ent-Kaurane Diterpenoids from *Isodon japonicus*

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Two new 6,7-seco-*ent*-kaurane diterpenoids, isojaponins A (**1**) and B (**2**), together with 18 known *ent*-kaurane diterpenoids were isolated from the aerial parts of *Isodon japonicus*. The structures of the two new compounds were elucidated by extensive 1D- and 2D-NMR spectroscopic methods in combination with MS experiments.

Introduction. – The genus *Isodon* is rich in *ent*-kaurane diterpenoids, most of which possess various important bioactivities [1][2]. In addition, a number of *ent*-kaurane diterpenoids in this genus have novel chemical structures [3–5]. The leaves of *Isodon japonicus* (BURMAN f.) H. HARA has been used as an antibacterial, anti-inflammatory, stomachic, and anthelmintic agent in China and Japan by local people [6]. Previous phytochemical studies on this plant resulted in the isolation of more than 30 *ent*-kaurane diterpenoids, and most of them possess highly oxygenated structures and antitumor bioactivities [7]. In search for new and bioactive diterpenoids from medicinal plant, we reinvestigated this species collected from the Qinling Mountain, Shanxi Province, People's Republic of China, and isolated 20 *ent*-kaurane diterpenoids including two new compounds, isojaponins A (**1**) and B (**2**), together with 18 known *ent*-kaurane diterpenoids. In this paper, we report the isolation and characterization of the two new compounds, isojaponins A (**1**) and B (**2**).

Results and Discussion. – Compound **1** was obtained as colorless needles. The molecular formula was determined to be $C_{21}H_{30}O_6$ by the HR-ESI-MS (*m*/*z* 401.1939 $[M+Na]^+$). Its ^{13}C-NMR data (*Table*) revealed 20 C-signals besides one MeO group. The IR spectrum showed the presence of OH groups (3384 cm^{-1}) and a C=O group (1726 cm^{-1}). Detailed analysis of the HMBC and ROESY data (*Fig. 1*) established the structure of **1** as (1α,6*R*,11α,15α)-6,20-epoxy-11,15-dihydroxy-6-methoxy-6,7-seco-*ent*-kaur-16-eno-1(7)-lactone[1]).

The ^{1}H- and ^{13}C-NMR spectra of **1** displayed the characteristic signals of an *ent*-kaurane diterpenoid, involving two Me and three unoxygenated CH groups, and three unoxygenated quaternary C-atoms (*Table*). In addition, according to the signals of an OCH_2 group at δ(C) 73.9, an acetal C-atom at δ(C)

[1]) Trivial numbering; for systematic names, see *Exper. Part.*

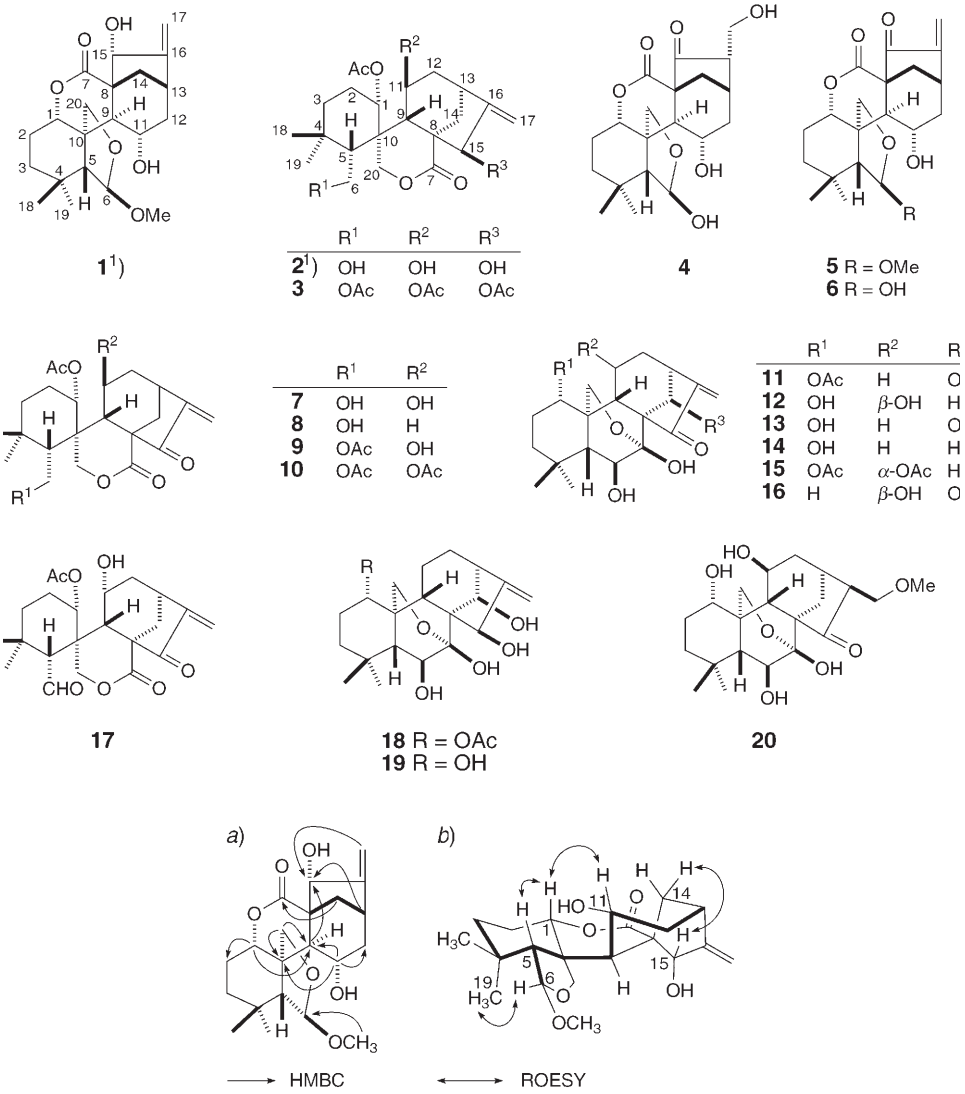

Fig. 1. a) *Key HMBC and* b) *key ROESY correlations for* **1**

109.1, and a carbonyl C-atom at δ(C) 175.2, compound **1** was inferred to be a 6,20-epoxy-6,7-seco-*ent*-kaurano-1,7-lactone diterpenoid. Detailed analysis of the NMR data revealed the existence of an additional MeO (δ(H) 3.15 (*s*, 3 H); δ(C) 54.3) group, and the usual signal of an α,β-unsaturated-ketone C=O group of *ent*-kaurane diterpenoids was replaced by an OCH signal at δ(C) 76.3. On the basis of the HMBC experiments (*Fig. 1,a*), the additional MeO group was located at C(6), and the OCH group (δ(C) 76.3) was attributed to C(15). Furthermore, the signals of C(1) (δ(C) 77.7), C(7) (δ(C) 175.2), and C(11) (δ(C) 63.7) as well as of C(20) (δ(C) 73.9) were also assigned. The orientations of H_β–C(1), H_α–C(6), H_β–C(11), H_β–C(15) were confirmed by the ROESY correlations H–C(1)/H_β–C(5), H–C(6)/Me_α(19), H–C(11)/H_β–C(1), and H–C(15)/H_α–C(14) (*Fig. 1,b*).

Table. *^{1}H- and ^{13}C-NMR Data* (400 MHz, C_5D_5N) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ (H)	δ (C)	δ (H)	δ (C)
H–C(1)	4.82 (*dd*, J = 5.2, 9.6, H_β)	77.7 (*d*)	5.68–5.70 (*m*, H_β)	77.4 (*d*)
CH_2(2)	1.75–1.86 (overlapped, 2 H)	24.1 (*t*)	1.97–2.01 (*m*, 2 H)	24.9 (*t*)
CH_2(3)	1.28–1.33 (*m*, 2 H)	37.0 (*t*)	1.34–1.46 (*m*, 2 H)	40.1 (*t*)
C(4)		31.5 (*s*)		34.1 (*s*)
H–C(5)	3.30 (*s*, H_β)	53.4 (*d*)	3.15 (*d*, J = 3.6, H_β)	54.1 (*d*)
H–C(6) or CH_2(6)	4.99 (br. *s*, H_α)	109.1 (*d*)	3.93 (br. *d*, J = 9.2, H_a), 4.43 (*dd*, J = 3.6, 9.2, H_b)	59.1 (*t*)
C(7)		175.2 (*s*)		175.9 (*s*)
C(8)		53.6 (*s*)		53.4 (*s*)
H–C(9)	3.27 (*d*, J = 8.0, H_α)	46.7 (*d*)	3.70 (*d*, J = 9.2, H_β)	41.1 (*d*)
C(10)		50.9 (*s*)		44.2 (*s*)
H–C(11)	4.27–4.33 (*m*, H_β)	63.7 (*d*)	4.33–4.35 (*m*, H_α)	65.8 (*d*)
CH_2(12)	2.90–2.92 (*m*, H_α), 1.90–1.86 (*overlapped*, H_β)	45.8 (*t*)	1.61–1.65 (*m*, H_β), 2.63–2.67 (*m*, H_α)	46.0 (*t*)
H–C(13)	2.69–2.72 (*m*, H_β)	37.3 (*d*)	2.70–2.72 (*m*, H_α)	36.2 (*d*)
CH_2(14)	1.65 (*dd*, J = 4.0, 9.6, H_α), 2.03 (*d*, J = 9.6, H_β)	34.5 (*t*)	1.87 (*d*, J = 9.6, H_α), 2.32 (*dd*, J = 4.0, 10.0, H_β)	30.7 (*t*)
H–C(15)	5.52 (br. *s*, H_β)	76.3 (*d*)	4.98 (*s*, H_α)	83.8 (*d*)
C(16)		158.5 (*s*)		160.3 (*s*)
CH_2(17)	5.21 (br. *s*, H_a), 5.47 (br. *s*, H_b)	108.5 (*t*)	5.24 (br. *s*, H_a), 5.47 (br. *s*, H_b)	109.1 (*t*)
Me(18)	0.96 (*s*)	32.9 (*q*)	1.01 (*s*)	34.3 (*q*)
Me(19)	0.92 (*s*)	23.3 (*q*)	0.81 (*s*)	21.6 (*q*)
CH_2(20)	4.18 (*d*, J = 7.2, H_a), 4.44 (*d*, J = 7.2, H_b)	73.9 (*t*)	5.13 (*s*, H_a), 5.14 (*s*, H_b)	67.8 (*t*)
MeO	3.15 (*s*)	54.3 (*q*)		
AcO				170.5 (*s*)
			2.20 (*s*)	21.6 (*q*)

Compound **2** was obtained as white amorphous powder. The HR-ESI-MS data revealed the $[M+Na]^+$ ion peak at m/z 431.2058, corresponding to the molecular formula $C_{22}H_{32}O_7$. Its ^{13}C-NMR data revealed 20 C-signals, besides those of one AcO group. Analysis of the 1D-NMR (*Table*) and 2D-NMR data as well as comparison with those of **3** allowed the elucidation of the structure of **2** as (1α,11β,15β)-1-(acetyloxy)-6,11,15-trihydroxy-6,7-seco-*ent*-kaur-16-eno-7(20)-lactone[1]).

The 6,7-seco-*ent*-kaurano-7(20)-lactone skeleton of **2** was deduced from the characteristic signals of two Me groups at δ(H) 1.01 (*s*, 3 H) and 0.81 (*s*, 3 H), a C=O group at δ(C) 175.9, and an OCH_2 group at δ(H) 5.13 and 5.14 (each *s*, 1 H) (*Table*). Detailed comparison of NMR data of **2** with those of **3** revealed that **1** was similar to **3** except for the presence of three OH groups in **1** instead of three AcO groups in **3**. The same conclusion was also drawn from the different MS data of **2** and **3**. The locations of the AcO and three OH groups in **1** were determined by HMBC experiments (*Fig. 2, a*). Furthermore, according to the ROESY correlations H–C(1)/H_β–C(5) and H–C(11)/H_α–C(13) (*Fig. 2, b*), and the obvious upfield signal of C(9) (δ(C) 41.1), which was caused by the *γ-gauche* steric compression effect from the 15β-positioned OH group, the orientations of H_β–C(1), H_α–C(11), and OH_β–C(15) were determined.

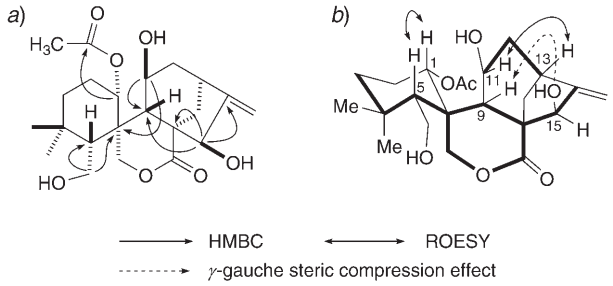

Fig. 2. a) *Key HMBC and* b) *key ROESYcorrelations for* **2** *and the* γ-gauche *steric compression effect*

The structures of the 18 known *ent*-kaurane diterpenoids were elucidated by comparison of their NMR with literature data as rabdosinate (**3**) [8], maoyecrystal D (**4**) [9], rabdosin A (**5**) [10], isodonoiol (**6**) [8], epinodosin (**7**) [11], rabdoternin H (**8**) [12], rabdosin B (**9**) [10], acetylexidonin (**10**) [13], lasiokaurin (**11**) [14], lasiodonin (**12**) [15], oridonin (**13**) [16], effusanin A (**14**) [17], shikokianin (**15**) [18], rosthorin A (**16**) [19], isodonal (**17**) [20], lasionkaurinol (**18**) [21], enmenol (**19**) [22], and lushanrubescensin F (**20**) [23].

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Experimental Part

General. Column chromatography (CC) and TLC: silica gel (200–300 mesh) from *Qingdao Marine Chemical Factory*, Qingdao, People's Republic of China. Melting points: *XRC-1* micro melting point apparatus; uncorrected. Optical rotations: *Jasco-DIP-370* digital polarimeter. UV Spectra: *UV-210A* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad-FtS-135* spectrophotometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-DRX-400* and *-DRX-500* instruments; $SiMe_4$ as an internal standard. MS: *VG-Auto-Spec-3000* spectrometer; in *m*/*z* (rel. %).

Plant Material. The aerial parts of *I. japonicus* were collected in the Qinling Mountain, Shanxi Province, People's Republic of China, in July 2005, and identified by Prof. *Xi-Wen Li*, Kunming Institute of Botany. A voucher specimen has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered aerial parts (7.5 kg) of *I. japonicus* were extracted with 70% aq. Me_2CO (3 × 30 l) at r.t. to yield the extract (427 g), which was dissolved in H_2O and then extracted successively with petroleum ether and AcOEt. The AcOEt extract (211 g) was subjected to CC (silica gel, $CHCl_3/Me_2CO$ 1:0 → 1:1): *Fractions I–V. Fr. IV* (82 g) was decolorized by passing it through a *MCI-gel CHP-20P* column (90% MeOH/H_2O) and subjecting it repeatedly to CC (silica gel and reversed-phase): **1** (5 mg), **2** (8 mg), and **3**–**20** in quantities of 2–21 mg each.

Isojaponin A (= *(1α,6*R*,11α,15α)-6,20-Epoxy-11,15-dihydroxy-6-methoxy-6,7-seco*-ent-*kaur-16-eno-1(7)-lactone* = *(5α)-13-Deoxy-1,*O*^{1}-dihydro-5-hydroxy-*O*^{10}-methylenmein*; **1**): Colorless needles. M.p. 228–230°. $[\alpha]_D^{26} = -130.5$ (*c* = 0.85, MeOH). UV (MeOH): 204 (2.50). IR (KBr): 3384, 2949, 1726, 1635, 1459, 1053, 1022, 918. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 401.1939 ([*M* + Na]$^+$, $C_{21}H_{30}O_6Na^+$; calc. 401.1940).

Isojaponin B (= *(1α,11β,15β)-1-(Acetyloxy)-6,11,15-trihydroxy-6,7-seco*-ent-*kaur-16-eno-7(20)-lactone* = *(1*S*,2*R*,4′a*S*,5′*S*,6*S*,7′*S*,9′*R*,9′a*S*)-6-(Acetyloxy)hexahydro-5′,9′-dihydroxy-2-(hydroxymethyl)-3,3-dimethyl-8′-methylenespiro[cyclohexane-1,4′(3′*H*)-[1*H*-7,9a]methanooxyclohepta[*c*]pyran]-1′-one*; **2**): White amorphous powder. $[\alpha]_D^{19} = +31.5$ (*c* = 0.59, MeOH). UV (MeOH): 204 (3.50). IR (KBr):

3423, 2952, 2877, 1736, 1639, 1369, 1238, 1124, 985. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS (pos.): 431.2058 ($[M + Na]^+$, $C_{22}H_{32}O_7Na^+$; calc. 431.2045).

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