A new *ent*-kaurane deterpenoid from *Isodon excisoides* (Sun ex C. H. Hu) C. Y. Wu et H. W. Li

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A new *ent*-kaurane diterpenoid, excisoidesin **(1)**, was isolated from the acetone extract of the leaves of *Isodon excisoides* (Sun ex C. H. Hu) C. Y. Wu et H. W. Li, along with kamebacetal B **(2)**, glaucocalyxin A **(3)**, leukamenin E **(4)**, kamebanin **(5)** and wangzaozin A **(6)**. The structure of the new compound was determined as 7α,14β,18-trihydroxy-*ent*-kaur-16-en-3,15-dione by spectroscopic methods. Compound **1–6** showed significant cytotoxic activity against human tumor Bel-7402 cells.

Keywords: *Isodon excisoides* (Sun ex C. H. Hu) C. Y. Wu et H. W. Li, *ent*-kaurane diterpenoid, excisoidesin, cytotoxicity

Isodon excisoides (Sun ex C. H. Hu) C. Y. Wu et H. W. Li, a perennial herb, is mainly found distributed in Yunnan, Sichuan and Gansu Provinces.[1] Leaves of the plant have been used in folk medicine for the treatment of digestive disorders, dyspepsia and ulcers. Although a large number of *ent*-kaurane diterpenoids with various biological activities have been isolated from the genus *Isodon*, the chemical constituents and biological activities of *Isodon excisoides* (Sun ex C. H. Hu) C. Y. Wu et H. W. Li have not been reported previously. Our search for bioactive diterpenoids from the leaves of *I. excisoides* (Sun ex C. H. Hu) C. Y. Wu et H. W. Li, collected in Zang County of Gansu Province, led to the isolation of a new diterpenoid, excisoidesin **(1),** together with the known, kamebacetal B **(2)**, glaucocalyxin A **(3)**, leukamenin E **(4)**, kamebanin **(5)** and wangzaozin A **(6)**.

Excisoidesin **(1)** was obtained as white needles. The EI-MS spectrum of **1** showed a molecular ion peak at *m/z* = 348 consistent with a molecular formula of $C_{20}H_{28}O_5$, which was confirmed by the HR-EIMS and NMR spectra. The NMR spectra (Table **1**) indicated the presence of two methyls, six methylenes (including one oxygen-bearing methylene), five methines (including two oxygen-bearing methines), three quaternary carbons, two olefinic carbons, one carbonyl carbon and an α, β-unsaturated ketonic carbon [δ_C 207.6 (s), δ_C150.1 (s), δ_C 116.3 (t), δ_H 6.28 (1H, s), δ_H 5.37 (1H, s)]. This corresponded to the basic skeleton of *ent*-kaurane diterpenoids previously described in the genus *Isodon*.[3–7] A careful analysis of the NMR spectral data revealed that the structure of compound **1** was similar to that of glaucocalyxin A **(3)**[4] except for one more oxygenated methylene and one less methyl group. Comparison of their ^{13}C NMR spectral data indicated that the difference between **1** and **3** was only in the ring A. This meant that two hydroxyl groups were located at C-7 and C-14, and a hydroxymethyl group and a carbonyl group were attached to the ring A in compound **1**. The hydroxymethyl group (δ_H 3.66 and δ_H 3.91, each 1H, AB *d*, *J* = 10.4 Hz; δ_C 68.5 *t*) was assigned to C-18 because there was a long-range correlation between the methylene carbon bearing an oxygen atom at δ_C 68.5 and the methyl protons at δ_H 1.01 (19-CH_3) in the HMBC spectrum. It was also supported by the upfield shifts of C-5 (δ_C 44.5) and C-19 (δ_C 17.6) due to a γ-gauche shielding shift effect on C-5 and C-19, and the downfield shift of C-4 (δ_C 52.4) due to a β-effect on C-4[8,9] when compared with those [C-5 (δ_C 51.5), C-19 (δ_C 20.9), C-4 (δ_C 46.8), respectively] of compound **3**.[11] Moreover the presence of the C7–OH, C14–OH and C3-oxo were proved by clear HMBC correlations of H-7β (δ_H 4.76) with C-5 (δ_C 44.5), C-9 (δ_C 53.1) and C-14 (δ_C 75.4); H-14α (δ_H5.10) with C-15 (δ_C 207.6), C-16 (δ_C 150.1) and C-17 (δ_C 116.3); C-3 (δ_C 216.4) with H-2β (δ_H 2.50) and H-19 (δ_H 1.01, Me), and by ^1H–^1H COSY cross-peaks between δ_H 4.76 (1H, *d*, *J* = 12.0 Hz, H-7β) with δ_H 2.08 (1H, *d*, *J* = 12.8 Hz, H-6α) and δ_H 2.23 (1H, *m*, H-6β); δ_H 5.10 (1H, *s*, H-14α) with δ_H 3.24 (1H, *br s*, H-13α).

The stereochemistry at C-7 and C-14 positions in **1** was determined by NOESY experiment. The NOESY spectra of **1** exhibited correlations for H-7β with H-5β and H-9β, H-14α with H-12α and Me-20 (Table 1). Thus, compound **1** was shown to be 7α,14β,18-trihydroxy-*ent*-kaur-16-en-3,15-dione (Fig.1).

Compounds **2–6** were identified by comparison of their ^1H and ^{13}C NMR, MS and IR spectroscopic data with those reported in the literature for kamebacetal B **(2)**, [10] glaucocalyxin A **(3)**,[11] leukamenin E **(4)**, [12] kamebanin **(5)**, [13] and wangzaozin A **(6)**.[14]

As shown in Table 2, compound **5** were found to exhibit the most significant cytotoxicity against human tumor Bel-7402 cells with IC_{50} 1.46 μM. Compound **1**, **2**, **4** and **6** demonstrated modest cytotoxic effect on Bel-7402 cells IC_{50} value of 4.10–5.50 μM.

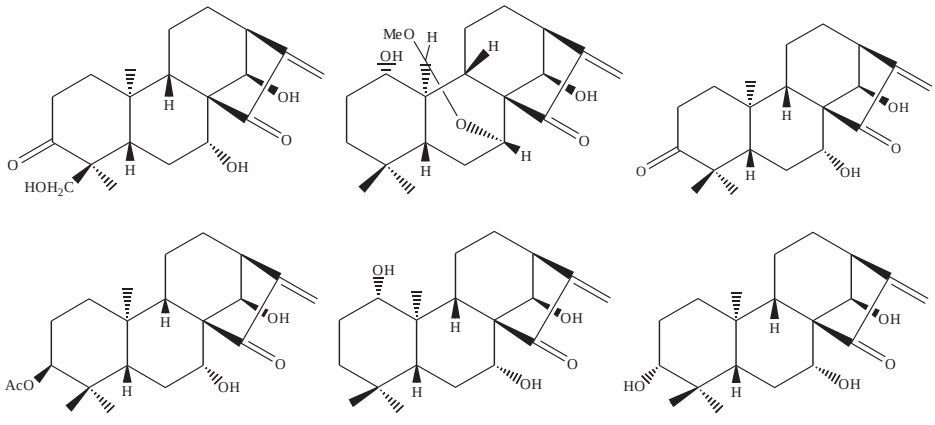

Fig. 1

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Table 1 ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of compound **1**[a]

No.	1 (δ_C)	δ_H (*J*, Hz)	H-H COSY	HMBC	NOESY
1	37.2 t	1.36 (β) d (12.8)	H-1α, H-2β, H-2α	H-20	H-5β, H-9β
		1.70 (α) m	H-2β, H-2α		H-11α
2	36.5 t	2.50 (β) m	H-1α, H-1β, H-2α		
		2.70 (α)	H-1α, H-1β, H-2β		H-19, H-20
3	216.4 s			H-19, H-2β	
4	52.4 s			H-19, H-5β	
5	44.5 d	2.66 (β) d (11.6)	H-6α, H-6β	H-6α, H-7β, H-19, H-20	H-1β, H-6β, H-7β, H-9β
6	30.9 t	2.23 (β) m	H-5β, H-7β	H-5β	H-5β
		2.08 (α) d (12.8)	H-5β, H-7β		
7	73.5 d	4.76 (β) d (12.0)	H-6α, H-6β	H-6α, H-6β, H-5β	H-5β, H-9β
8	61.7 s			H-6α	
9	53.1 d	1.56 (β) br s	H-11 α, H-11β	H-1β, H-5β, H-7β, H-20	H-1β, H-5β, H-7β,
10	38.6 s			H-20, H-1β, H-5β, H-6α	
11	18.4 t	1.44 (β) m	H-9 β, H-12α, H-12β	H-9β,	H-5β, H-9β
		1.41 (α) m	H-9 β, H-12α, H-12β		H-1α, H-12α, H-20
12	31.0 t	1.64 (β) dd (12.6, 4.8)	H-11α, H-11β, H-12α		
		1.62 (α) m	H-11α, H-11β, H-12β		H-13α, H-14α
13	46.8 d	3.24 (α) br s	H-12α, H-14α	H-11, H-17a, H-17b	H-12α, H-14α, H-17a
14	75.4 d	5.10 (α) s	H-13α	H-7β, H-17a	H-12α, H-20
15	207.6 s			H-14α, H-17a, H-17b	
16	150.1 s			H-14α, H-17b	
17	116.3 t	5.37 (a) s	H-17b, H-13β	H-14α	H-17b, H-13α
		6.28 (b) s	H-17a, H-13β		H-17a
18	68.5 t	3.66 (a) d (10.4)	H-18b	H-19, H-5β	
		3.91 (b) d (10.4)	H-18a		
19	17.6 q	1.01 (3H) s		H-5β	H-2α, H-20
20	18.1 q	1.09 (3H) s			H-19,H-14α, H-11α, H-2α

[a]Determined in C_5D_5N, ^{13}C NMR multiplicities were established by DEPT.

Table 2 Cytotoxicity of compound 1–6

		IC_{50} (μ M)[a]
Test substance	MW	Bel-7402
1	348	4.80±1.06
2	362	4.99±1.03
3	332	2.74±0.64
4	376	5.50±1.03
5	334	1.46±0.49
6	334	4.10±1.00

[a]IC_{50}: 50% inhibition concentration.

Experimental

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR-spectra were taken on an IFS-120H IR spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on an INOVA-400 (Varian) spectrometer with TMS as internal standard. HRMS and EIMS spectra were obtained on an Autospec 3000 and HP 5988 MS spectrometer respectively.

Extraction and isolation procedures: The air-dried leaves of Isodon excisoides (Sun ex C. H. Hu) C. Y. Wu et H. W. Li were extracted with 70% Me_2CO and filtered. The filtrate was concentrated and extracted with EtOAc. The EtOAc extract (120 g) was applied to a silica gel column and eluted with $CHCl_3$–Me_2CO gradient system to yield fractions *I–V*. All fractions were collected and combined by monitoring with TLC. Each fraction was further purified by recrystallisation obtaining excisoidesin **(1,** 15 mg**)**, kamebacetal B **(2,** 20 mg**)**, glaucocalyxin A **(3,** 32 mg**)**, leukamenin E **(4,** 305 mg**),** kamebanin **(5,** 255 mg**)**, and wangzaozin A **(6,** 18 mg**)**.

Excisoidesin (**1**): White needles, m.p. 211–213 °C, [α] –107° (*c* 0.15, MeOH). IR ν_{max} cm^{-1}: 3312, 1721, 1711, 1640, 1432, 1248, 1088, 939. EI-MS *m/z* (rel. int.): 348 (M+, 9), 330 (16), 312 (15), 297 (8), 281 (15), 194 (57), 176 (100), 105 (62). HR-FABMS *m/z*: 349.1913 $[M+1]^+$, Calcd. 349.1937. ^{1}H and ^{13}C NMR data see Table 1.

Cytotoxicity against human tumor Bel-7402 cells: The cytotoxicity assay was performed in a method of SRB, the experimental details of which have been reported previously.[15]

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