Two new diterpenoids and other constituents from *Isodon serra*

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Two new ent-6,7-seco-kaurane-type diterpenoids, 15α,20β-dihydroxy-6β-methoxy-6,7-seco-6,20-epoxy-ent-kaur-16-en-1,7-olide (**1**), 6α,15α-dihydroxy-20-aldehyde-6,7-seco-6,11α-epoxy-ent-kaur-16-en-1,7-olide (**2**), together with seven known diterpenoids, enmein (**3**), nodosin (**4**), isodocarpin (**5**), nervosin (**6**), epinodosin (**7**), epinodosinol (**8**) and rabdosin (**9**) were isolated from the aerial part of *Isodon serra.* Their structures were elucidated by spectroscopic means.

Keywords: labiatae, *Isodon serra,* ent-kaurane, diterpenoid

Isodon serra (Labiatae) is widely distributed in China and has been used for the treatment of acute jaundice, hepatitis and acute cholecystitis in folk mediãne.[1] Investigation of this plant led to the isolation of two new ent-6,7-seco-kaurane-type diterpenoids, 15α,20β-dihydroxy-6β-methoxy-6,7-seco-6,20-epoxy-ent-kaur-16-en-1,7-olide (**1**), 6α,15α-dihydroxy-20-aldehyde-6,7-seco-6,11α-epoxy-ent-kaur-16-en-1,7-olide (**2**), and seven known diterpenoids enmein (**3**), nodosin (**4**), isodocarpin (**5**), nervosin (**6**), epinodosin (**7**), epinodosinol (**8**) and rabdosin (**9**).

The leaves of *Isodon serra* was collected in the Henan Province of China and identified by Professor Changshan Zhu, Henan Agriculture University. A voucher speãmen (no. 200611) was deposited in the Pharmacy College, Xinxiang Medical University. The leaves were extracted with Me_2CO/H_2O (7 : 3 v/v), and the extract was separated by silica gel column chromatography to give compounds **1**–**9**.

Compound **1**, was obtained as colourless needles, m.p. 196–197ºC, $[\alpha]_D^{23}$–141.0° (*c* 0.19, CH_3OH). Its HR–ESI–MS showed $[M + Na]^+$ at *m/z* 401.1939 (calcd. 401.1940), corresponding to a molecular formula of $C_{21}H_{30}O_6$ and suggesting seven degrees of unsaturation. The IR spectrum has absorptions at 3359 (hydroxyl group), 2830 (methoxyl group), 1717 (lactone carbonyl group) cm^{-1}. The ^{13}C NMR (Dept) spectra (Table 1) showed 21 carbon signals, consisting of three Me (including a methoxyl group), six CH_2 (including an olefinic one), seven CH (including four oxygenated carbons), a lactone carbonyl group and four quaternary C-atoms. The ^1H NMR spectrum (Table 1) indicated the presence of a methoxyl group (δ 3.33) and a terminal double bond (δ5.04, 5.14). In the light of the structural types of diterpenoids which have been isolated from the genus *Isodon*, compound **1** was assigned as an ent-6,7-seco-kaurane diterpenoid with two hydroxyl groups. On examination of the HMQC and HMBC spectra of compound **1**, the methoxyl group at δ_C 55.2(q) (δ_H 3.33) was located at C-6 based on the HMBC correlation of OCH_3 with C-6. On the other hand, the positions of hydroxyl groups were determined by the HMBC correlations between the H-15 (δ 5.12) and C-7 (δ 176.7), C-9 (δ 31.4), C-14 (δ 33.7), C-17 (δ 108.9); H-20 (δ 5.60) and C-1 (δ 76.0), C-6 (δ 104.9), C-9 (δ 31.4), C-10 (δ 49.4).

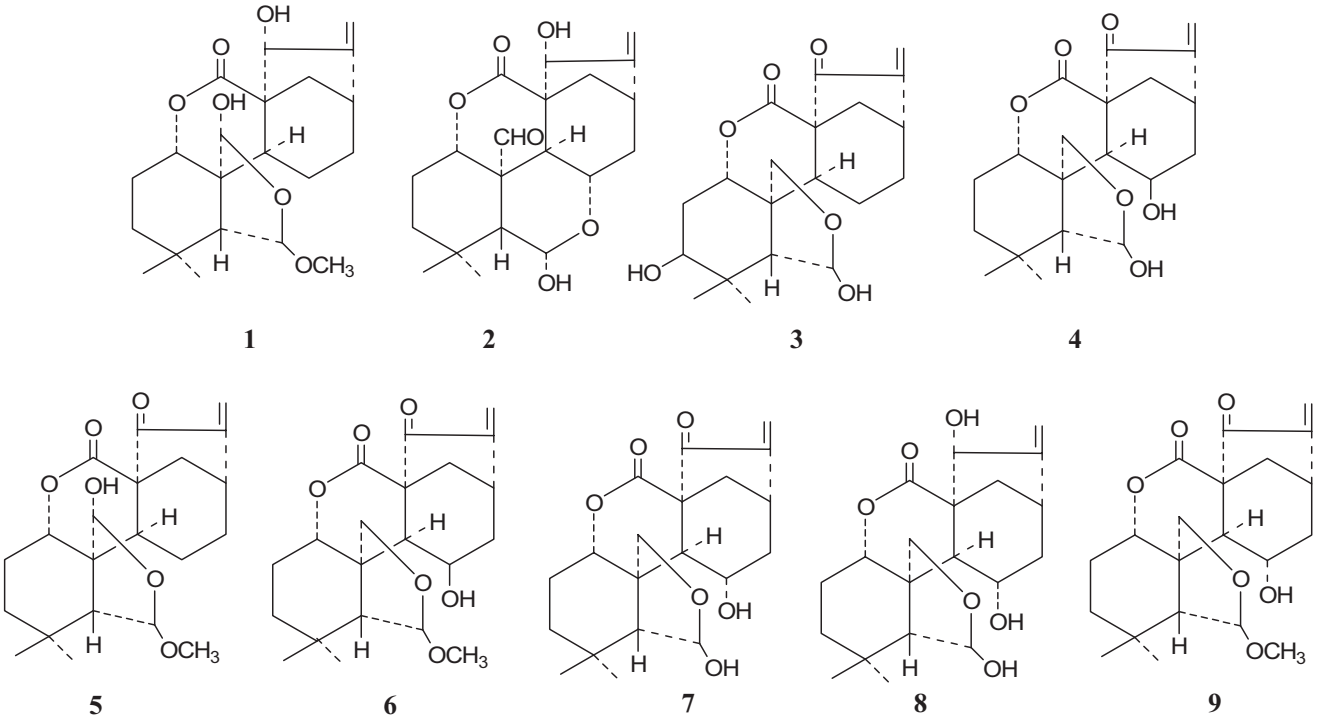

Fig. 1 The structures of compounds **1–9**.

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The relative stereochemistry of **1** was shown to be 15(R), 20(S)-configuration by a NOESY experiment. In its NOESY spectrum, H-15 (δ 5.12) showed a strong correlation with H-13 (δ 2.70 m) and H-14 (δ 1.78, 1.82 m); H-20 (δ 5.60 s) with H-2 (δ1.86, 1.95 m) and H-19 (δ 0.96 s). It showed that the H-15 had the β-orientation and H-20 had the α-orientation. Based on the above analysis and by comparison of its spectral data with the literature values for similar structures,[2,3] the structure of compound **1** was established as 15α,20β-dihydroxy-6β-methoxy-6,7-seco-6,20-epoxy-kaur-16-en-1,7-olide-ent (Fig. 1).

Compound **2**, was obtained as colourless needles, m.p. 221–222ºC, $[\alpha]_D^{23}$–11.0° (*c* 0.12, CH_3OH). Its molecular formula, $C_{20}H_{26}O_6$, was deduced from the HR-ESI-MS (found 385.1627, calcd for $[M + Na]^+$ 385.1625), suggesting eight degrees of unsaturation. The IR spectrum exhibited the presence of a lactone carbonyl group (1705 cm^{-1}), an aldehyde group (1733 cm^{-1}) and hydroxyl groups (3392 cm^{-1}). The ^{13}C NMR (DEPT) spectrum of compound **2** (Table 1) exhibited 20 signals: two Me, five CH_2 (including an olefinic one), eight CH (including four oxygenated ones and one aldehyde), a lactone carbonyl group and four quaternary C-atoms, respectively. The ^1H NMR spectrum (Table 1) indicated the presence of an aldehyde group (δ 10.71, s) and a terminal double bond (δ5.57, 5.58, brs). In the light of the structures of diterpenoids previously isolated from the genus Isodon,[4] along with the characteristic lactone-type C=O signal at δ_C 173.8 (s) assignable to C-7, and a terminal double bond signal at δ_C 109.5 (t) and 158.8 (s) assignable to C-17 and C-16, as well as four oxygenated CH (δ_C 62.9, 77.3, 80.2, 92.3), compound **2** was assigned an 6,7-seco -kauranoid-1,7-olide structure.[5] On examination of the HMQC and HMBC spectra of compound **2**, the structure of the 6,11-epoxy moiety was established by the HMBC correlations between the H-6 (δ 5.93) and C-11 (δ 62.9), C-10 (δ 50.7), C-5 (δ 50.4). The positions of hydroxyl groups were determined by the HMBC correlations between the H-6 (δ 5.93) and C-11 (δ 62.9), C-10 (δ 50.7), C-5 (δ 50.4); H-15 (δ 5.85) and C-17 (δ 109.5), C-14 (δ 32.3), C-9 (δ 33.2). This was also supported by the observed cross peaks in its ^1H–^1H COSY spectrum between H-6 (δ 5.93 s) and H-5 (δ 1.92 s) and 6-OH (δ 8.80 s) as well as H-9 (δ 3.59 d) and H-11 (δ 4.30 dt).

The observed NOESY correlations from β-oriented H-5 to both H-6 and H-11, established that the H-6 and H-11 were in β-orientation; H-15 had no correlation with H-9, showing that the H-15 was in β-orientation.

Based on the above evidence, the structure of compound **2** was eluãdated as 6α, 15α-dihydroxy-20-aldehyde-6,7-seco-6,11α-epoxy-ent-kaur-16-en-1,7-olide (Fig. 1).

Compounds **3**–**9** were identified by comparison of their ^1H and ^{13}C NMR, MS and IR spectroscopic data with those reported in literatures as enmein (**3**),[6] nodosin (**4**),[7] isodocarpin (**5**),[8] nervosin (**6**),[9] epinodosin (**7**),[4,10] epinodosinol (**8**)[4,11] and rabdosin (**9**).[12]

Experimental

Melting points were determined with a Kofler melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were taken on a Nicolet 170 SX FT–IR spectrometer. ^1H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM-400 NMR spectrometer with TMS as internal standard. HR–ESI–MS was obtained on a Waters HPLCQ-Tof HR–MS spectrometer. Silica gel (200–300 mesh) was used for column chromatography and silica gel GF_{254} for TLC were carried out by the Qing-dao Marine Chemical Factory of China.

Extraction and isolation procedures

The air dried and powdered leaves of *Isodon serra* (Maxim.) (11 kg) were extracted four times with Me_2CO/H_2O (7 : 3 v/v) at room temperature for five days. The combined extract was filtered and the solvent was removed under reduced pressure. The extract was suspended in water, and then partitioned successively with EtOAc and concentrated to obtain residue (300 g), which was then subjected to column chromatography over silica gel (3000 g, 200–300 mesh) and eluted with a gradient of $CHCl_3$/MeOH (1 : 0, 20 : 1, 10 : 1, 5 : 1, 3 : 1, 2 : 1, 1 : 1, 0 : 1,) to give eight factions accordin g to their TLC analysis. After repeated chromatography over silica gel and eluted with a gradient of $CHCl_3$/MeOH and $CHCl_3/Me_2CO$ it gave **1** (55 mg), **2** (15 mg), **3** (760 mg), **4** (953 mg), **5** (66 mg), **6** (21 mg), **7** (23 mg), **8** (16 mg), **9** (11 mg). The structures of two new compounds **1** and **2** were identified on the basis of HR–MS, ^1H, ^{13}C and 2D NMR spectroscopic methods. The structures of compounds **3**–**9** were characterised by comparing their m.p., IR, MS, ^1H and ^{13}C NMR chemical shifts with those reported in the literature.

15α,20β-dihydroxy-6β-methoxy-6,7-seco-6,20-epoxy-ent-kaur-16-en-1,7-olide (**1**): colourless needles, m.p. 196–197ºC, $[\alpha]_D^{23}$–141.0° (*c* 0.19, CH_3OH). IR (KBr) ν_{max}/cm^{-1}: 3359, 2830, 1717, 1662, 1450,

Table 1 ^1H NMR(400 MHz), ^{13}C NMR (100 MHz), Dept and HMBC data of compounds **1** and **2**

1				**2**			
No.	δ_H	δ_C	HMBC	No.	δ_H	δ_C	HMBC
1	4.44 (dd, *J* = 6.0, 11.6 Hz)	76.0 d	H-9, 20	1	4.50 (dd, *J* = 4.8, 8.4 Hz)	77.3 d	H-3,5,9
2	1.86, 1.95 m	23.7 t	H-1, 3, 5	2	1.76,1.98 m	25.6 t	H-3
3	1.20 m	37.7 t	H-1, 2, 18, 19	3	1.41,2.69 m	39.8 t	H-2,18,19
4		31.3 s	H-2, 5, 18, 19	4		33.4 s	H-2,3,5,18,19
5	2.02 s	54.2 d	H-2, 6, 18, 19	5	1.92 s	50.4 d	H-6,9,18,19
6	4.53 s	104.9 d	H-5, 20	6	5.93 s	92.3 d	H-5,11
7		176.7 s	H-14, 15	7		173.8 s	H-2,14
8		52.3 s	H-9, 13	8		49.8 s	H-9,13,14,15
9	2.75 (dd, *J* = 5.6, 12.4 Hz)	31.4 d	H-1, 12, 14, 15,20	9	3.59 (d, *J* = 11.6 Hz)	33.2 d	H-12,14,15
10		49.4 s	H-5, 6, 9, 18, 19, 20	10		50.7 s	H-2,5,6,9
11	1.24, 1.75 m	19.4 t	H-9, 13	11	4.30 (dt, *J* = 8.4, 11.6 Hz)	62.9 d	H-6,9,12,13,14
12	2.16, 2.19 m	31.8 t	H-12, 14	12	1.42, 2.72 m	40.8 t	H-9,13,14
13	2.70 m	37.2 d	H-12, 14, 17	13	2.83 m	37.3 d	H-12,14,17
14	1.78, 1.82 m	33.7 t	H-9, 12,15	14	1.15,1.70 m	32.3 t	H-9,12,15
15	5.12 s	77.9 d	H-9, 13, 14, 17	15	5.85 s	80.2 d	H-9,13,14,17
16		157.7 s	H-12, 14, 15, 17	16		158.8 s	H-15,17
17	5.14, 5.04 s	108.9 t	H-13, 15	17	5.57, 5.58 brs	109.5 t	H-15
18	0.98 s	33.2 q	H-3, 5, 19	18	0.94 s	30.4 q	H-2,3,5,19
19	0.96 s	23.6 q	H-3, 5, 18	19	1.13 s	21.9 q	H-3,5,18
20	5.60 s	102.0 d	H-1, 5, 6, 9	20	10.71 s	203.1 d	H-5,9
21	3.33 s	55.2 q	H-6				

1243, 1145, 1104, 1032, 973. HR–ESI–MS: Found: 401.1939, Calcd. for $C_{21}H_{30}O_6$ + Na: 401.1940. For ^1H and ^{13}C NMR data see Table 1.

6α,15α-dihydroxy-20-aldehyde-6,7-seco-6,11α-epoxy-ent-kaur-16-en-1,7-olide (**2**)**:** Colourless needles, m.p. 221–222ºC, $[\alpha]_D^{23}$–11.0° (*c* 0.12, CH_3OH). IR (KBr) ν_{max}/cm^{-1}: 3392, 1733, 1705, 1614, 1461, 1244, 1121, 1069, 984. HR–ESI–MS: Found: 385.1627, Calcd. for $C_{20}H_{26}O_6$ + Na: 385.1625. For ^1H and ^{13}C NMR data see Table 1.

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