# Two new diterpenoids from *Isodon japonica*

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Two new diterpenoids, maoyecrystal J (**1**) and maoyecrystal K (**2**), together with five known diterpenoids, effusanin A (**3**), isodonal (**4**), isodonoiol (**5**), rabdosinate (**6**) and rabdosin B (**7**) were isolated from *Isodon japonica* (Burman f.) Hara. The structures of the two new compounds were assigned 1α,6β,7β-trihydroxy-7α,20-epoxy-*ent*-kaur-11(12),16(17)-dien-15-one (**1**) and 6β,11α-dihydroxy-16β-methoxymethyl-6,20-epoxy-6,7-*seco*-*ent*-kaur-15-one-1α,7-olide (**2**) on the basis of HR–MS, $^1$H, $^{13}$C and 2D NMR spectroscopic methods.

**Keywords:** diterpenoids, *Isodon japonica*

*Isodon japonica* (Burman f.) Hara (Labiatae) has been used as folk medicines in China and Japan as an antibacterial, anti-inflammatory, stomachic and anthelmintic for long time.[1] Phytochemical studies on this plant collected in different regions have led to identification of over thirty *ent*-kauranoids.[2-13] In our effort to find biologically active components from Chinese medicinal plants[14] we found that *I. japonica* (Burman f.) Hara collected in Jiyuan prefecture of Henna province, China, afforded two new diterpenoids, *i.e.*, 1α,6β,7β-trihydroxy-7α,20-epoxy-*ent*-kaur-11(12),16(17)-dien-15-one (**1**), (maoyecrystal J), and 6β,11α-dihydroxy-16β-methoxymethyl-6,20-epoxy-6,7-*seco*-*ent*-kaur-15-one-1α,7-olide (**2**), (maoyecrystal K), together with five known diterpenoids, *i.e.*, effusanin A (**3**), isodonal (**4**), isodonoiol (**5**), rabdosinate (**6**) and rabdosin B (**7**). We report the isolation and structure elucidation of the two new compounds and their total $^1$H and $^{13}$C NMR chemical shifts assignments (Fig 1).

The dried and powdered leaves of *I. japonica*, which were collected from Jiyuan prefecture of Henan province, China and identified as *Isodon japonica* (Burman f.) Hara by Professor Changshan Zhu, Henan Agriculture University, China, were extracted with 70% $Me_2CO$ followed by silica gel column chromatographic separation to give compounds **1–7**.

Compound **1** was obtained from the MeOH eluant as colourless plates. The HR–ESI–MS spectrum exhibited an M+Na ion peak at *m/z* 369.1665, corresponding to a molecular formula of $C_{20}H_{26}O_5$ (calcd. for M+Na 369.1672). Its $^1$H, $^{13}$C and DEPT NMR spectra coupled with the IR spectrum revealed the presence of an exocyclic double bond conjugated with a carbonyl group [IR: 1630 and 1707 cm$^{-1}$; $\delta_H$ 5.92 and 5.21 (each 1H, s); $\delta_C$ 114.4, 150.6 and 209.3], an endocyclic double bond [IR: 1593 cm$^{-1}$; $\delta_H$ 6.42 (1H, dd, *J*= 9.2, 2.8 Hz) and 6.25 (1H, ddd, *J*= 9.2, 9.2, 2.8 Hz); $\delta_C$ 128.2 and 132.9], two methines containing oxygen [$\delta_H$ 4.27 (1H, dd, *J*= 8.4, 4.0 Hz) and 3.88 (1H, dd, *J*= 8.0, 6.8 Hz); $\delta_C$ 74.4 and 71.9], a methylene containing an oxygen [$\delta_H$ 4.61 and 4.35 (each 1H, AB, d, *J*= 10.1 Hz); $\delta_C$ 64.9 (t)], two methyls [$\delta_H$ 1.15 (3H, s) and 1.01 (3H, s); $\delta_C$ 32.5 and 21.1], three methylenes ($\delta_C$ 39.2, 32.9 and 29.7), three methines ($\delta_C$ 63.4, 52.5 and 37.7), and four quarternary carbons including a ketalic carbon ($\delta_C$ 60.8, 43.9, 33.9, and 97.4). The signals at $\delta_H$ 9.04 (1H, br), 6.77 (1H, d, *J* = 8.4 Hz) and 6.14 (1H, br) in the $^1$H NMR spectrum and the absorption at 3265 cm$^{-1}$ in the IR spectrum suggest the existence of three hydroxyl groups. With reference to the known structures of diterpenoids from the *Isodon japonica*,[2-13,15] this suggested that compound **1** might be an epoxy-*ent*-kaurane diterpenoid with three hydroxyl groups and an endocyclic double bond. Careful comparison of the $^{13}$C NMR data of **1** with those of effusanin A (**3**)[15] revealed a close similarity between the two molecules except that the C-11 and C-12 methylenes ($\delta_C$ 20.2 and 30.1) in **3** were replaced by the endocyclic double bond ($\delta_C$ 128.2 and 132.9) in **1**. The location of this double bond can be confirmed by the clear HMBC correlations of H-11 with C-8, C-9, C-13, and H-12 with C-9, C-13. The location of the 1-OH was confirmed by the HMBC correlations of H-2α, H-2β and H-20b with C-1 ($\delta_C$ 71.9), and that of H-1 with C-20. The location of the 6-OH was confirmed by the HMBC correlations of H-6/C-4, H-6/C-7, H-6/C-8, along with the H-H COSY correlation of 6β-OH with H-6α. The relative configurations of the 1-OH and 6-OH are deduced to be α- and β-oriented, respectively, from their coupling constants ($\delta_H$ 3.88, 1H, dd, *J*= 8.0, 6.8 Hz for H-1β, and $\delta_H$ 4.27, 1H, dd, *J*= 8.4, 4.0 Hz for H-6α), and confirmed by the NOESY correlations as shown in Fig. 2. Therefore, the structure of **1** was assigned as 1α,6β,7β-trihydroxy-7α,20-epoxy-*ent*-kaur-11(12),16(17)-dien-15-one, and named maoyecrystal *J*. The total $^1$H and $^{13}$C NMR chemical shift assignments together with the HMBC correlations of **1** are listed in Table 1. Also listed are $^{13}$C chemical shifts of **3** for comparison.

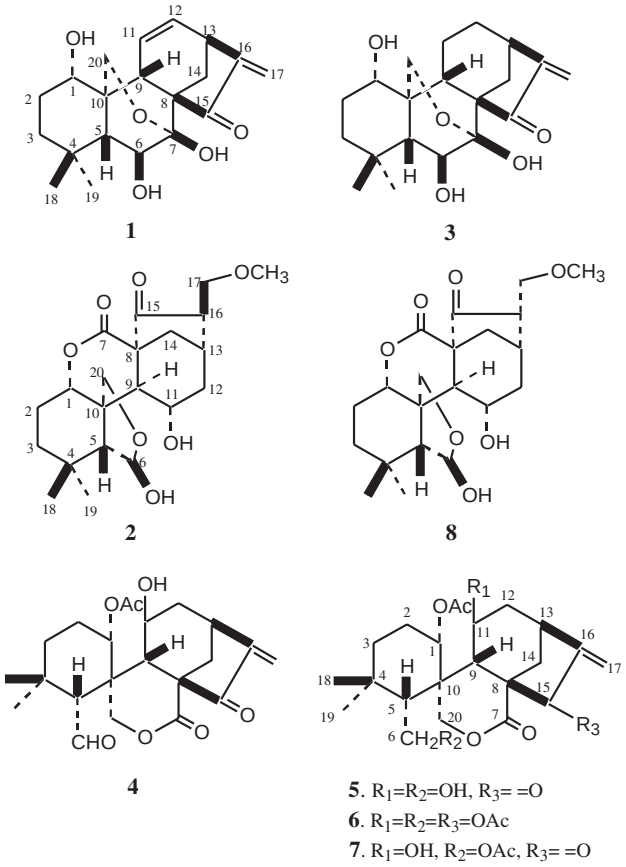


**Fig. 1** Molecular structures.

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**Table 1** $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR chemical shifts of **1** and **3** and HMBC correlations of **1**[a]

| No. | $\delta_C$ | | $\delta_H$ (*J* in Hz) | HMBC (H→C) |
|---|---|---|---|---|
| | **1** | **3** | **1** | **1** |
| 1 | 71.9, d | 73.3, d | 3.88 (dd, 8.0, 6.8, H-1β)<br>6.14 (br. s, OH-1α) | H-2α, H-2β, H-20b |
| 2 | 29.7, t | 30.4, t | 1.88 (m, H-2α, H-2β) | H-3α, H-3β |
| 3 | 39.2, t | 39.1, t | 1.34 (m, H-3α), 1.40 (m, H-3β) | H-2α, H-2β, Me-18, Me-19 |
| 4 | 33.9, s | 34.0, s | | H-2α, H-2β, H-5β, H-6α, Me-18, Me-19 |
| 5 | 63.4, d | 61.3, d | 1.57 (d, 4.0, H-5β) | H-3β, H-9β, Me-18, Me-19, H-20a |
| 6 | 74.4, d | 74.8, d | 4.27 (dd, 8.4, 4.0, H-6α)<br>6.77 (d, 8.4, OH-6β) | H-5β |
| 7 | 97.4, s | 95.8, s | 9.04 (br. s, OH-7β) | H-6α, H-14β, H-20b |
| 8 | 60.8, s | 60.5, s | | H-6α, H-9β, H-11, H-13α, H-14α, H-14β |
| 9 | 52.5, d | 52.0, d | 2.41 (m, H-9β) | H-5β, H-11, H-12, H-14α, H-14β, H-20a, H-20b |
| 10 | 43.9, s | 41.7, s | | H-2α, H-2β, H-5β, H-9β, H-20a, H-20b |
| 11 | 128.2, d | 20.2, t | 6.42 (dd, 9.2, 2.8, H-11) | H-9β, H-13α |
| 12 | 132.9, d | 30.1, t | 6.25 (ddd, 9.2, 9.2, 2.8, H-12) | H-9β, H-13α, H-14β |
| 13 | 37.7, d | 35.1, d | 3.28 (m, H-13α) | H-11, H-12, H-14α, H-14β, H-17a, H-17b |
| 14 | 32.9, t | 26.4, t | 2.44 (d, 11.0, H-14α)<br>2.86 (dd, 11.0, 4.1, H-14β) | H-9β |
| 15 | 209.3, s | 210.9, s | | H-9β, H-13α, H-14α, H-17a, H-17b |
| 16 | 150.6, s | 154.3, s | | H-14α, H-17a |
| 17 | 114.4, t | 115.7, t | 5.92, 5.21 (each 1 H, s, H-17a, H-17b) | |
| 18 | 32.5, q | 33.2, q | 1.15 (s, Me-18) | H-3α, H-3β, H-5β, Me-19 |
| 19 | 21.1, q | 22.1, q | 1.01 (s, Me-19) | H-3α, H-3β, H-5β, Me-18 |
| 20 | 64.9, t | 63.7, t | 4.61, 4.35 (each 1 H, AB, d, 10.1, H-20a, H-20b) | H-1β, H-5β, H-9β |

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

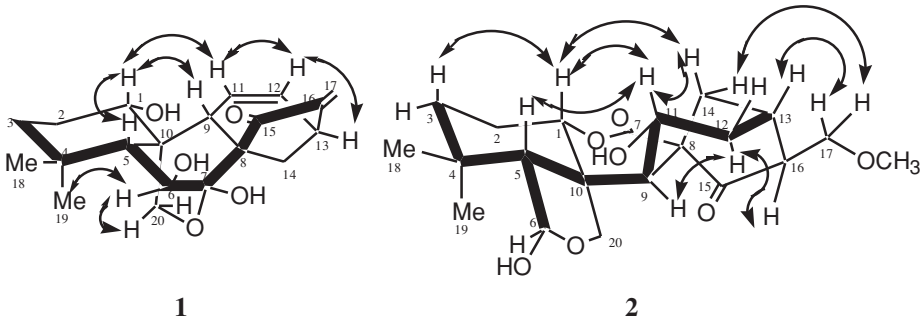


**Fig. 2** Significant NOESY correlations of **1** and **2**.

Compound **2** was obtained from the MeOH eluant as colourless needles. The HR–ESI–MS spectrum exhibited an M+Na ion peak at *m/z* 417.1879, corresponding to a molecular formula of $C_{21}H_{30}O_7$ (calcd. for M+Na 417.1884). Its $^1$H, $^{13}$C and DEPT NMR spectra coupled with the IR spectrum revealed the presence of a carbonyl group [IR: 1760 cm$^{-1}$; $\delta_C$ 212.5], a δ-lactone [IR: 1716 cm$^{-1}$; $\delta_H$ 4.88 (1H, dd, *J* = 10.5, 7.2 Hz); $\delta_C$ 76.7 and 171.0], a hemiacetal group [$\delta_H$ 5.72 (1H, s) and 9.04 (1H, br); $\delta_C$ 102.1], another hydroxyl group [IR: 3306 cm$^{-1}$; $\delta_H$ 4.50 (1H, m); $\delta_C$ 63.6], two methylenes carrying oxygen [$\delta_H$ 4.38, 4.26 (each 1H, AB, d, *J* = 9.6 Hz) and 3.57, 3.48 (each 1H, AB, dd, *J*= 9.2, 4.8 Hz); $\delta_C$ 73.6 and 71.3], a methoxy group [$\delta_H$ 3.14 (3H, s); $\delta_C$ 58.6], two methyls [$\delta_H$ 0.95 (6H, s); $\delta_C$ 32.9 and 23.1], four methylenes ($\delta_C$ 41.6, 37.3, 33.8, and 24.0), four methines ($\delta_C$ 58.2, 53.9, 52.3, and 31.7), and three quarternary carbons ($\delta_C$ 57.0, 51.0, and 31.6). With reference to the known structures of diterpenoids from the genus *Isodon*, this suggested that compound **2** might be an enmein type diterpenoid with two hydroxyl groups and one methoxy group. Comparison of the $^{13}$C chemical shifts of **2** with those of taibaijaponicain A (**8**)[13] indicated that the two molecules were almost identical except for the notable down-field shift of C-12 ($\delta_C$ 41.6 and 32.6 for **2** and **8** respectively) and an appreciable up-field shift of C-14 ($\delta_C$ 33.8 and 35.0 for **2** and **8** respectively) in **2** in comparison with those in **8**. These facts suggest that the 16-$CH_2OCH_3$ of **2** was β-oriented, due to the absence of γ–gauche interaction of the 16α-$CH_2OCH_3$ with H-12α and the presence of γ–gauche interaction between 16β-$CH_2OCH_3$ and H-14α. This configuration was supported by the NOESY correlations of H-16α with H-12α, and H-17a with H-13β, and H-17b with H-14α (see Fig. 2). Therefore, compound **2** was assigned as the 16-epimer of **8,** namely, 6β,11α-dihydroxy-16β-methoxymethyl-6,7-*seco*-6,20-epoxy-*ent*-kaur-15-one-1α,7-olide and named as maoyecrystal K. The structure and configuration of the molecule were confirmed by HMBC and NOESY as shown in Table 2 and Fig. 2 respectively. The complete $^1$H and $^{13}$C NMR chemical shift assignments together with HMBC correlations of **2** are listed in Table 2. Also listed are $^{13}$C chemical shifts of **8** for comparison.

Compounds **3**–**7** were identified by comparison of their $^1$H and $^{13}$C NMR, MS and IR spectroscopic data with those reported in literatures as effusanin A (**3**),[15] isodonal (**4**),[16] isodonoiol (**5**),[4,8] rabdosinate (**6**)[8] and rabdosin B (**7**).[7]

## Experimental

Melting points were determined with a Kofler melting point apparatus and uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Shimadzu UV-260 instrument. IR spectra were taken on a Nicolet 170 SX FT-IR spectrometer. $^1$H, $^{13}$C and 2D NMR spectra were recorded

**Table 2** $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR chemical shifts of **2** and **8** and HMBC correlations of **2**[a]

| No. | $\delta_C$ | | $\delta_H$ (*J* in Hz) | HMBC (H→C) |
|---|---|---|---|---|
| | **2** | **8** | **2** | **2** |
| 1 | 76.7, d | 77.3, d | 4.88 (dd, 10.5, 7.2, H-1β) | H-9α |
| 2 | 24.0, t | 24.2, t | 1.86 (m, H-2α, H-2β) | |
| 3 | 37.3, t | 37.4, t | 1.31 (m, H-3α, H-3β) | Me-18, Me-19 |
| 4 | 31.6, s | 32.1, s | | H-5β, Me-18, Me-19 |
| 5 | 53.9, d | 54.3,d | 3.25 (s, H-5β) | Me-18, Me-19, H-20b |
| 6 | 102.1, d | 102.6,d | 5.72 (s, H-6α) | H-5β, H-20a |
| | | | 9.04 (br. s, OH-6β) | |
| 7 | 171.0, s | 171.2, s | | H-14α |
| 8 | 57.0, s | 57.7, s | | H-9α |
| 9 | 52.3, d | 53.1, d | 2.88 (d, 11.2, H-9α) | H-5β, H-14α, H-20a, H-20b |
| 10 | 51.0, s | 50.9, s | | H-5β, H-9α, H-20a, H-20b |
| 11 | 63.6, d | 63.3, d | 4.50 (m, H-11β) | H-9α, H-12α |
| 12 | 41.6, t | 32.6, t | 1.55 (dd, 14.0, 9.6, H-12α) | H-14α |
| | | | 2.94 (m, H-12β) | |
| 13 | 31.7, d | 31.3, d | 2.60 (m, H-13β) | H-17a, H-17b |
| 14 | 33.8, t | 35.0, t | 2.32 (dd, 12.0, 4.4, H-14α) | H-9α |
| | | | 2.68 (d, 12.0, H-14β) | |
| 15 | 212.5, s | 212.3, s | | H-9α, H-13β, H-14β, H-16α, H-17a, H-17b |
| 16 | 58.2, d | 58.8, d | 2.57 (m, H-16α) | H-12β, H-17a, H-17b |
| 17 | 71.3, t | 69.3, t | 3.57, 3.48 (each 1 H, AB, dd, 9.2, 4.8, H-17a, H-17b) | -$OCH_3$ |
| 18 | 32.9, q | 33.1, q | 0.95 (s, Me-18) | H-5β, Me-19 |
| 19 | 23.1, q | 23.5, q | 0.95 (s, Me-19) | H-5β, Me-18 |
| 20 | 73.6, t | 73.9, t | 4.38, 4.26 (each 1 H, AB, d, 9.6, H-20a, H-20b) | H-5β, H-9α |
| $OCH_3$ | 58.6, q | 56.4, q | 3.14 (s) | H-17a, H-17b |

[a]Determined in $C_5D_5N$ (for **2**) and acetone-$d_6$ (for **8**). $^{13}$C NMR multiplicities were established by DEPT.

on a Bruker AM-400 NMR spectrometer with TMS as internal standard. HR-ESI-MS was obtained on a Bruker APEX II FT-MS spectrometer.

*Extraction and isolation procedures*

The dried and crushed leaves of *Isodon japonica* (7.5kg) were extracted three times with $Me_2CO/H_2O$ (7:3 v/v) at room temperature for 5 days. The extract was filtered and the solvent was removed under reduced pressure, and the residue was partitioned between $H_2O$ and AcOEt. The AcOEt fraction gave 131 g of residue after removing the solvent. This residue was separated by silica gel (200–300 mesh) column chromatography with gradient elution of $CHCl_3$/MeOH (1:0 to 0:1) to give seven fractions which were subjected repeated chromatography (silica gel, gradient elution with $CHCl_3/Me_2CO$), giving pure maoyecrystal J (**1**, 4 mg), maoyecrystal K (**2**, 2 mg), effusanin A (**3**, 12 mg), isodonal (**4**, 60 mg), isodonoiol (**5**, 600 mg), rabdosinate (**6**, 8 mg) and rabdosin B (**7**, 30 mg). The structures of the two new compounds **1** and **2** were identified as mentioned above. The structures of compounds **3**–**7** were characterised by comparing their m.p., IR, MS, $^1$H and $^{13}$C NMR chemical shifts with those reported in literatures.[4,7-8,15-16]

*Maoyecrystal J* (**1**)*:* Colourless plates, m.p. 252–254 °C, $[\alpha]_D^{19}$–39.8°(*c* 0.25, $C_5H_5N$). UV $\lambda_{max}$ (MeOH) 230 nm (log ε, 0.86); IR (KBr) $\nu_{max}/cm^{-1}$: 3265, 2925, 2867, 1707, 1630, 1593, 1422, 1391, 1182, 1069, 1050, 1024, 972, 946, 877. HR-ESI-MS: Found: 369.1665, Calcd. for $C_{20}H_{26}O_5$ + Na: 369.1672. For $^1$H and $^{13}$C NMR data see Table 1.

*Maoyecrystal K* (**2**): Colourless needles, m.p. 200–202 °C, $[\alpha]_D^{19}$–210°(*c* 0.18, $C_5H_5N$). IR (KBr) $\nu_{max}/cm^{-1}$: 3306, 2930, 2892, 1760, 1716, 1456, 1253, 1110, 1050, 981, 906, 731. HR-ESI-MS: Found: 417.1879, Calcd. for $C_{21}H_{30}O_7$ + Na: 417.1884. For $^1$H and $^{13}$C NMR data see Table 2.

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