

New *ent*-kaurane-type diterpenoids from *Isodon nervosus*Fu Lin Yan^{a,*}, Lan Qing Guo^a, Ji Xia Zhang^a, Su Ping Bai^a, Han Dong Sun^b^a Pharmacy College, Xinxiang Medical University, Xinxiang 453003, China^b Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China

Received 8 October 2007

Abstract

Two new *ent*-kaurane-type diterpenoids, 6 β ,7 β ,13 α -trihydroxy-1 α -acetoxo-7 α ,20-epoxy-*ent*-kaur-16-en-15-one (**1**) and 15 β -hydroxy-6,7-*seco*-6,11 β :6,20-diepoxy-1 α ,7-olide-*ent*-kaur-16-ene (**2**) were isolated from the *Isodon nervosus*, and the structures were elucidated by spectroscopic analysis.

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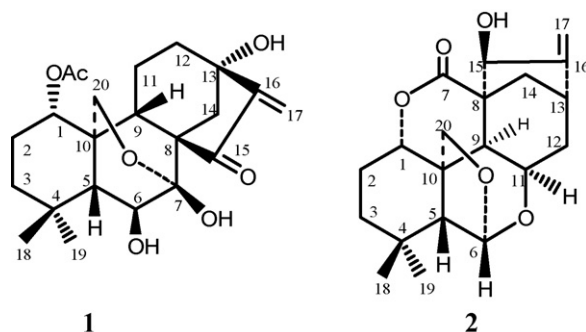
Keywords: Labiatae; *Isodon nervosus*; *ent*-Kaurane diterpenoid

Isodon nervosus (Labiatae), a perennial herb of the *Isodon* genus, which is a rich natural source of bioactive *ent*-kaurane-type diterpenoids. The leaves and stem of this plant have been used as a folk medicine in the treatment of acute hepatitis, sore and eczema in China [1]. From *I. nervosus* collected in Tongbai Prefecture of Henan Province, twelve *ent*-kaurane-type diterpenoids including two new compounds (**1** and **2**) were isolated. Herein, we report the structural elucidation of the two new compounds.

Compound **1**, obtained as colorless needles, mp 265–266 °C, $[\alpha]_D^{23}$ -97 (c 0.11, CH₃OH), the molecular formula of C₂₂H₃₀O₇ was determined on the basis of HRESIMS at m/z 429.1879 [$M + Na$]⁺ (calcd. 429.1889). The IR spectrum exhibited the presence of carbonyl groups (1714 cm⁻¹) and hydroxyl groups (3400, 3318, 3268 cm⁻¹). In the ¹H, ¹³C and DEPT NMR spectra, in addition to the signals of an acetoxo group at δ 169.7 (s), 21.4 (q), 1.91 (3H, s), there were twenty carbon signals which consist of two tertiary methyls, seven methylenes (including one oxygenated at δ 68.8 (t) and one olefinic carbon at δ 116.7 (t)), four methines (including two oxygenated at δ 71.1 (d), 74.5 (d)), seven quaternary carbon (including one oxygenated at δ 74.1 (s), one carbonyl carbon at δ 208.5 (s), one olefinic quaternary carbon at δ 156.4 (s)), one hemiketalic carbon at δ 96.2 (s)), suggesting **1** to be a 7,20-epoxy-*ent*-kauranoid by comparison with reported ¹³C NMR spectral data [2,3]. The position of the acetoxo was confirmed at C-1 (δ 71.1 d) from the long-range correlation of H-1 (δ 5.56 m) with C-21 (δ 169.7 s) in the HMBC spectrum, and three hydroxyl groups were attached to C-6, C-7 and C-13 from the HMBC correlations of H-6 (δ 4.3 br s) with C-5 (δ 60.3 d), C-7 (δ 96.2 s) and C-8 (δ 60.7 s); H-20 (δ 4.23, 4.45 d) with C-7 (δ 96.2 s); H-11 (δ 1.17 m), H-14 (δ 2.97, 2.75 d) and H-17 (δ 6.22, 5.89 br s) with C-13 (δ 74.1 s), respectively. Reported literatures [4–6] showed that the H-5 and H-9 were in β -orientation, H-13 was in α -orientation in the 7,20-epoxy-*ent*-kaurane-type diterpenoids. So, the 13-OH should be α -orientation in compound **1**. The observed NOESY correlations from H-1 to H-9 and OAc to H-20, established that the

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Fig. 1. The structures of compounds **1** and **2**.

H-1 was in β -orientation; the observed NOESY correlation from H-6 to H-19, H-6 no correlation with H-5, it showed that the H-6 was in α -orientation (Fig. 2). Therefore, the structure of **1** was determined to be 6 β ,7 β ,13 α -trihydroxy-1 α -acetoxy-7 α ,20-epoxy-*ent*-kaur-16-en-15-one (Fig. 1).

Compound **2**, obtained as colorless needles, mp 292–293 °C, $[\alpha]_D^{23}$ -11 (*c* 0.22, CH₃OH), was determined to possess the molecular formula C₂₀H₂₆O₅ by the HRESIMS [found (*M* + Na)⁺ 369.1681, calcd. 369.1678]. The IR spectrum exhibited the presence of a lactone carbonyl group (1743 cm⁻¹) and a hydroxyl group (3530 cm⁻¹). The ¹³C and DEPT NMR spectra of **2** (Table 1) showed 20 carbon signals which were indicated to be composed of one lactonic carbonyl carbon, four quaternary carbon (including an olefinic one), seven methines including four oxygenated ones, six methylenes including oxygenated one and olefinic one, and two methyls, which suggested **2** as an *ent*-kauranoid, combined with the consideration of the similar structure of diterpenoids previously isolated from this genus [7,8]. The absence of HMBC cross-peaks between H-5 or H-6 and C-7 indicated a 6,7-*seco* structure, and lactone formation of C-7 to C-1 was supported by an H-1/C-7 correlation in the HMBC spectrum, compound **2** was determined to possess an enmein-type skeleton. However, the extra degree of unsaturation required by the molecular formula indicated the presence of an additional ring. The correlation between H-6 (δ 5.55 br s) and C-11 (δ 63.8 d) in the HMBC spectra unambiguously proved that the additional ring was an ether bridge from C-6 to C-11. Comparison of the structure of compound **2** with Isodoacetal [8], revealed that **2** was 15-deacetyl-isodoacetal, which was confirmed by HMBC and

Table 1
NMR data of compounds **1** and **2** (C₅D₅N, δ in ppm)

Position	1 δ_C	1 δ_H	2 δ_C	2 δ_H
1	71.1 d	5.56 (1H, m)	76.8 d	5.05 (dd, <i>J</i> = 4.0, 12 Hz)
2	31.0 t	1.47, 1.23 (2H, m)	24.1 t	1.81 m
3	41.6 t	1.35 (2H, m)	40.8 t	1.59 m
4	33.9 s		30.6 s	
5	60.3 d	1.42 (1H, d, <i>J</i> = 7.2 Hz)	53.0 d	2.48 s
6	74.5 d	4.30 (1H, brs)	99.9 d	5.55 s
7	96.2 s		174.5 s	
8	60.7 s		54.5 s	
9	53.0 d	1.79 (1H, d, <i>J</i> = 4.4 Hz)	43.3 d	3.42 (d, <i>J</i> = 8.8 Hz)
10	37.1 s		45.3 s	
11	18.8 t	1.17 (2H, m)	63.8 d	4.73 m
12	47.0 t	2.50, 2.46 (2H, d, <i>J</i> = 5.2 Hz)	42.2 t	1.91 m
13	74.1 s		39.3 d	2.83 m
14	36.8 t	2.97, 2.75 (2H, d, <i>J</i> = 11.6, 16 Hz)	34.6 t	2.24 (dd, <i>J</i> = 5.6, 12 Hz)
15	208.5 s		76.6 d	5.52 s
16	156.4 s		154.1 s	
17	116.7 t	6.22, 5.89 (2H, brs)	107.6 t	5.15, 5.49 brs
18	34.5 q	1.29 (3H, s)	34.2 q	1.01 s
19	22.7 q	1.07 (3H, s)	24.5 q	0.99 s
20	68.8 t	4.23, 4.45 (2H, d, <i>J</i> = 8.8 Hz)	75.0 t	3.66, 4.27 (d, <i>J</i> = 8 Hz)
OAc	169.7 s, 21.4 q	1.91 s		

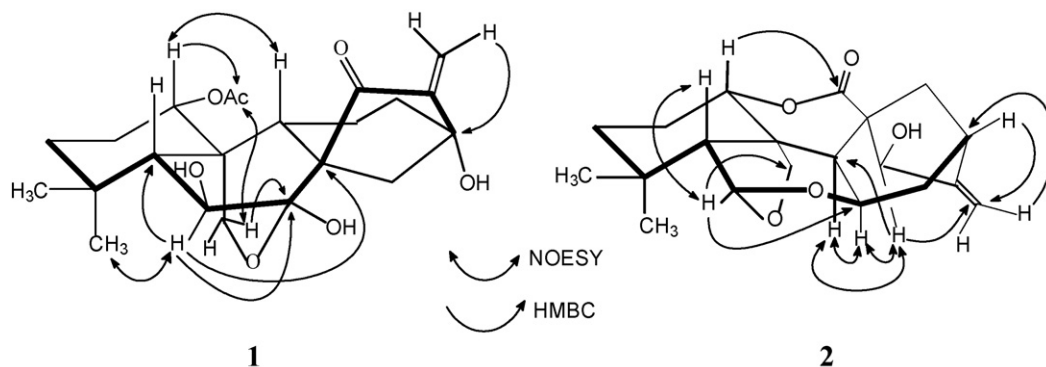


Fig. 2. Selective HMBC and NOESY correlations of compounds **1** and **2**.

NOESY experimental results. NOESY interactions of H-6 with H-5 β , H-9 α with H-11 and H-15 were observed (Fig. 2), which indicated the β -orientation of H-6, the α -orientation of H-11 and H-15, respectively. Therefore, the structure of **2** was determined to be 15 β -hydroxy-6,7-*seco*-6,11 β :6 α ,20-diepox-1 α ,7-olide-*ent*-kaur-16-ene (Fig. 1).

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