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Two New *ent*-Kaurane Diterpenoids from *Isodon japonica*

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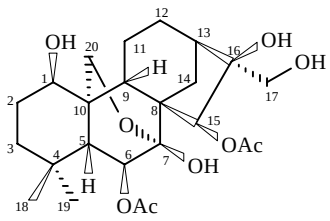
Abstract: Two new *ent*-kauranoids, named maoyecrystals A (**1)** and B (**2)**, were isolated from the EtOAc extract of the dried leaves of *Isodon japonica* (Burman f.) Hara collected in Tongbai mountains, Henan Province. Their structures were determined on the basis of spectral data, especially by 2D NMR.

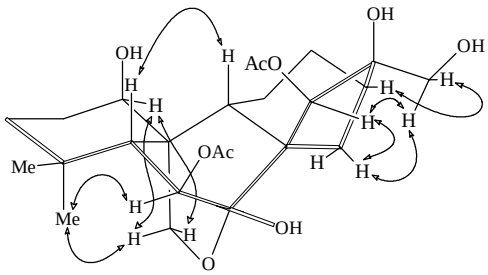
Keywords: *Isodon japonica*, *ent*-kauranoids, maoyecrystals A and B.

Isodon japonica (Burman f.) Hara has been used in folk medicine as anti-bacterial, inflammation disminishing and stomachic agents, even anthelmintic in many places of China and Japan[1]. Moreover, its anticancer activities[2-4] had been proved early in 1960's. Continuous phytochemical studies[5-17] on this plant collected in different regions had revealed more than thirty *ent*-kauranoids. To find more active substances, we have carefully reinvestigated the chemical components of *I. japonica* (Burman f.) Hara collected in Tongbai mountains, Henan Province, which resulted to obtain two new *ent*-kauranoids, named maoyecrystals A (**1)** and B (**2)**. Their structures were determined as 1β, 7β, 16β, 17-tetrahydroxy-6β, 15β-diacetoxy-7α, 20-epoxy-*ent*-kaurane (**1**) and 1β, 7β-dihydroxy-6β, 15β-diacetoxy-16β, 17: 7α, 20-diepoxy-*ent*-kaurane (**2**) by spectral methods. In this paper, the structure elucidation is presented.

Figure 1 Key ROESY correlations of compound **1**



1



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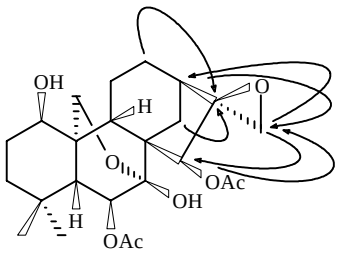
Maoyecrystal A 1, colorless rhombic crystals (from acetone), $[\alpha]_D^{20.0}$ –63.64 (*c* 1.10, MeOH), mp: 149-151°C, had a molecular formula as $C_{24}H_{36}O_9$ determined by EIMS ($[M]^+$ *m/z* 468), HREIMS (calcd. 468.2359, found 468.2333) and ^{13}C NMR spectrum. Comparison of the ^1H and ^{13}C NMR spectra (see **Table 1**) with the reference's data[18] indicated that **1** owned not only the 7β-hydroxy-7α, 20-epoxy-*ent*-kaurane skeleton including additionally oxygenated three methines, one methylene and one quaternary carbon, but also two acetoxyl groups which were located at C-6 and C-15 respectively, based on the HMBC correlations of H-6 (δ_H 6.23) with one quaternary carbon at δ_C 171.2, and H-15 (δ_H 5.91) with the other at δ_C 171.6. Similarly, the clearly observed ^1H-^{13}C long-range correlations of H-1/C-5, H-1/C-9 and H-1/C-20 suggested the presence of OH-1. In the same way, the existence of OH-16 was proved by the coupling signals from H_2-12 and H_2-14 to the quaternary carbon (δ_C 80.8, due to C-16), and the presence of OH-17 was also established according to the obvious correlations of H_2-17 with C-15, and H-15 with C-17 in turn, which was confirmed by the ones of H_2-17 with C-13, and H-13 with C-17.

Finally, the relative configurations of the oxy-substituents were deduced by key interactions appeared in ROESY spectrum as exhibited in **Figure 1**. In detail, OH-1 was established to be β-oriented by the coupling of H-1 with H_2-20, and OAc-6 to be also in β-orientation by the clear cross peak of H-6/H_3-19. As for OH-16, it could be designated as β-orientation because the interactions of H_2-17/H-13 appeared distinctly while H-13 was absolutely α-oriented in all *ent*-kauranoids obtained so far, which was affirmed by the NOE correlations between H_2-17 with H-14 at δ_H 2.60. Furthermore, the interaction of the same H-14 with H-15 indicated that H-15 must be α-oriented. Therefore, **1** was elucidated as 1β, 7β, 16β, 17-tetrahydroxy-6β, 15β-diacetoxy-7α, 20-epoxy-*ent*-kaurane, and named maoyecrystal A.

Figure 2 Key HMBC correlations of compound **2 (**from H to C)



2

Maoyecrystal B 2, colorless prismatic crystals (in MeOH), $[\alpha]_D^{20.0}$ –64.44 (*c* 0.90, MeOH), mp: 201-203°C, gave a molecular ion peak at *m/z* 450 $[M]^+$ in EIMS, corresponding to a molecular formula of $C_{24}H_{34}O_8$ which was confirmed by HREIMS (calcd. 450.2254, found 450.2246). By comparing the ^{13}C NMR data of **2** with those of **1** (see **Table 1**), it was found that the two compounds were so similar that just few notable differences existed among the data for rings C and D, mainly at C-12, 16 and 17.

The signal of C-12 was recognized at δ_C 20.4 in the ^{13}C NMR data of **1**, but replaced by a downfield shift at δ_C 25.7 in **2**. Moreover, the chemical shifts of C-16 (δ_C 80.8) and C-17 (δ_C 69.4) in **1** moved upfield to δ_C 74.6 and δ_C 48.5 in **2**, respectively. These alterations indicated that the condensation of neighboring 16β-OH and 17-OH, which lost a molecule of H_2O, had taken place. That was why **2** was 18 amu less than **1**, and why the chemical shift of C-12 in **2** was obviously downfield than that in **1** and the chemical shifts of H_2-12 in **2** were obviously upfield than those in **1**, without γ-steric compression from 16β-OH. At the same time, careful contrast of **2** to its analog, jiuhuanin A[19], showed up that the two compounds had the similar structure unit of ring D, especially the unit of an oxirane between C-16 and C-17. In fact, the chemical shifts of

Table 1 ^1H and ^{13}C NMR data[a] of **1** and **2** (δ in ppm, J in Hz, C_5D_5N)

Position	**1**		**2**	
	δ_H (400MHz)	δ_C[b]	δ_H (500MHz)	δ_C[b]
1	4.16 (br. s, 1H)	65.4 (CH)	3.73 (br. s, 1H)	65.0 (CH)
2	2.17 (overlap, 2H)	27.8 (CH_2)	1.76 (overlap, 1H)	27.5 (CH_2)
			1.74 (overlap, 1H)	
3	2.65 (overlap, 1H)	34.5 (CH_2)	2.28 (m, 1H)	34.2 (CH_2)
	1.63 (overlap, 1H)		1.20 (overlap, 1H)	
4		34.1 (C)		33.8 (C)
5	2.73 (br. s, 1H)	51.0 (CH)	2.24 (d, 1H, 6.0)	50.8 (CH)
6	6.23 (br. s, 1H)	75.3 (CH)	5.83 (d, 1H, 6.0)	74.9 (CH)
7		96.1 (C)		95.6 (C)
8		51.9 (C)		52.5 (C)
9	3.81 (m, 1H)	39.0 (CH)	3.26 (d, 1Hd, 9.6, 4.4)	38.8 (CH)
10		41.0 (C)		40.7 (C)
11	2.50 (overlap, 1H)	15.5 (CH_2)	2.10 (m, 1H)	15.6 (CH_2)
	2.10 (overlap, 1H)		1.67 (overlap, 1H)	
12	3.00 (overlap, 1H)	20.4 (CH_2)	1.97 (m, 1H)	25.7 (CH_2)
	2.13 (overlap, 1H)		1.59 (m, 1H)	
13	3.03 (overlap, 1H)	36.8 (CH)	1.78 (overlap, 1H)	37.5 (CH)
14	2.60 (overlap, 1H)	26.0 (CH_2)	2.51 (dd, 1H, 12.5, 9.0)	28.1 (CH_2)
	2.50 (overlap, 1H)		2.19 (d, 1H, 12.5)	
15	5.91 (br. s, 1H)	75.9 (CH)	5.97 (s, 1H)	77.0 (CH)
16		80.8 (C)		74.6 (C)
17	4.88 (d, 1H, 8.0)	69.4 (CH_2)	2.94 (d, 1H, 5.0)	48.5 (CH_2)
	4.57 (d, 1H, 8.0)		2.77 (d, 1H, 5.0)	
18	1.43 (s, 3H)	33.1 (CH_3)	1.00 (s, 3H)	32.8 (CH_3)
19	1.63 (s, 3H)	23.5 (CH_3)	1.22 (s, 3H)	23.1 (CH_3)
20	4.59 (br. d, 1H, 12.4)	66.3 (CH_2)	4.13 (br. s, 2H)	66.0 (CH_2)
	4.55 (br. d, 1H, 12.4)			
6-$COCH_3$		171.2 (C)		170.4 (C)
6-$COCH_3$	2.50 (s, 3H)	21.6 (CH_3)	2.03 (s, 3H)	21.2 (CH_3)
15-$COCH_3$		171.6 (C)		171.0 (C)
15-$COCH_3$	2.55 (s, 3H)	22.0 (CH_3)	2.16 (s, 3H)	21.5 (CH_3)

[a]The data were measured with reference to TMS

[b]The ^{13}C NMR data were measured in 125MHz

C-12, 16, and 17 in jiuhuanin A were similar to those in **2** and occurred in file at δ_C 26.3, δ_C 73.8 and δ_C 48.0. So, it was further evidenced that the condensation existed. This conclusion could be also confirmed by HMBC cross-peaks of H_2-17/C-13 and C-15, as

well as by those from H_2-12 and H_2-14 to C-16 (see **Figure 2**). Thus, **2** was finally elucidated as 1β, 7β-dihydroxy-6β, 15β-diacetoxy-16β, 17: 7α, 20-diepoxy-*ent*-kaurane, and named maoyecrystal B.

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