Two New Diterpenoids and other Constituents from *Isodon nervosus*

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Two new *ent*-kaurane diterpenoids, 15β-hydroxy-6,7-seco-6,11β:6,20-diepoxy-1α,7-olide-*ent*-kaur-16-ene (**1**), 11α,15α-dihydroxy-6β-methoxy-6,7-seco-6,20-epoxy-1α,7-olide-*ent*-kaur-16-ene (**2**), together with four known diterpenoids, nodosin (**3**), isodocarpin (**4**), odonicin (**5**) and maoyecrystal F (**6**) were isolated from the aerial parts of *Isodon nervosus.* The structures of the new compounds were elucidated on the basis of their spectral evidence, especially on 2D NMR.

Keywords: Labiatae; *Isodon nervosus*; *ent*-Kaurane diterpenoid.

INTRODUCTION

Isodon nervosus (Labiatae) is widely distributed in China and has long been used as a Chinese folk medicine for the treatment of acute jaundice, hepatitis and acute cholecystitis.[1] Previous phytochemical studies on this species obtained *ent*-kaurane diterpenoids, most of which have been shown to have antitumor and anti-inflammatory activities.[2] We have re-examined the leaves of *Isodon nervosus* collected in Tongbai prefecture of Henan Province of China and obtained two new *ent*-kaurane type diterpenoids (**1** and **2**) and four known compounds (**3**-**6**).

RESULTS AND DISCUSSION

Six *ent*-kaurane diterpenoids (**1**-**6**) were obtained by repeated column chromatography over silica gel from the EtOAc soluble part of Me_2CO/H_2O (7:3 v/v) extract of *Isodon nervosus*. The known compounds were determined as nodosin (**3**),[3] isodocarpin (**4**),[4] odonicin (**5**)[5] and maoyecrystal F (**6**)[6] by comparison of their spectral data with those reported in the literature.

Compound **1**, obtained as colorless needles, mp 292-293 °C, $[\alpha]_D^{23}$ -11 (*c* 0.22, CH_3OH), was determined to possess the molecular formula $C_{20}H_{26}O_5$ by the HRESIMS [found $(M+Na)^+$ 369.1681, calcd. 369.1678]. The IR spectrum exhibited the presence of a lactone carbonyl (1743 cm^{-1}) and a hydroxyl group (3530 cm^{-1}). The ^{13}C and DEPT NMR spectra of **1** showed 20 carbon signals which were indicated to be composed of one lactonic carbonyl carbon, four quaternary carbon (including an olefinic one), seven methines including four oxygenated ones, six methylenes including an oxygenated one and an olefinic one, and two methyls, which suggested **1** as an *ent*-kauranoid, combined with the consideration of a similar structure to diterpenoids previously isolated from this genus.[7,8] The absence of HMBC cross-peaks between H-5 or H-6 and C-7 indicated a 6,7-*seco* structure, and lactone formation of C-7 to C-1 was supported by an H-1/C-7 correlation in the HMBC spectrum (Fig. 2); therefore, compound **1** was determined to possess an enmein-type skeleton.[8] However, the extra degree of unsaturation required by the molecular formula indicated the presence of an additional ring. The correlation between H-6 (δ 5.55 br s) and C-11 (δ 63.8 d) in the HMBC spectra unambiguously proved that the additional ring was an ether bridge from C-6 to C-11. Comparison of the structure of compound **1** with Isodoacetal[9] revealed that **1** was 15-deacetyl-Isodoacetal, which was confirmed by HMBC and NOESY experimental results. NOESY interactions of H-6 with H-5β, H-9α with H-11 and H-15 were observed (Fig. 3), which indicated the β-orientation of H-6 and the α-orientation of H-11 and H-15, respectively. Therefore, the structure of **1** was determined to be 15β-hydroxy-6,7-seco-6,11β:6α,20-diepoxy-1α,7-olide-*ent*-kaur-16-ene (Fig. 1).

Compound **2**, obtained as colorless crystals, mp 196-198 °C, $[\alpha]_D^{23}$ -14 (*c* 0.23, CH_3OH), was determined to possess the molecular formula $C_{21}H_{30}O_6$ by the HRESIMS [found $(M+Na)^+$ 401.1924, calcd. 401.1939]. The IR spectrum exhibited the presence of a lactone carbonyl (1727

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cm^{-1}) and hydroxyl groups (3372 cm^{-1}). The ^{13}C NMR (DEPT) spectrum showed 21 carbon signals, consisting of three Me (including an oxygenated one), six CH_2 (including an olefinic one and an oxygenated one), seven CH (including four oxygenated ones), a lactone carbonyl group and four quaternary C-atoms. The ^{1}H NMR spectra indicated the presence of a methoxyl group (δ 3.15) and a terminal double bond (δ 5.20, 5.47). The NMR spectra of compound **2** suggested an *ent*-kaurane-type diterpenoid. On examination of the HMQC and HMBC spectra (Fig. 2) of compound **2**, the methoxyl group at δ_C 54.3 (q) (δ_H 3.15) was located at C-6 based on the HMBC correlation of H-21 with C-6. On the other hand, the positions of hydroxyl groups were determined by the HMBC correlations between the H-15 (δ 5.52) and C-7 (δ 175.2), C-9 (δ 46.7), C-14 (δ 34.5), C-17 (δ 108.5); H-11 (δ 4.31) and C-9 (δ 46.7), C-10 (δ 50.9), C-13 (δ 37.0). The relative stereochemistry of **2** was confirmed by NOESY experiment. In its NOESY spectrum (Fig. 3), H-15 (δ 5.52 s) showed a strong correlation with H-14 (δ 2.03 d) but no correlation with H-9 (δ 3.28 d); H-11 (δ 4.31, m) with H-1 (δ 5.52 m), H-5 (δ 3.33 s). It showed that the H-11 and H-15 were both in β-orientation. The observed NOESY correlation from H-6 (δ 5.00, s) to H-19 (δ 0.91, s) showed that the H-6 was in α-orientation. Based on the above spectral analysis and by comparison of its spectral data with the literature values of epinodosinol,[10] the structure of compound **2** was established as 11α,15α-dihydroxy-6β-methoxy-6,7-seco-6,20-epoxy-1α,7-olide-*ent*-kaur-16-ene (Fig. 1).

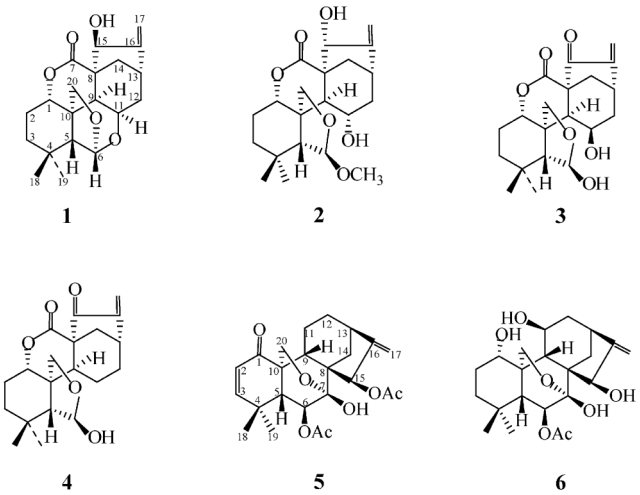

Fig. 1. The structures of compounds **1**-**6**.

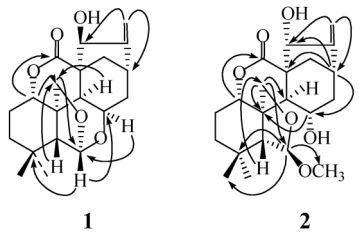

Fig. 2. The key HMBC correlations of compounds **1** and **2**.

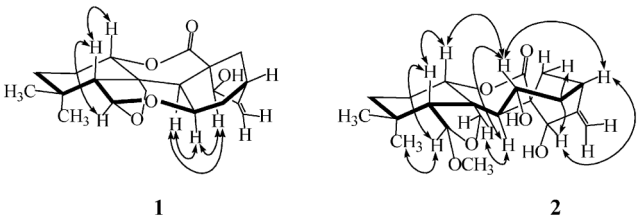

Fig. 3. The key NOESY correlations of compounds **1** and **2**.

EXPERIMENT SECTION

General

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. ^{1}H, ^{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) used for column chromatography and silica gel GF_{254} for TLC were made by the Qing-dao Marine Chemical Factory of China.

Plant Material

The leaves of *Isodon nervosus* were collected in Tongbai County, Henan province, P. R. China, in September 2006, and identified by Professor Changshan Zhu (朱長山), Henan Agriculture University, P. R. China. A voucher specimen (No. 200609) has been deposited in the Pharmacy College, Xinxiang Medical University.

Extraction and Isolation

The air-dried and powdered leaves of *Isodon nervosus* (14 kg) were extracted with Me_2CO/H_2O (7:3 v/v) at room temperature and were filtered. The combined Me_2CO/H_2O (7:3 v/v) extract was concentrated under pressure to remove acetone and then partitioned with EtOAc and concentrated to obtain residue (300 g), which then was subjected to column chromatography over silica gel (3000 g, 200-300 mesh) and eluted with a gradient of $CHCl_3$-CH_3OH (1:0, 30:1, 20:1, 10:1, 5:1 3:1, 0:1) to give seven fractions according to their TLC analysis. From fractions 3

($CHCl_3$-CH_3OH 20:1) and 4 ($CHCl_3$-CH_3OH 10:1), compounds **1** (41 mg), **2** (18 mg), **3** (174 mg), **4** (25 mg), **5** (88 mg) and **6** (12 mg) were obtained by repeated silica gel columns with $CHCl_3$-$(Me)_2CO$ or $CHCl_3$-CH_3OH.

15β-Hydroxy-6,7-seco-6,11β:6,20-diepoxy-1α,7-olide-*ent*-kaur-16-ene (1)

Colorless needles, molecular formula: $C_{20}H_{26}O_5$; mp 292-293 °C; $[\alpha]_D^{23}$ -11 (*c* 0.22, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ nm: 210 (log ε 3.73); IR (KBr) ν_{max} cm^{-1}: 3530 (OH), 1743 (C=O), 1255, 1216, 1083; HRESI MS *m/z* 369.1681 $[M+Na]^+$ (calcd. for $C_{20}H_{26}O_5Na$ 369.1678); ^1H and ^{13}C NMR data (Table 1).

11α,15α-Dihydroxy-6β-methoxy-6,7-seco-6,20-epoxy-1α,7-olide-*ent*-kaur-16-ene (2)

Colorless crystals, molecular formula: $C_{21}H_{30}O_6$; mp 196-197 °C; $[\alpha]_D^{23}$ -14 (*c* 0.23, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ nm: 218 (log ε 3.62); IR (KBr) ν_{max} cm^{-1}: 3372 (OH), 1727 (C=O), 1236, 1109, 1054; HRESI MS *m/z* 401.1924 $[M+Na]^+$ (calcd. for $C_{21}H_{30}O_6$ Na 401.1939); ^1H and ^{13}C NMR data (Table 1).

Nodosin (3)

Colorless solids, molecular formula: $C_{20}H_{26}O_6$; mp 274-276 °C (Me_2CO); $[\alpha]_D^{23}$: -213° (*c* 0.23 CH_3OH); HRESI MS *m/z* 385.1632 $[M+Na]^+$ (calcd. for $C_{20}H_{26}O_6$ Na 385.1627); ^1H NMR δ ppm (C_5D_5N, 400 MHz): 8.29 (1H, br s, 6-OH), 5.94, 5.28 (each 1H, brs, 17-H), 5.79 (1H, s, 6-H), 5.78 (1H, m, 1-H), 5.15 (1H, m, 11-H), 4.55, 4.31 (each 1H, ABd, *J* = 8.8 Hz, 20-H), 3.70 (1H, d, *J* = 10.8 Hz, 14β-H), 3.13 (1H, m, 13β-H), 3.05 (1H, s, 5β-H), 1.00 (3H, s, 18-H), 0.97 (3H, s, 19-H); ^{13}C NMR δ ppm (C_5D_5N, 100 MHz): 78.6 (d, C-1), 24.2 (t, C-2), 37.6 (t, C-3), 31.7 (s, C-4), 55.9 (d, C-5), 102.2 (d, C-6), 172.0 (s, C-7), 56.7 (s, C-8), 48.8 (d, C-9), 50.2 (s, C-10), 66.2 (d, C-11), 41.5 (t, C-12), 35.7 (d, C-13), 34.3 (t, C-14), 201.2 (s, C-15), 151.4 (s, C-16), 117.5 (t, C-17), 33.2 (q, C-18), 23.5 (q, C-19), 74.2 (t, C-20).

Isodocarpin (4)

Colorless crystals, molecular formula: $C_{20}H_{26}O_5$; mp 268-270 °C (Me_2CO); $[\alpha]_D^{23}$: -164° (*c* 0.43 CH_3OH); HRESI MS *m/z* 369.1685 $[M+Na]^+$ (calcd. for $C_{20}H_{26}O_5Na$ 369.1678); ^1H NMR δ ppm ($CDCl_3$, 400 MHz): 6.07, 5.48 (each 1H, s, 17-H), 5.34 (1H, s, 6-H), 4.41 (1H, m, 1-H), 4.03, 3.97 (each 1H, ABd, *J* = 9.0 Hz, 20-H), 3.12 (1H, m, 13-H), 2.53 (1H, m, 9-H), 1.01 (3H, s, 18-H), 0.96 (3H, s, 19-H); ^{13}C NMR δ ppm (C_5D_5N, 100 MHz): 76.8 (d, C-1), 23.8 (t, C-2), 37.3 (t, C-3), 32.9 (s, C-4), 54.5 (d, C-5), 102.0 (d, C-6), 171.7 (s, C-7), 56.8 (s, C-8), 45.9 (d, C-9), 50.1 (s, C-10), 19.9 (t, C-11), 35.3 (t, C-12), 31.1 (d, C-13), 29.8 (t, C-14), 200.7 (s, C-15), 151.4 (s, C-16), 117.5 (t, C-17), 32.9 (q, C-18), 23.1 (q, C-19), 74.2 (t, C-20).

Table 1. NMR data of compounds **1** and **2** (C_5D_5N, δ in ppm)

Position	**1** δ_C	**1** δ_H	**2** δ_C	**2** δ_H
1	76.8 d	5.05 (dd, *J* = 4.0, 12 Hz)	76.3 d	4.81 m
2	24.1 t	1.81 m	24.1 t	1.88 m
3	40.8 t	1.59 m	37.2 t	1.31 m
4	30.6 s		31.5 s	
5	53.0 d	2.48 s	53.6 d	3.33 s
6	99.9 d	5.55 s	109.1 d	5.00 s
7	174.5 s		175.2 s	
8	54.5 s		53.4 s	
9	43.3 d	3.42 (d, *J* = 8.8 Hz)	46.7 d	3.27 (d, *J* = 10 Hz)
10	45.3 s		50.9 s	
11	63.8 d	4.73 m	63.7 d	4.31 m
12	42.2 t	1.91 m	45.8 t	2.92, 1.86 m
13	39.3 d	2.83 m	37.0 d	2.71 m
14	34.6 t	2.24 (dd, *J* = 5.6, 12 Hz)	34.5	2.03, 1.65 (d, *J* = 11.6 Hz)
15	76.6 d	5.52 s	77.7 d	5.52 s
16	154.1 s		158.5 s	
17	107.6 t	5.15, 5.49 brs	108.5 t	5.47, 5.20 brs
18	34.2 q	1.01 s	32.9 q	0.95 s
19	24.5 q	0.99 s	23.3 q	0.91 s
20	75.0 t	3.66, 4.27 (d, *J* = 8 Hz)	73.9 t	4.43, 4.17 (d, *J* = 8.8 Hz)
OMe			54.3 q	3.15 s

Odonicin (5)

Colorless crystals, molecular formula: $C_{24}H_{30}O_7$; mp 197-199 °C (Me_2CO); $[\alpha]_D^{23}$: -178° (*c* 0.19 CH_3OH); HRESI MS *m/z* 453.1881 $[M+Na]^+$ (calcd. for $C_{24}H_{30}O_7Na$ 453.1889); ^1H NMR δ ppm ($CDCl_3$, 400 MHz): 6.69 (1H, d, *J* = 10 Hz, 3-H), 5.89 (1H, d, *J* = 10 Hz, 2-H), 5.63 (1H, m, 15-H), 5.29 (1H, d, *J* = 8.8 Hz, 6-H), 5.06, 4.83 (each 1H, br s, 17-H), 4.33, 4.00 (each 1H, d, *J* = 11.2 Hz, 20-H), 2.14, 2.05 (each 3H, s, 2xOAc), 1.20 (3H, s, 18-H), 1.07 (3H, s, 19-H); ^{13}C NMR δ ppm ($CDCl_3$, 100 MHz): 197.4 (s, C-1), 128.3 (d, C-2), 159.6 (d, C-3), 35.9 (s, C-4), 51.8 (d, C-5), 75.1 (d, C-6), 96.8 (s, C-7), 51.3 (s, C-8), 42.9 (d, C-9), 46.9 (s, C-10), 17.9 (t, C-11), 32.1 (t, C-12), 35.7 (d, C-13), 26.4 (t, C-14), 74.4 (d, C-15), 157.7 (s, C-16), 109.8 (t, C-17), 30.0 (q, C-18), 25.1 (q, C-19), 65.7 (t, C-20), 173.7 (s, OAc), 170.6 (s, OAc), 22.2 (q, OAc), 21.4 (q, OAc).

Maoyecrystal F (6)

Colorless needles, molecular formula: $C_{22}H_{32}O_7$; mp 215-217 °C (Me_2CO); $[\alpha]_D^{23}$: -7.6° (*c* 0.21 CH_3OH); HRESI MS *m/z* 431.2034 $[M+Na]^+$ (calcd. for $C_{22}H_{32}O_7Na$ 431.2045); ^1H NMR δ ppm (C_5D_5N, 400 MHz): 5.70 (1H, d, *J* = 5.6 Hz, 6-H), 5.38, 5.18 (each 1H, s, 17-H), 5.04 (1H, s, 15-H), 4.76, 4.45 (each 1H, d, *J* = 10 Hz, 20-H), 4.67 (1H, m, 11-H), 4.23 (1H, m, 1-H), 2.84 (1H, d, *J* = 9.8 Hz, 9-H), 1.12 (3H, s, 18-H), 0.80 (3H, s, 19-H); ^{13}C NMR δ ppm (C_5D_5N, 100 MHz): 73.4 (d, C-1), 28.2 (t, C-2), 39.3 (t, C-3), 34.1 (s, C-4). 55.2 (d, C-5), 75.6 (d, C-6), 95.5 (s, C-7), 53.4 (s, C-8), 49.7 (d, C-9), 42.7 (s, C-10), 63.2 (d, C-11), 41.5 (t, C-12), 37.1 (d, C-13), 28.0 (t, C-14), 74.7 (d, C-15), 161.4 (s, C-16), 107.2 (t, C-17), 31.5 (q, C-18), 22.3 (q, C-19), 64.4 (t, C-20), 169.3 (s, OAc), 21.3 (q, OAc).

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