione 2β, 19-hemiketal.

Experimental

General

Column chromatography (CC): Silica gel (SiO_2; 300–400 mesh; Qingdao Marine Chemical Plant, Qingdao, China), MCI gel (CHP20P, 75–150 μmol·L^{-1}, Mitsubishi Chemical Industries, Ltd., Tokyo, Japan), and C_{18} reversed-phase silica gel (250 mesh, Merck, Darmstadt, Germany). TLC: pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Plant, Qingdao, China). Semi-prep HPLC: Waters 515 pump with a Waters 2487 detector (254 nm, Milford, United States), and an YMC−Pack ODS−A column (250 mm × 10 mm, S-5 μm, 12 nm, Kyoto, Japan). Optical rotations: Perkin-Elmer 341 polarimeter, Shelton, United States. UV Spectra: Varian Cary 50 UV spectrophotometer (Santa Clara, United States). IR spectra: Perkin-Elmer 577 IR spectrometer (Shelton, United States). NMR spectra: Bruker AM-400 NMR spectrometer (Billerica, United States) with TMS as internal standard. HRESI-MS: Waters Q-TOF Ultima mass spectrometer (Milford, United States).

Plant material

The aerial part of *Vernonia bockiana* was collected from Chishui County, Guizhou Province, China and were authenticated by Professor CHEN Qian-Hai of Guizhou Biological Research Institute, Guizhou Academy of Science. A voucher specimen (2008-Verboc-1Y) has been deposited in Shanghai Institute of Materia Medica.

Extraction and isolation

The air-dried powder of aerial part of *V. bockiana* (5 kg) was extracted three times with 95% EtOH (each 10 L, three days) at room temperature to give an ethanolic extract (650 g), which was dissolved in 1 L water to form a suspension. After defatted with petroleum ether, the aquatic phase was partitioned with EtOAc to obtain the EtOAc-soluble fraction (90 g), which was subjected to passage over an MCI gel column chromatography ($MeOH/H_2O$, 30 : 50 to 90 : 10) to produce four fractions, A–D. Fraction C (20 g) was chromatographed over a silica gel CC, eluted with petroleum ether/

acetone in a gradient (20 : 1 to 1 : 1), to afford five sub-fractions (C1–C5). Fraction C4 was subjected to a reversed-phase C_{18} silica gel CC, eluted with MeOH/H_2O (6 : 4 to 9 : 1), to give two major fractions, C4a and C4b. Fraction C4a was purified by a silica gel CC, eluted with petroleum ether/EtOAc (4 : 1 to 1 : 1), to give compound **1** (5 mg). Fraction C4b was separated by semi-preparative HPLC (CH_3CN/H_2O, 65 : 15, 3 mL·min^{-1}), to give compounds **2** (8 mg) and **3** (7 mg), respectively.

Vernobockolide C (15-carboxygermacra-1(10)E, 4Z-diene-8α, 12-olide; **1**). White gum. $[\alpha]_D^{20}$ +111.7 (*c* 0.12, MeOH). UV (MeOH): 249 (3.60). IR (KBr): 3147, 3113, 2937, 1739, 1707, 1460, 1340, 1217, 1192, 987, 760 cm^{-1}. ^{1}H and ^{13}C NMR: see Table 1. HRESI-MS: 287.1284 ([M + Na]$^+$, $C_{15}H_{20}NaO_4^+$; Calcd. 287.1259).

Vernobockone A (2α-hydroxypregnan-3, 16-dione 2β, 19-hemiketal; **2**). White amorphous powder. $[\alpha]_D^{20}$ −55.2 (*c* 0.13, MeOH). IR (KBr): 3435, 2931, 1736, 1462, 1192, 1174, 1130, 1020, 1005 cm^{-1}. ^{1}H and ^{13}C NMR: see Table 1. HR ESI-MS: 369.2055 ([M + Na]$^+$, $C_{21}H_{30}NaO_4^+$; Calcd. 369.2042).

Vernobockone B (2α-hydroxypregnan-17(20)-ene-3, 16-dione 2β, 19-hemiketal; **3**). White amorphous powder. $[\alpha]_D^{20}$ −18.6 (*c* 0.07, MeOH). UV (MeOH): 243 (3.00). IR (KBr): 3437, 3390, 1728, 1705, 1643, 1192, 1136, 1032, 1009 cm^{-1}. ^{1}H and ^{13}C NMR: see Table 1. HRESI-MS: 367.1872 ([M + Na]$^+$, $C_{21}H_{28}NaO_4^+$; Calcd. 367.1885).

Acknowledgment

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Supporting Information

IR, HRESI-MS, 1D and 2D NMR spectra of compounds **1**–**3** are available free of charge *via* the Internet at http: // onlinelibrary.wiley.com.

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