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Chinese Chemical Letters 21 (2010) 200–202
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

A new *ent*-kaurane diterpenoid from *Isodon japonica*

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Received 23 June 2009

Abstract

One new *ent*-kaurane diterpenoid, named maoyecrystal L was isolated from the EtOAc extract of the dried leaves of *Isodon japonica*. Its structure was established by various spectroscopic means and confirmed by X-ray crystallographic analysis.
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Keywords: Labiatae; *Isodon japonica*; Ent-kaurane diterpenoid; Maoyecrystal L

Isodon japonica (Burm. f.) Hara is widely distributed in China and has various bioactivities [1]. The leaves of *Isodon japonica* have been used as an antibacterial, anti-inflammatory, stomachic, and anthelmintic agent in China, Korea and Japan by local people [2,3]. In our research for biologically active constituents, we examined the leaves of *Isodon japonica*, and a new ent-kaurane diterpenoid named maoyecrytal L (**1**) was isolated. Its structure was elucidated by spectroscopic technique (1D NMR, 2D NMR, HR-MS and IR) and confirmed by X-ray crystallographic analysis.

The leaves of *Isodon japonica* were collected in Tongbai County of Henan province, in August 2007, and identified by Professor Changshan Zhu, Henan Agriculture University.

The air-dried leaves of *Isodon japonica* (9 kg) were pulverized and extracted three times with Me_2CO/H_2O (7:3, v/v) at room temperature for 6 days and filtered. The filtrate was concentrated and partitioned with EtOAc. The EtOAc layer was evaporated under reduced pressure to obtain residue (350 g), which was subjected to silica gel column (12 cm × 150 cm, 3000 g, 200–300 mesh) gradiently eluted with $CHCl_3$–CH_3OH (1:0, 30:1, 20:1, 10:1, 5:1 3:1, 0:1) to give seven factions according to their TLC analysis. From the faction 4 ($CHCl_3$–CH_3OH 10:1) compound **1** (62 mg) was obtained by repeated silica gel column with $CHCl_3$–Me_2CO.

Compound **1** was obtained as colorless crystals (methanol), mp 232–234 °C, $[\alpha]_D^{23}$ − 79.6 (*c* 0.45, CH_3OH). The IR (KBr) spectrum showed absorption at 3533, 3424 cm^{-1} (hydroxyl group), 1710 cm^{-1} (ester group). Its molecular formula of $C_{24}H_{34}O_8$ was determined on the basis of HRESIMS at *m/z* 473.2149[M+Na]$^+$(calcd. 473.2151). The ^1H and ^{13}C NMR spectrum (Table 1) exhibited the presence of two acetate groups at δ_C171.1 (s), 171.2 (s), 21.3 (q), 21.9 (q), 2.10 (s, 3H), 2.16 (s, 3H) and the other twenty carbon signals which consist of two tertiary methyls, seven

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doi:10.1016/j.cclet.2009.10.029

Table 1
^{1}H NMR and ^{13}C NMR data of compounds **1** and **2**.

Position	[a]1 δ_H	[a]1 δ_C	[b]2 δ_H	[a]2 δ_C
1	3.73 (br s, 1H)	65.2 d	3.58 (t, 1H, J = 2.0 Hz)	65.5 d
2	1.76 (m, 2H)	27.6 t		27.9 t
3	1.19 (m, 2H)	34.3 t		34.4 t
4		33.8 s		33.9 s
5	2.31 (d, 1H, J = 6.8 Hz)	50.4 d	1.93 (d, 1H, J = 7.0 Hz)	50.1 d
6	5.85 (d, 1H, J = 6.8 Hz)	75.1 d	5.21 (d, 1H, J = 7.0 Hz)	75.6 d
7		95.6 s		95.9 s
8		49.7 s		51.3 s
9	3.20 (dd, 1H, J = 4.8, 12.8 Hz)	38.2 d		39.0 d
10		40.8 s		41.0 s
11	2.23 (m, 2H)	17.2 t		15.4 t
12	2.56, 2.19 (m, 2H)	40.0 t		27.6 t
13		75.5 s		37.1 d
14	2.54, 2.39 (d, 2H, J = 11.2 Hz)	36.8 t		32.0 t
15	6.41 (br s, 1H)	73.6 d	5.62 (t, J = 2.0 Hz, 1H)	75.6 d
16		162.1 s		160.0 s
17	5.69,5.45 (d, each 1H, J = 1.6 Hz)	107.8 t	5.04, 4.91 (dd, each 1H, J = 2.0, 1.0 Hz)	108.9 t
18	1.02 (s, 3H)	33.0 q	0.87 (s, 3H)	33.3 q
19	1.22 (s, 3H)	23.4 q	1.13 (s, 3H)	23.8 q
20	4.13 (s, 2H)	66.1 t	3.93 (s, 2H)	66.5 t
OAc	2.10 (s, 3H)	171.1 s, 21.3 q	2.06 (s, 3H)	171.7 s, 21.6 q
OAc	2.16 (s, 3H)	171.2 s, 21.9 q	2.19 (s, 3H)	171.7 s, 22.2 q

[a] ^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz; pyridine-d_5; δ in ppm.
[b] ^{1}H NMR (60 MHz) were measured in $CDCl_3$.

methylenes (including an olefinic one and an oxygenated one), five methines (including three oxygenated ones), six quaternary carbon (including an olefinic one and two oxygenated ones). Considering the structures of diterpenoids previously isolated from the genus Isodon [4–6], compound **1** was assigned an *ent*-kauranoid. Comparison of the spectroscopic data of **1** and Thichokaurin (**2**) [7,8] revealed close similarities, and the only difference was that **1** had one more OH group. The additional OH group was positioned at C-13 due to the signals shifted to lower field at 40.0 (C-12), 75.5 (C-13) and 36.8 (C-14) in **1**, compared to those of compound **2** showing the corresponding signals at 27.6 (C-12), 37.1 (C-13) and 32.0 (C-14). The HMBC correlations of H-11 (δ 2.23, m), H-14 (δ 2.54, 2.39 d) and H-17 (δ 5.69, 5.45 d) with C-13 (δ 75.5 s) confirmed the above assignment. On the basis of the above evidence, the structure of compound **1** was determined to be 1β,7β,13α-trihydroxy-6β,15β-diacetoxy-7α,20-epoxy-*ent*-kaur-16-ene, and named maoyecrystal L (Fig. 1).

The single crystal X-ray diffraction analysis confirms the molecular structure of **1** proposed by spectroscopic methods. The crystal data were as follows: Colorless block crystal from MeOH, crystal size: 0.63 mm × 0.63 mm × 0.60 mm; crystal data: a = 10.2945(18), b = 13.696(3), c = 15.802(3) Å, V = 2228.0(7) Å^3, space group $P2_12_12_1$, Z = 4, D_{cacl} = 1.343 g/cm^3, λ = 0.71073 Å, μ(Mokα) = 0.10 mm^{-1}, F(0 0 0) = 968, T = 293(2) K; a total of 18,422 reflections were collected on a Rigaku SPIDER with a rotating anode tube. Refinement with 2878 reflections (2819 with $I > 2\sigma$) led to final R, R (all), GOF values of 0.0298, 0.0311, 1.002. Ring A adopts

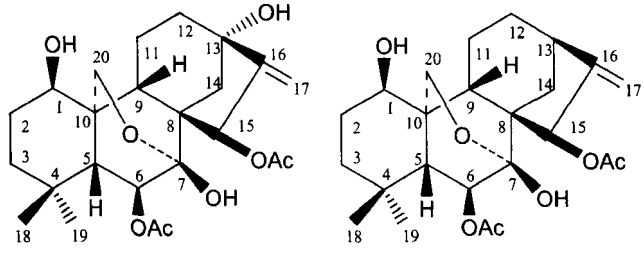

Fig. 1. The structure of compounds **1** and **2**.

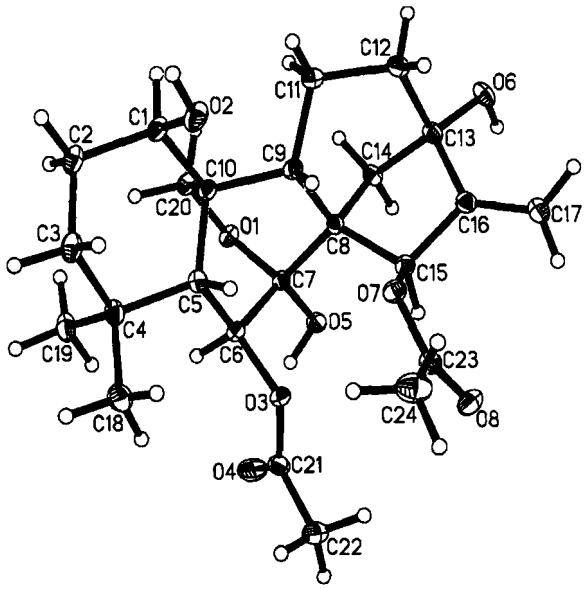

Fig. 2. The crystal structure of compound **1**.

chair conformation, with an average torsion angle of 51.43°. Rings B and ring C adopt boat conformation because of the formation of the oxygen bridge at C-7 and C-20. Ring D shows an envelope conformation; the flap atom, C14, lies 0.7551 Å from the plane defined by atoms C8, C15, C16 and C13 (Fig. 2). Crystallographic data for the structure in this paper were measured by the Beijing Institute of Technology.

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