

## A New Diterpenoid Isolated from *Isodon japonica* Structure Elucidation by NMR Spectroscopy

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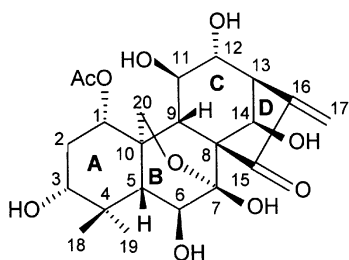
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From the plants of the genus *Isodon* (Labiatae), many diterpenoids having *ent*-kaurene, 7,20-epoxy-*ent*-kaurene, 6,7-*seco-ent*-kaurene, 8,9-*seco-ent*-kaurene, 6-*epi-ent*-gibberelene, *ent*-isopimanthrene and abietene skeletons have been isolated and characterized.<sup>1</sup> These substances have shown various biological activities such as antibacterial, antitumor and cytotoxic effects<sup>2-3</sup> etc. In a previous study of the chemical constituents of *Isodon*, we reported on the isolation of two new *ent*-kaurene diterpenoids, taibairubescensins A and B.<sup>1</sup> During the course of our systematic search for biologically active substances from plants of the genus *Isodon*, recently, we examined diterpenoid constituents of *Isodon japonica* (Burm. f.) Hara (Labiatae) collected from Taibai Mountain, China, and isolated a new diterpenoid, named taibaijaponicain E (**1**), along with four known compounds, norhendosin A (**2**), nodosin (**3**), rabdophyllin G (**4**), lasiodin (**5**). We report here the isolation and structure elucidation of new compound by a combination of NMR techniques, including DEPT, HMBC and NOESY.



Taibaijaponicain E (**1**) has a molecular formula as C<sub>22</sub>H<sub>30</sub>O<sub>10</sub> that was determined by FABMS and elemental analysis. The <sup>1</sup>H, <sup>13</sup>C and DEPT NMR spectra of **1** (see Table 1) showed the signals for three methyl groups (including a methyl of acetyl group), two methylene groups, nine methine groups, four quaternary carbons, two olefinic carbons, one ketonic carbon and one ester carbonyl carbon. Its UV and IR spectra showed characteristic absorption bands for a five-membered ring ketone conjugated with an *exo*-methylene (233.2 nm; 1737, 1644 cm<sup>-1</sup>). The IR spectrum also revealed hydroxyl and acetyl bands at 3438, 3337 and 1712 cm<sup>-1</sup>. One of four quaternary carbons had a chemical

shift signal at  $\delta$  98.3 ppm, and the <sup>1</sup>H NMR spectrum exhibited an AB peak signals at  $\delta$  4.26, 4.10 ppm. Considering the structures of diterpenoids from the genus *Isodon*, these data suggested that **1** was a 7,20-epoxy-*ent*-kaurene type diterpenoid with one acetoxy group and six hydroxyl groups.

In HMBC spectrum of **1** (see Table 1), the carbon signal at  $\delta$  40.5 (s) and the proton signal at  $\delta$  3.02 (1H, br d,  $J$  = 9.7 Hz) were assigned to the resonances of C-10 and H-13 $\alpha$ , respectively. The carbon signal at  $\delta$  40.5 correlated with the proton signal at  $\delta$  4.63 ppm (1H, dd,  $J$  = 11.3, 5.6 Hz) which showed cross-peaks with the carbon signals at  $\delta$  170.1 (s, CH<sub>3</sub>COO-) and 25.8 (t, C-2). These indicated that the acetoxy group was located at the C-1 position. The coupling constants between H-1 and 2H-2 were 11.3 and 5.6 Hz, which suggested that the dihedral angles between H-1 and H-2 approximated 180° and 60°, and the relative configuration of the acetoxy group at C-1 was in a  $\alpha$ -orientation. Meanwhile, HMBC correlations from the carbon signal at  $\delta$  76.0 (d, C-1) to the proton signals at  $\delta$  1.44 (1H, m, H-2 $\alpha$ ) and 1.38 (1H, m, H-2 $\beta$ ), and its DEPT spectrum indicated that C-2 was a methylene group. Since there was not hydroxyl group at C-13 deduced from the chemical shifts of C-13 (44.1d) and H-13, and one out of two methylene groups was accounted for C-20, six hydroxyl groups should be unambiguous located at C-3, C-6, C-7, C-11, C-12, C-14, respectively. Previous studies of the NMR spectra of 7,20-epoxy-*ent*-kaurene type diterpenoids showed that ring A adopts the chair conformation, ring B has a boat conformation, ring D is in an envelope conformation, and ring C is in either a chair or a boat conformation depending on the substituents in ring C; and that HO-6 and HO-7 are in the  $\beta$ -orientations.<sup>5</sup> In compound **1**, the ring C should be in a boat conformation and the HO-14 should be in a  $\beta$ -orientation by considering the coupling constants of H-14 $\alpha$  and H-13 $\alpha$ . The relative configurations of the hydroxyl groups at C-3, C-11 and C-12 were established by a NOESY experiment, most of the NOESY correlations are shown in Figure 1. The following cross-peaks were observed: from the proton signal at  $\delta$  3.67 (H-3) to the signals at  $\delta$  1.35 (H-5 $\beta$ ) and 4.63 (H-1 $\beta$ ); 5.41 (H-11) to the signals at  $\delta$  4.92 (H-14 $\alpha$ ) and 3.02 (H-13 $\alpha$ ); 4.93 (H-12) to the signal at  $\delta$  2.00 (H-9 $\beta$ ). On the basis of above evidences, the three hydroxyl groups at C-3, C-11 and C-12 were assigned to be in the  $\alpha$ , $\beta$ , $\alpha$ -orientations, respectively. Therefore, taibaijaponicain E (**1**) was elucidat-

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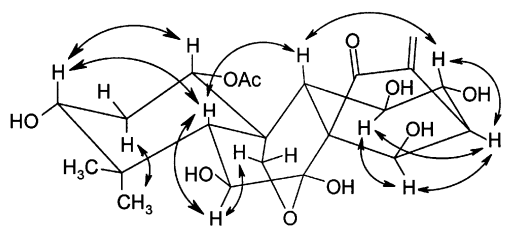


Figure 1. Major NOE correlations in **1**.

ed to be 1 $\alpha$ -aectoxy-3 $\alpha$ ,6 $\beta$ ,7 $\beta$ ,11 $\beta$ ,12 $\alpha$ ,14 $\beta$ -hexahydroxy-7,20-epoxy-*ent*-kaur-16-en-15-one.

Compounds **2**, **3**, **4** and **5** were identified as diterpeneoids, norhendosin A, nodosin, rabdophyllin G and lasiodin by comparison of the physical and  $^{13}\text{C}$ -NMR data with reported ones [6]-[10].

### Experimental Section

**General Methods.** FAB mass spectrum was recorded on a ZAB-HS Instrument (data system: MASPEC II). Elementary analysis was performed on a PE2400 CHN elemental ana-

Table 1.  $^{13}\text{C}$ ,  $^1\text{H}$  NMR data and principal HMBC correlations of **1** in acetone- $d_6$ .

Proton	$\delta^a$	$\delta_{\text{H}}$ , mult. integr. (J, Hz)	HMBC (carbon) <sup>b</sup>
1 $\beta$	76.0d	4.63, dd, 1H. (11.3, 5.6)	(2), 9, (10), 20, AcO (170.1 ppm)
2 $\alpha$	25.8t	1.44, m, 1H	(1), (3)
2 $\beta$		1.38, m, 1H	(1), (3)
3 $\beta$	74.6d	3.67, m, 1H	(4)
4	34.1s	—	—
5 $\beta$	61.0d	1.35, overlap, 1H	(4), (6), (10), 19
6 $\alpha$	74.4d	3.67, overlap, 1H	(5), (7), 10
7	98.3s	—	—
8	62.8s	—	—
9 $\beta$	53.5d	2.00, br s, 1H	(8), (10), 20
10	40.5s	—	—
11 $\alpha$	73.7d	5.41, br d, 1H, (9.2)	8, (12), 13
12 $\beta$	74.5d	4.93, m, 1H	9
13 $\alpha$	44.1d	3.02, br d, 1H, (9.7)	11, (12), (14), 15, (16), 17
14 $\alpha$	73.9d	4.92, overlap, 1H	(8), 12, 15, 16, 17
15	209.1s	—	—
16	153.1s	—	—
17a	119.4t	6.02, s, 1H	13, 15, (16)
17b		5.52, s, 1H	13, 15, (16)
18	22.0q	1.07, s, 3H	(4), 19
19	32.9q	1.11, s, 3H	(4), 18
20a	63.6t	4.26, 4.10, AB, dd, each 1H, (10.7, 1.3)	5, 9, (10)
20b			5, 9, (10)
OAc	21.3(q) 170.1(s)	1.94, s, 3H	AcO (170.1 ppm)
HO-6		6.15, d, 1H, (10.5)	(6), 7
HO-7		6.38, s, 1H	6, (7), 8

<sup>a</sup>The  $^{13}\text{C}$  NMR multiplicities were obtained by the DEPT spectrum.

<sup>b</sup>Two-bond correlations are indicated in parentheses.

lyzer. UV spectrum was recorded in MeOH on a HITACHI U-2000 spectrophotometer. IR spectrum was recorded in KBr pellets on a Nicolet AVATAR 360 FT-IR spectrometer. The optical rotation was measured with a JASCO-20C polarimeter. NMR spectra were recorded on a Bruker AM-400 Instrument. The chemical shift values are given in ppm using TMS as the internal standard.

**Plant Material.** The plant material was collected in Taibai Mountain, Shaanxi Province, P. R. China, in August 1997. It was identified as *Isodon japonica* (Burm. f.) Hara. A voucher specimen (SNU 97-08-03, 1.i) was deposited in the Herbarium of Life Science College, Shaanxi Normal University.

**Extraction and Isolation.** The dried powdered leaves and tender branches of *Isodon japonica* (7.0 kg) were extracted with 95% EtOH (18 L  $\times$  2) at room temperature for 7 days. After removal of the solvent in vacuo, the residue was partitioned in H<sub>2</sub>O and extracted with petroleum ether (4 L  $\times$  3) and EtOAc (4 L  $\times$  3), respectively. The EtOAc extract (130 g) was subjected to column chromatograph (CC) on silica gel (3 kg, 200-300 mesh), eluting with CHCl<sub>3</sub> and increasing proportions of Me<sub>2</sub>CO (CHCl<sub>3</sub>/Me<sub>2</sub>CO: from 10:0 to 0:10). The eluate from 8:2 (CHCl<sub>3</sub>/Me<sub>2</sub>CO) was further submitted to repeated CC to give **1** (224 mg, 0.0032%), **2** (273 mg, 0.0039%), **3** (110 mg, 0.0016%), **4** (67 mg, 0.00096%), **5** (3.3 g, 0.047%).

**Taibaijaponicain E (1):** An amorphous white powder. FABMS  $m/z$ : 461 [M-Li]<sup>+</sup>, 477 [M+Na]<sup>+</sup>, and elemental analysis: C 58.0%, H 6.7% (calcd. C 58.1%, H 6.6%) for C<sub>22</sub>H<sub>30</sub>O<sub>10</sub>.  $[\alpha]_{\text{D}}^{22}$ : -42.3° (c = 0.50, acetone); UV  $\lambda_{\text{max}}$  (MeOH): 233.2 nm (log  $\epsilon$  = 3.38), IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3438, 3337, 2963, 1737, 1712, 1644, 1245 and 1071.  $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT NMR spectral data and principal HMBC correlations are listed in Table 1. Most of NOESY correlations are shown in Figure 1.

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### References

- Guo, Y. W.; Chen, P. Y. *Zhong Cao Yao* **1991**, *22*, 371-375.
- Fujita, T.; Takeda, Y.; Sun, H. D.; Minami, Y.; Marunaka, T.; Takeda, S.; Yamada, Y.; Iogo, T. *Planta Medica* **1988**, *54*, 414-417.
- Nagao, Y.; Ito, N.; Kohno, T.; Kuroda, H.; Fujita, E. *Chem. Pharm. Bull.* **1982**, *30*, 727-729.
- Li, B. L.; Chen, S. N.; Shi, Z. X.; Chen, Y. Z. *Phytochemistry* **2000**, *53*, 855-859.
- Xu, G. Y. *Acta Chimica Sinica* **1985**, *43*, 35-43.
- Meng, X. J.; Chen, Y. Z.; Cui, Y. X.; Cheng, J. L. *J. Nat. Prod.* **1988**, *51*, 812-816.
- Osawa, K.; Yasuda, H.; Maruyama, T.; Morita, H.; Iakeya, K.; Itokawa, H.; Okuda, K. *Chem. Pharm. Bull.* **1994**, *42*, 922-925.
- Chen, Y. Z.; Wu, Z. W.; Cheng, P. Y. *Acta Chimica Sinica* **1984**, *42*, 645-649.
- Cheng, P. Y.; Xu, M. J.; Lin, Y. L.; Shi, J. C. *Acta Pharmaceutica Sinica* **1982**, *17*, 917-921.
- Li, G. Y.; Song, W. Z.; Ji, Q. Y.; Wang, Y. L. *Zhong Yao Tong Bao* **1984**, *9*, 221-222.