Two new *ent*-kaurane diterpenoids from *Isodon macrophyllus*

Ji Xia Zhang[a]*, Zheng Yue Chen[a], Yong Xue Wang[a], Pei Yong Qiu[a], Sheng Xiong Huang[b], Han Dong Sun[b]

[a]*Pharmaceutical Laboratory, Xinxiang Medical College, Xinxiang, Henan Province 453003*

[b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204*

Two new *ent*-kaurane diterpenoids, dayecrystals A (**1**) and B (**2**), together with six known relatives, were isolated from the leaves of *Isodon macrophyllus*. Their structures were determined by spectroscopic means.

Keywords: *Isodon macrophyllus*, *ent*-kaurane, diterpene, dayecrystals A and B

Plants belonging to the genus *Isodon*, are known to be a rich source of *ent*-kaurane diterpenoids, most of which have been shown to have antitumor and anti-inflammatory activities.[1] A reinvestigation of the constituents of *I. macrophyllus* (Migo) H. Hara[2,3] collected in Tongbai prefecture of Henan province of China, resulted in the isolation of two new diterpenoids, dayecrystals A (**1**) and B (**2**), together with six known diterpenoids, epinodosin (**3**),[4,5] isodonoiol (**4**),[6] lasiodonin (**5**),[7,8] oridonin (**6**),[9,10] lasiodin (**7**)[11] and rabdophyllin H (**8**).[2] This paper describes the isolation and structural elucidation of the two new diterpenoids by spectroscopic methods. (Fig. 1).

Compound **1** was obtained from methanol MeOH as colourless needles, $[\alpha]_D^{19}$ – 42.0 (*c* 1.16, MeOH). Its molecular formula, $C_{20}H_{32}O_3$, was deduced from HRESIMS (found 343.2248, calcd for $[M + Na]^+$ 343.2249), suggesting five degrees of unsaturation. Its NMR data in combination with the IR spectrum revealed the presence of an aldehyde group [IR: 2765, 1709 cm^{-1}, δ_H10.30 (1H, s), δ_C 207.7], three methyl carbons [δ_H 1.25, 0.61 and 0.44 (each 3H, s); δ_C 24.2, 32.3, and 21.2], eight methylenes [δ_C 18.0, 18.0, 20.0, 25.9, 33.8, 35.4, 42.1, and 56.0], four methines including one which was oxygenated (δ_C 54.7, 55.7, 59.9, and 81.2), and four quaternary carbons including one oxygenated carbon (δ_C 34.0, 51.4, 54.8, and 80.1). The resonances at δ_H 6.20 (1H, br d, J = 6.4 Hz) and 5.85 (1H, br s) in the ^1H NMR spectrum and the absorption at 3387 cm^{-1} in the IR spectrum suggested the existence of two hydroxyl groups. Considering the structural types of diterpenoids isolated from the genus *Isodon*, compound **1** was assigned tentatively as an *ent*-kaurane diterpenoid with two hydroxyl and an aldehyde group. The downfield chemical shift of C-10 (δ_C 54.8) and the normal shift of C-4 (δ_C 34.0) suggested that the aldehyde carbon might be located at C-20, which was confirmed by the HMBC correlations of H-20 with C-1 and C-5 (Fig. 2). The clear HMBC correlations of H-14 with C-8, C-9, C-15, and C-16 suggested the presence of a hydroxyl group at C-14. The existence of a hydroxyl group at C-16 was established by the cross-peaks of H_2-12, H-15 and H_3-17 with C-16 in the HMBC spectrum. The relative configuration of **1** was established by the NOESY spectrum. The proton of H-14 showing NOESY correlations with H-12α, H-13, and H-20 which suggested a β orientation for the hydroxyl group at C-14. The β-orientation of the hydroxyl group at C-16 was suggested by the clear cross-peaks of H_3-17 with H-13 and H-14α in NOESY spectrum as shown in Fig. 2. Hence, **1** was elucidated as 14β,16β-dihydroxy-20-al-*ent*-kaurane, and named dayecrystal A.

Compound **2** was obtained from methanol MeOH as colourless needles, $[\alpha]_D^{19}$ – 31.0 (*c* 2.52, MeOH) and had a molecular formula of $C_{21}H_{32}O_7$ which was deduced from HRESIMS (found 419.2041, calcd for $[M + Na]^+$, 419.2046).

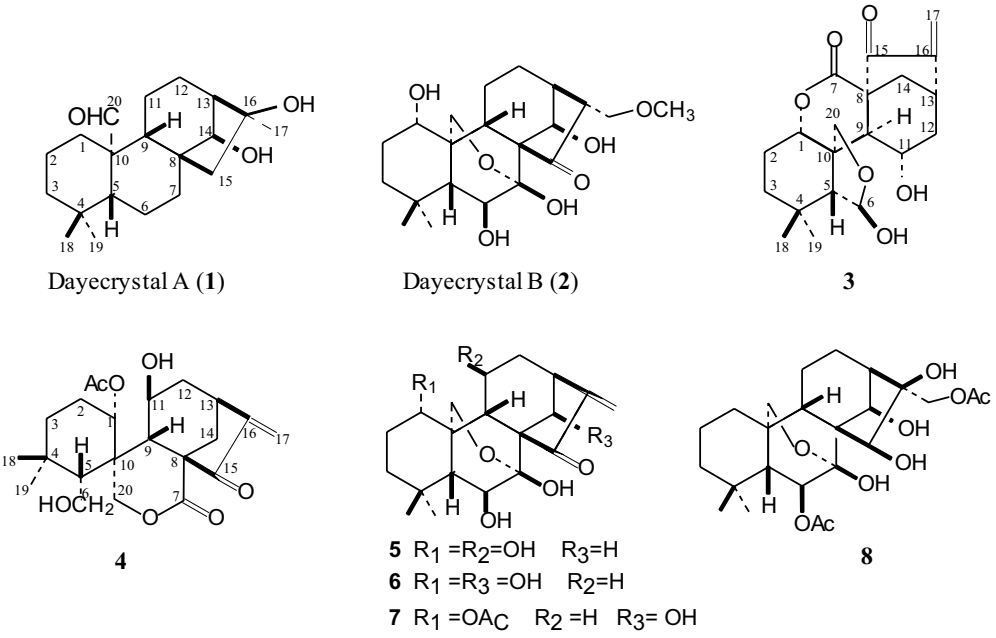

Fig. 1 Molecular structures of compounds **1–8**.

* Correspondent. E-mail: zjx@xxmc.edu.cn

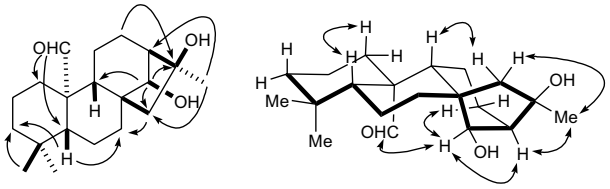

Fig. 2 Key HMBC (left) and NOESY (right) correlations of **1**.

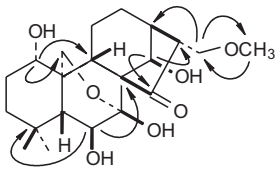

Fig. 3 Key HMBC correlations of **2**.

Comparison of the spectroscopic data of **2** with those of rubescensin G[3] showed similarities except that a hydroxyl group at C-17 in rubescensin G was replaced by a methoxyl group (δ_C 57.6, q) in **2**. This was confirmed by the downfield chemical shift of C-17 from δ_C 63.4 in rubescensin G to δ_C 74.6 in **2**. Examination of its 2D NMR data (Fig. 3) showed that **2** was 16(*R*)-1α,6β,7β,14β-tetrahydroxy-17-methoxy-7α,20-epoxy-*ent*-kaur-15-one. It was named dayecrystal B.

Experimental

Melting points were determined with a Kofler melting point apparatus and uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Shimadzu UV-2550 instrument. IR spectra were taken on a Nicolet 170SX FT-IR spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM-400 NMR spectrometer with TMS as internal standard. HR-ESI-MS was obtained on a Waters HPLCQ-Tof HR-MS spectrometer.

Extraction and isolation procedure

The dried and crushed leaves of *Isodon macrophyllus* (10.0 kg) were extracted three times with Me_2CO/H_2O (7: 3 v/v) at room temperature for 3 days. The extract was filtered and the solvent was removed under reduced pressure. The residue was partitioned between H_2O and AcOEt. The AcOEt fraction gave 360 g of residue after removing the solvent. This residue was separated by silica gel (200–300 mesh) column chromatography with gradient elution of $CHCl_3$/MeOH (1: 0 to 0: 1) to give seven fractions which were subject to repeated chromatography (silica gel, gradient elution with $CHCl_3/Me_2CO$), giving pure compounds: dayecrystal A (**1**, 10 mg), dayecrystal B (**2**, 16 mg), epinodosin (**3**, 43 mg), isodonoiol (**4**, 28 mg), lasiodonin (**5**, 30 mg), oridonin (**6**, 8 g), lasiodin (**7**, 76 mg) and rabdophilin H (**8**, 8 mg). Compounds **3**–**8** were identified by comparing their m.p., IR, MS, ^{1}H and ^{13}C NMR chemical shifts with those reported in the literature.[2,4-11]

Dayecrystal A (**1**): Colourless needles, m.p.173–175°C,$[\alpha]_D^{20}$–42° (*c* 1.16, MeOH), UV λ_{max} (MeOH) 261 nm (logε, 4.52); IR (KBr) ν_{max}/cm^{-1}: 3387, 3217, 2927, 2869, 2765, 1709, 1447, 1066, 1050, 1024, 977, 937, 876. HR-ESI-MS: Found: 343.2248, Calcd. for $C_{20}H_{32}O_3$ + Na: 343.2249. For ^{1}H and ^{13}C NMR data see Table 1.

Dayecrystal B (**2**): Colourless needles, m.p.176–178°C, $[\alpha]_D^{20}$–31° (*c* 2.52, MeOH), IR(KBr) ν_{max}/cm^{-1}: 3231, 2935, 2867, 2833, 1713, 1456, 1253, 1181, 1060. HR-ESI-MS: Found: 419.2041, Calcd. for $C_{21}H_{32}O_7$ + Na: 419.2046. For ^{1}H and ^{13}C NMR data see Table 1.

Received 31 December 2005; accepted 30 January 2006
Paper 05/3726

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Table 1 ^{1}H and ^{13}C NMR data of compounds 1, 2 and rubescensin G (C_5D_5N, δ ppm)

	δ_C			δ_H (J/Hz)	
No	**1**	**2**	rubescensin G	**1**	**2**
1	35.4 t	75.3 d	75.3 d	2.48 (m, H-α) 0.38 (m, H-β)	3.43 (m)
2	18.0 t	30.4 t	31.2 t	1.58 (m), 1.28 (overlap)	1.37-1.45 (2H, overlap)
3	42.1 t	39.1 t	39.4 t	1.07 (m), 0.89 (m)	1.18-1.21 (2H, overlap)
4	34.0 s	33.6 s	33.9 s		
5	55.7 d	59.7 d	60.7 d	0.94 (dd, 15.4, 2.4)	1.25 (d, 7.6)
6	20.0 t	72.9 d	73.2 d	1.44 (m), 1.17 (m)	4.05 (dd, 10.4, 7.6)
7	25.9 t	97.9 s	98.1 s	1.28–1.32 (2H, overlap)	
8	51.4 s	63.3 s	61.4 s		
9	59.9 d	53.0 d	53.5 d	1.29 (overlap)	1.75 (m, H-9)
10	54.8 s	41.3 s	41.6 s		
11	20.0 t	19.9 t	20.2 t	1.71 (m, H-α) 1.15 (m, H-β)	2.18 (overlap, H-α) 1.86 (m, H-β)
12	33.8 t	30.2 t	30.5 t	2.57 (m, H-α) 1.04 (m, H-β)	2.20 (overlap, H-α) 1.64 (overlap, H-β)
13	54.7 d	38.9 d	38.9 d	2.04 (br s)	2.53 (br d, 8.0)
14	81.2 d	74.6 d	74.8 d	3.77 (br d, 6.4)	5.05 (br s,)
15	56.0 t	221.9 s	222.2 s	1.53 (d, 14.4, H-α) 1.98 (d, 14.4, H-β)	
16	80.1 s	57.9 d	60.0 d		2.67 (m)
17	24.2 q	74.6 t	63.4 t	1.25 (s)	3.74 (2H, d, 7.2)
18	32.3 q	33.2 q	33.4 q	0.61 (s)	1.09 (3H, s)
19	21.2 q	22.0 q	22.3 q	0.44 (s)	0.92 (3H, s)
20	207.7 d	63.5 t	63.7 t	10.30 (s)	4.56 and 4.11 (2d, 10.0)
OCH_3		57.6 q			3.05 (3H, s)

^{13}C NMR multiplicities were established by DEPT spectrum.