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A new labdane diterpene from *Leonurus heterophyllus*

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A new labdane diterpene, heteronone B (**1**), together with a known labdane diterpene, heteronone A (**2**), have been isolated from the aerial part of *Leonurus heterophyllus*. Their structures were established mainly by 1D and 2D NMR analysis and the stereochemistry of **2** was confirmed by single-crystal X-ray diffraction analysis.

Keywords: *Leonurus heterophyllus*; Labdane diterpene; Heteronone B

1. Introduction

The whole plant of *Leonurus heterophyllus*, also known as Yimucao in traditional Chinese medicine, is mainly used for the treatment of gynaecological problems, such as amenorrhea, irregular menstruation, and postpartum haemorrhage, as well as edema in chronic and acute nephritis [1]. Many labdane diterpenes have been isolated from related species of the same family over the past decade [1–9]. Our recent phytochemical investigation of *Leonurus heterophyllus* has resulted in the isolation of two labdane diterpenes, heteronone B (**1**) and heteronone A (**2**), of which **1** is new.

2. Results and discussion

Compound **1** was obtained as colorless crystals. ARCIMS revealed a quasimolecular ion peak at m/z 329 $[M + H]^+$. Its molecular formula, $C_{20}H_{24}O_4$, was confirmed by HRESIMS (at m/z 328.1658 $[M]^+$). The ^{13}C -NMR spectrum of **1** contained 20 carbon signals, including four tertiary methyls, two keto functionalities (δ_C 214.6 s, 182.0 s), and eight olefinic carbons. These data allowed the skeleton of **1** to be deduced as a labdane diterpene. The IR

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spectrum showed absorption at 3379 (OH), 1710 (keto), and 1630 (conjugated double bond). Of the nine degrees of unsaturation, six were accounted for by double bonds, of which four were carbon–carbon double bonds, and two were carbon–oxygen double bonds, indicating that **1** was a tricyclic molecule. Its $^1\text{H-NMR}$ spectrum contained resonances for a typical β -substituted furan ring (δ_{H} 7.40, 7.30, 6.33, 1H each, H-15, H-16 and H-14, respectively). The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data for **1** showed remarkable similarities to those of a known compound, heteronone A (**2**) [10]. The significant differences were the absence of four signals, which were assigned to H-5 (δ_{H} 2.31, 1H, d), H-6 (δ_{H} 4.24, 1H, dd), C-5 (δ_{C} 54.0 d) and C-6 (δ_{C} 71.3 d), respectively, in **2** and the presence of two olefinic carbons (δ_{C} 137.5 s, 142.0 s) in the NMR spectrum of **1**. Thus a $\Delta^{5,6}$ double bond was suggested in **1**, which is the only difference between these two compounds. $^1\text{H}-^{13}\text{C}$ correlations observed from C-5 to H-18, H-19 and H-20, and from C-6 to 6-OH as well as from C-7 to 6-OH proved this assumption (figure 1). Full assignment of **1** was finally achieved on the basis of 2D NMR analysis. Consequently, compound **1** was elucidated as 15,16-epoxy-6-hydroxyabd-5(6),8,13(16),14-tetraen-3,7-dione.

Compound **2** was obtained as colorless crystals. The NMR spectrum data were consistent with those reported for heteronone A [10]. As high-quality crystals of **2** can readily be grown, a single-crystal X-ray crystallographic analysis was undertaken to determine the configuration of **2** (figure 2).

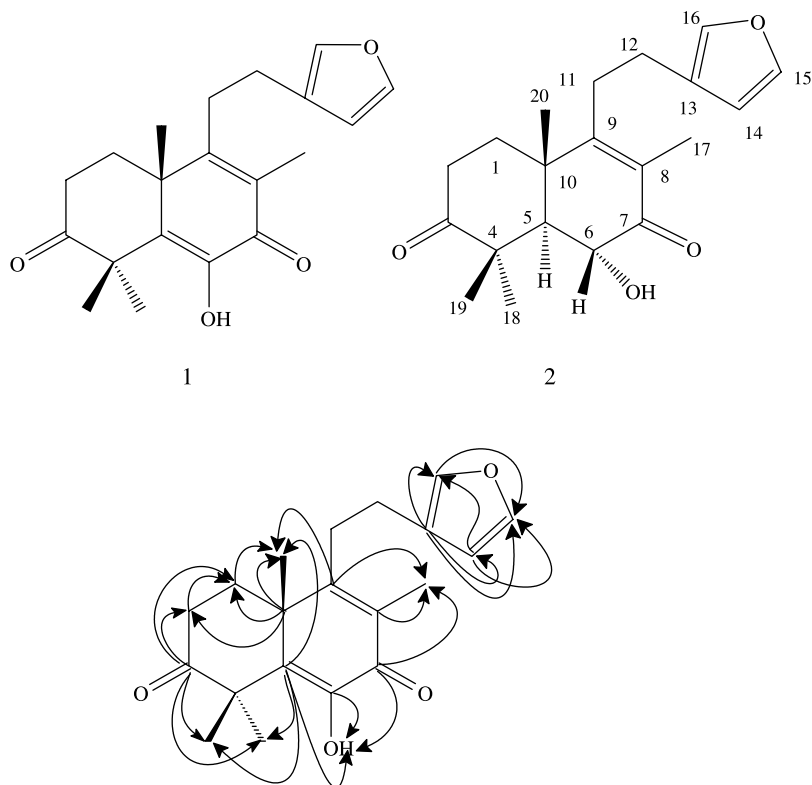
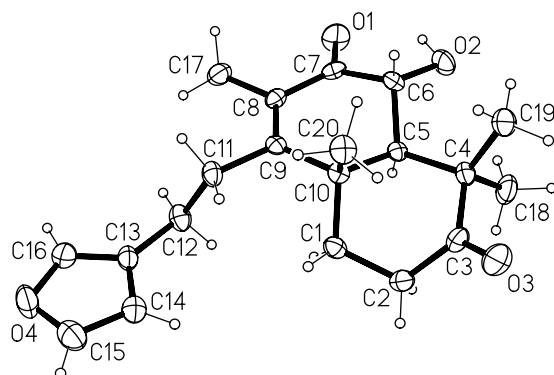


Figure 1. The HMBC correlations of compound **1**.

Figure 2. X-ray crystal structure of heteronone A (**2**).

3. Experimental

3.1 General experimental procedures

Melting points were obtained using a Fisher micro-melting point apparatus and are uncorrected. The IR spectra were recorded on a Bruker IFS-55 instrument (KBr). UV spectra were measured in MeOH using an Agilent 1100 series DAD. MS spectra were recorded on a VG Auto spec-3000 spectrometer. 1D- and 2D-NMR spectra were recorded on a Bruker ARX-600 instrument with TMS as internal standard.

3.2 Plant material

The aerial part of *Leonurus heterophyllus* Sweet were donated by Changchun Huakang Pharmaceutical Company and identified by Professor Qi-shi Sun (Shenyang Pharmaceutical University). A sample has been deposited at the School of Chinese Medicine, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

Dried and powdered aerial parts of *Leonurus heterophyllus* Sweet (10 kg) were extracted three times with EtOH (70%). After evaporation of EtOH *in vacuo*, the concentrated extract was suspended in water and partitioned with EtOAc. The EtOAc portion (30 g) was chromatographed on a silica gel (300 g, Merck) column and eluted with CHCl₃–MeOH (from 100:0 to 2:1) to give 18 fractions monitored by TLC (Merck). Fraction 1 (0.5 g) was rechromatographed over silica gel (30 g) and eluted with cyclohexane–EtOAc (25:1), yielding compound **1** (20 mg). Fraction 2 (2 g) was rechromatographed over silica gel (30 g) and eluted with petroleum ether–acetone (10:1) to yield heteronone A (**2**) (10 mg).

3.3.1 15,16-Epoxy-6-hydroxy-labd-5(6),8,13(16),14-tetraen-3,7-dione (1). Compound **1** was obtained as white needles from methanol, mp 133–135°C. $[\alpha]_D^{20} + 28.90$. UV (MeOH) λ_{\max} : 210 nm, 236 nm, 260 nm. IR (KBr) ν_{\max} (cm⁻¹): 3379 (OH), 2938, 1710 (keto), 1628

Table 1. ^1H - and ^{13}C -NMR data for compound **1** (600 MHz for ^1H , 150 MHz for ^{13}C , CDCl_3).

Position	<i>I</i>		
	δ_{H}	δ_{C}	HMBC
1	1.82–1.87 (1H, m), 2.28–2.32 (1H, m)	28.1 t	H-20
2	2.63–2.77 (2H, overlap)	32.6 t	H-1
3		214.6 s	H-1, H-2, H-18, H-19
4		48.1 s	H-18, H-19
5		137.5 s	H-18, H-19, H-20, OH-6
6		142.0 s	6-OH
7		182.0 s	6-OH, H-17
8		129.4 s	H-17
9		162.8 s	H-17, H-20
10		42.9 s	H-1, H-2, H-20
11	2.52–2.69 (2H, overlap)	31.5 t	
12	2.52–2.62 (2H, overlap)	23.8 t	
13		124.0 s	H-14, H-15, H-16
14	6.33 (1H, t)	110.5 d	H-15, H-16
15	7.40 (1H, t)	143.3 d	H-14, H-16
16	7.30 (1H, br s)	138.8 d	H-15
17	2.05 (3H, s)	11.8 q	
18	1.53 (3H, s)	20.9 q	H-19
19	1.50 (3H, s)	24.4 q	H-18
20	1.14 (3H, s)	21.5 q	
6-OH	6.95 (1H, s)		

(conjugated double bond), 1330. ^1H -NMR and ^{13}C -NMR, see table 1. ARCIMS: 329 $[\text{M} + \text{H}]^+$; ESI-MS (positive mode) m/z 329 $[\text{M} + \text{H}]^+$, 274, 235, 95, 61; HRESIMS m/z 328.1658 $[\text{M}]^+$ (calcd for $\text{C}_{20}\text{H}_{24}\text{O}_4$, 328.1674).

3.3.2 Heteronone A (2). Heteronone A was obtained as colorless crystals from methanol, mp 161–163°C. UV (MeOH) λ_{max} : 208 nm, 248 nm, 266 nm, 304 nm. IR (KBr) ν_{max} (cm^{-1}): 3437 (OH), 2925, 1701 (keto), 1659 (conjugated double bond), 1303, 1318, 1126.

3.3.3 Single-crystal X-ray analysis of heteronone A (2). Crystal data for heteronone A: $\text{C}_{20}\text{H}_{26}\text{O}_4$, $M = 330.41$, orthorhombic, space group $P2_12_12_1$ (No. 19), $a = 7.7879(6)$, $b = 10.3664(8)$, $c = 21.4076(16)$ Å, $V = 1728.3(2)$ Å³, $T = 293$ K, $Z = 4$, 3051 unique Mo $K\alpha$ reflections ($R_{\text{int}} = 0.0656$), final $R_1 = 0.0663$, $wR_2 = 0.1629$ for 2240 observed reflections [$I \geq 2\sigma(I)$]. Intensity data for compound **2** were collected at 293 K in the variable ϕ - and ω -scan mode on a Bruker SMART 1000 CCD diffractometer equipped with an area detector using Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å). Data collection and reduction were performed using SMART and SAINTPLUS (Bruker, 1998) (SMART and SAINTPLUS: Bruker Analytical Instrumentation, Madison, Wisconsin, USA, 1998). The structure of compound **2** was solved by direct methods, yielding the positions of all non-hydrogen atoms, which were refined with anisotropic thermal parameters. Hydrogen atoms attached to carbon atoms were generated assuming idealized geometry (C–H bond lengths fixed at 0.96 Å), assigned appropriate isotropic thermal parameters and allowed to ride on their parent carbon atoms. Hydrogen atoms involved in the hydrogen-bonding scheme were unambiguously located from difference electron density maps, assigned isotropic thermal parameters 1.2–1.5 times those of their parent non-hydrogen atoms and refined using the riding model. All computations were performed on an IBM-compatible PC with SHELXTL97 (Sheldrick,

1997) for full-matrix least-squares refinement against F^2 and XP-Interactive Molecular Graphics, Version 5.1 (Bruker AXS, 1998), was used to prepare the diagrams. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-252690. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK) (. E-mail: deposit@ccdc.cam.ac.uk).

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