Diterpenoids from *Isodon excisoides*

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Two new *ent*-kaurane diterpenoids taihangexcisoidesin A and B (**1** and **2**), together with 10 known diterpenoids, were isolated from the EtOAc extract of the leaves of *Isodon excisoides*. Their structures were determined on the basis of spectroscopic methods.

Keywords: *Isodon excisoides, ent-kaurane,* diterpenoid, taihangexcisoidesin A and B

Isodon excisoides (Sun ex C.H. Hu) C.Y. Wu et H.W. Li, a perennial shrub, is distributed in the Yunnan, Sichuan, Hubei and Henan Provences of China. It has been used in Chinese folk medicine to treat sore throat and inflammation. Previous investigations have shown that many bioactive diterpenoids have been isolated from this species, collected in different regions.[1-3] To search more bioactive constituents, we reinvestigated this plant, collected in the Taihang Mountains, Henan Province. From the leaves of *I. excisoides,* two new *ent-kaurane* diterpenoids Taihangexcisoidesin A and B (**1** and **2**), were isolated together with ten known diterpenoids, lasiokaurin[4] (**3**), lasiodonin[5,6] (**4**), isodonoiol[7] (**5**), oridonin[8,9] (**6**), sodoponin[10] (**7**), lasiokaurinol[11] (**8**), enmenol[12] (**9**), rabdosinate[13] (**10**), rabdosin B[14] (**11**) and epinodosinol[10] (**12**). We describe here the isolation and structure elucidation of the two new diterpenoids.

Compound **1**, obtained as colourless needles from MeOH, has a molecular formula $C_{20}H_{34}O_3$ based on its HR-ESI-MS (*m/z* 345.2391 $[M + Na]^+$, Calcd 345.2406) and the ^1H and ^{13}C NMR data, suggesting four degrees of unsaturation. The ^{13}C NMR (DEPT) spectrum showed signals from four methyls, seven methylenes, five methines including two oxymethines [δ_C 78.1 (d), δ_H 3.44 (1H, m) and δ_C 78.9 (d), δ_H 4.46 (1H, d, J = 8.4 Hz)], and four quaternary carbons including one which was oxygenated [δ_C 79.2 (s)]. On the basis of other compounds isolated from the *Isodon* genus, compound **1** was assigned a 20-non-oxygenated-*ent-kaurane* diterpene skeleton. The signals at δ_H 6.32 (1H, d, J = 6.4 Hz), δ_H 5.98 (1H, s) and 5.70

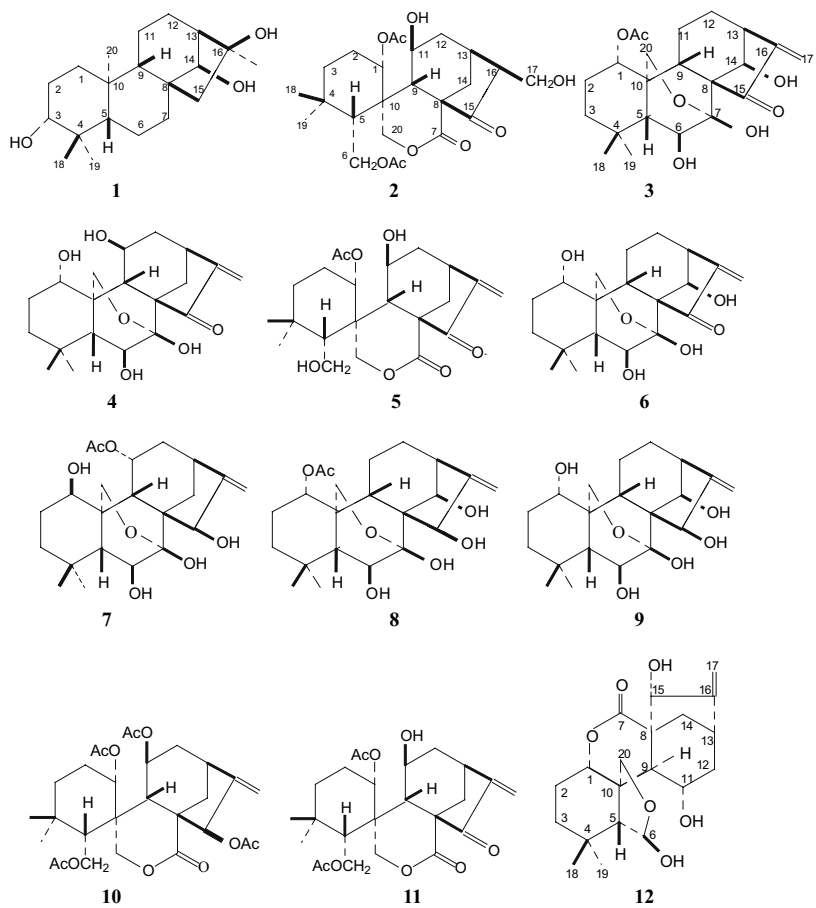

Fig. 1 Molecular structures of compounds **1–12**.

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Table 1 ^{13}C (100 MHz) NMR spetral date of **1** and **2** in C_5D_5N (δ in ppm)

Position	δ_C		Position	δ_C	
	1	**2**		**1**	**2**
1	39.2 t	77.1 d	12	27.8 t	33.1 t
2	28.4 t	24.5 t	13	54.5 d	31.5 d
3	78.1 d	40.1 t	14	78.9 d	31.5 t
4	39.4 s	34.3 s	15	57.3 t	212.4 s
5	55.5 d	49.3 d	16	79.2 s	57.4 d
6	20.2 t	61.9 t	17	24.4 q	59.1 t
7	34.1 t	170.3 s	18	28.0 q	34.0 q
8	51.3 s	59.5 s	19	18.0 q	24.1 q
9	59.6 d	44.1 d	20	16.3 q	67.6 t
10	39.4 s	44.7 s	$OCOCH_3$		170.4 s, 170.5 s
11	18.0 t	65.9 d	$OCOCH_3$		21.5 q, 21.2 q

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

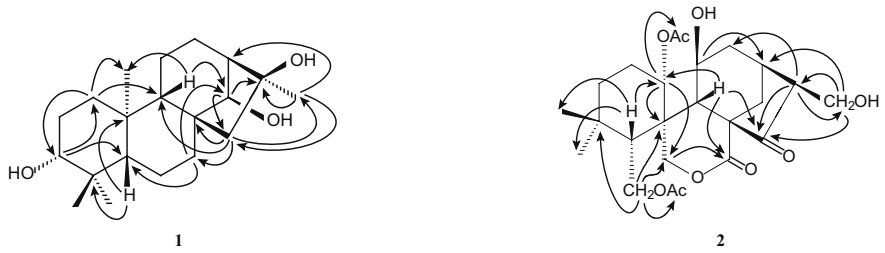

Fig. 2 Key HMBC correlations of compounds **1** and **2**.

(1H, br s) in the ^1H NMR spectrum and the absorption at 3351 and 3318 cm^{-1} in the IR spectrum suggested the presence of three hydroxyl groups. In the HMBC spectrum, correlations were clearly observed between H-14 with C-8, C-9, C-15 and C-16, H-3 with C-1 and C-5, H_2-12, H_2-15 and H_3-17 with C-16 (Fig. 2). Meanwhile, according to the cross-peaks in the HMBC and ^1H–^1H COSY spectra, the three hydroxyl groups were obviously located at C-3, C-14 and C-16, respectively.

The relative configuration of the substituents were revealed by NOESY experments (Fig. 3). In the NOESY spetrum, there were correlations between H-3 and H-1β, H-5β and between Me-18, H-14 with H-6α, H-12α, H-13α and Me-20. Thus, the 3-OH and 14-OH have the α and β orientation, respectively. The β-orientation of the 16-OH was suggested by the clear cross-peaks of H_3-17 with H-13α in the NOESY spectrum as shown in Fig 3. Therefore, **1** was elucidated as 3α, 14β, 16β-trihydroxy-*ent*-kaurane, and named taihangexcisoidesin A.

Compound **2** was obtained from MeOH as colourless needles. It possessed a molecular ion at *m/z* 489.2083 $[M + Na]^+$ in its HR-ESI-MS, consistent with the molecular formula $C_{24}H_{34}O_9$. This was confirmed by its ^{13}C NMR spectrum which showed signals for the 24 carbons in the molecular formula including four carbons of two acetoxyl groups. On the basis of the characteristic lactone carbonyl signal at δ_C 170.3 (s) due to C-7 and significant oxygenated methylene signals [δ_C 67.6 (t), C-20; δ_H 5.08 and 4.79 (each 1H, ABd, J = 12.4 Hz), H-20a/b], compound **2** was assumed to be a 6, 7-seco-*ent*-kauran-7,20-olide. Comparison of the spectroscopic data of **2** with those of rabdosin B[14] (**11**, one known major constituents of this plant) indicated that **2** was almost identical with **11** except for C-16 and C-17. The exomethylene group at C-16 of **11** was replaced by a methine (δ_C 57.4, C-16; δ_H 3.11, 1H, m, H-16α) and a hydroxymethyl group (δ_C 59.1, C-17; δ_H 4.39, 2H, m, H_2-17) in **2**. This was confirmed by the HMBC spectral evidence (Fig. 2). The 16β-CH_2OH was identified by the HMBC correlations of H_2-17 with C-13 and C-15, and the NOEs of H-16α with H-13α, H_2-17 with H-12β and H-9β (Fig. 3), which was also confirmed by the obvious up-field shift of C-12 (δ_C 33.1 in **2**, δ_C 41.5 in **11**) caused by the steric compression between H_2-17 and H-12β[15]. The β-orientation of H-1 was proven by the NOE of H-1 with H-5β. Similarly, H-11α was confirmed by the NOE between H-11α with H-14α. Thus, compound **2** was shown to be 1α,6-diacetoxy-11β-hydroxy-16(*S*)-hydroxymethyl-6,7-seco-*ent*-kauran-15-one-7,20-olide, and named taihangexcisoidesin B.

Experimental

Melting points were determined with a Kofler melting point apparatus and are 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. ^1H, ^{13}C and 2D NMR spectra were recorded

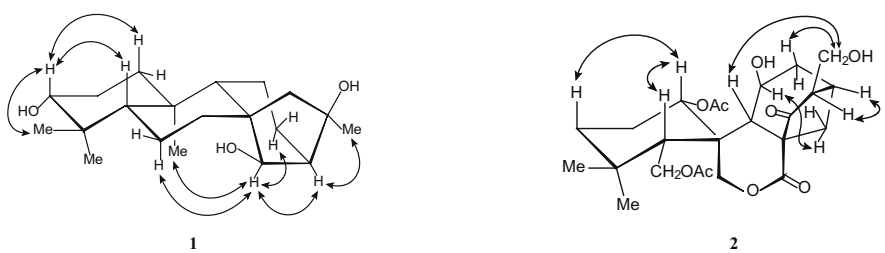

Fig. 3 Key NOESY correlations of compounds **1** and **2**.

on a Bruker AM-400 NMR spectrometer with TMS as the internal standard. HR-ESI-MS was obtained on a Waters HPLCQ-Tof HR-MS spectrometer.

Extraction and isolation procedures

The dried and crushed leaves of *Isodon excisoides* (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 290 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: taihangexcisoidesin A (**1**, 4 mg), taihangexcisoidesin B (**2**, 46 mg), lasiokaurin (**3**, 33 mg), lasiodonin (**4**, 25 mg), isodonoiol (**5**, 19 mg), oridonin (**6**, 7 g), sodoponin (**7**, 13 mg), lasiokaurinol (**8**, 57 mg), enmenol (**9**, 31 mg), rabdosinate (**10**, 7 mg), rabdosin B (**11**, 21 mg), and epinodosinol (**12**, 6 mg) Compounds **3**–**12** were identified by comparing their m.p., IR, MS, ^1H and ^{13}C NMR chemical shifts with those reported in the literature.[4-14]

1: $C_{20}H_{34}O_3$, colourless needles, m.p. 237–239 °C, IR ν (KBr) cm^{-1}: 3351, 3318, 2946, 2864, 1453, 1066, 1040, 1028, 976, 934. ^1H NMR (C_5D_5N, 400 MHz, δppm): 4.46 (1H, d, *J* = 8.4 Hz, H-14α), 3.44 (1H, m, H-3β), 2.78 (1H, d, *J* = 12.8 Hz, H-7a), 2.32 (1H, br s, H-13α), 2.22 and 1.72 (each 1H, d, *J* = 13.6 Hz, H-15a/b), 1.88 (2H, m, H_2-2), 1.74 (1H, m, H-1a), 1.67 (2H, m, H_2-12), 1.60 (2H, m, H_2-11), 1.52 (3H, s, Me-17), 1.45 (2H, m, H_2-6), 1.14 (1H, m, H-9β), 1.13 (1H, m, H-7b), 1.20, 0.98 and 0.97 (each 3H, s, 3 × Me), 0.92 (1H, m, H-1b), 0.85 (1H, dd, *J* = 11.6, 2.0 Hz, H-5β). HR-ESI-MS *m/z*: 345.2391 $[M + Na]^+$(Calcd 345.2406). ^{13}C NMR data see Table 1.

2: $C_{24}H_{34}O_9$, colourless needles, m.p.180–182 °C, $[\alpha]_D^{20}$ + 53.8 (*c* 0.02, MeOH), IR ν (KBr) cm^{-1}: 3572, 3471, 3322, 2993, 2944, 2837, 2822, 1726, 1708, 1640, 1409, 1375, 1301, 1262, 1233, 1126, 1054. ^1H NMR (C_5D_5N, 400 MHz, δppm): 5.57 (1H, dd, *J* = 5.6, 9.6 Hz, H-1β), 5.08 and 4.79 (each 1H, ABd, *J* = 12.4 Hz, H-20a/b), 4.57 and 4.47 (each 1H, dd, *J* = 12.8, 4.0 Hz, H-6a/b), 4.41 (1H, m, H-11α), 4.39 (2H, m, H_2-17), 3.29 (1H, t, *J* = 7.6, 3.6 Hz, H-5β), 3.11 (1H, m, H-16α), 3.06 (1H, d, *J* = 9.6 Hz, H-9β), 2.85 (1H, br s, H-13α), 2.62 (1H, dd, *J* = 12.8, 4.0 Hz, H-14a), 2.32 (1H, d, *J* = 12.8 Hz, H-14b), 2.24 (2H, m, H_2-12), 2.17 and 1.98 (each 3H, s, 2 × OAc), 1.92 (2H, m, H_2-2), 1.33 (2H, m, H_2-3), 0.91 and 0.87 (each 3H, s, 2 × Me). HR-ESI-MS *m/z*: 489.2083 $[M + Na]^+$ (Calcd 489.2101). ^{13}C NMR data see Table 1.

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