Ent-Kaurane Diterpenoids from *Isodon rubescens* var. *rubescens*

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Five new *ent*-kaurane diterpenoids xindongnins H—L (1—5), together with five known ones, xindongnins A and B (6, 7), melissoidesins G (8), dawoensin A (9), and glabcensin V (10) were isolated from *Isodon rubescens* var. *rubescens*. Their structures were elucidated by spectroscopic methods including extensive 2D NMR techniques.

Key words *Isodon rubescens* var. *rubescens*; *ent*-kauranoid; xindongnin H; xindongnin I; xindongnin J; xindongnin L

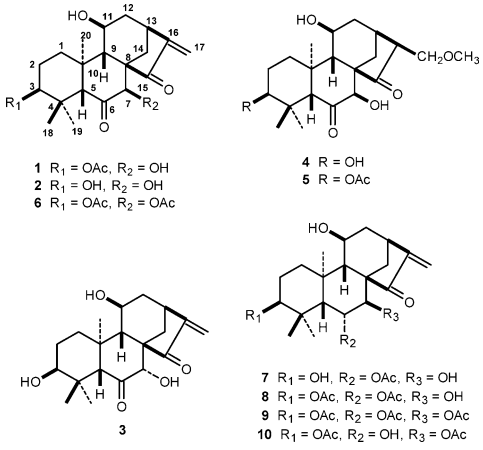
It was revealed by our previous phytochemical investigations on *Isodon rubescens* complex, a well-known antitumor herb in China, that the structural types of secondary metabolites changed with the different ecological environment of habitat for this plant.[1—6] Xindongnins A and B, two 20-non-oxygenated *ent*-kaurane diterpenoids, were regarded as the major constituents of *I. rubescens* var. *rubescens*,[7] which was collected in Xin Prefecture, the northwestern part of the Dabie Mountains. To further examine the chemistry of this species in the Dabie Mountains, we collected some plant materials in the southeastern mountains. Besides xindongnins A and B (**6**, **7**), five new 20-non-oxygenated *ent*-kauranoids (**1**—**5**) and three known analogues melissoidesin G (**8**),[8] dawoensin A (**9**),[9] and glabcensin V (**10**),[10] were isolated from this plant. The structures of the five new compounds were determined by analysis of their spectral data, especially two dimensional (2D) NMR spectra. Herein, we report the isolation and structural elucidation of these compounds.

Results and Discussion

Compound **1** was obtained as white amorphous powder, and showed a molecular ion peak at *m*/*z* 390.2030 in its high resolution electron impact (HR-EI)-MS corresponding to the molecular formula $C_{22}H_{30}O_6$. This was confirmed by ^{13}C- and distortionless enhancement by polarization transfer (DEPT)-NMR spectra, which exhibited all 20 carbon signals for the diterpene skeleton in addition to an acetoxy group.

On the basis of the characteristic signals of three methines (δ_C 52.8, 59.5, 37.6 due to C-5, 9, 13), three quaternary carbons (δ_C 54.3, 45.0, 35.7 assignable to C-8, 10, 4), three methyls (δ_C 26.9, 22.3, 18.6 attributable to C-18, 19, 20), an α,β-unsaturated ketone group (δ_C 210.3 s, 151.1 s, 113.4 t, assigned as C-15, 16, 17), along with the structures of the known diterpenoids also isolated from this plant, we assumed that compound **1** should be one of the analogues of *ent*-kaur-16-en-15-one. Comparison of the NMR data of **1** with those of the major component xindongnin A (**6**) clearly revealed that the structure of **1** was very similar to that of **6**. The only difference between these two compounds was the group at C-7 position, and there was an hydroxy group at C-7 in **1** instead of a acetoxyl group in **6**. It was also evident from the heteronuclear multiple bond connectivity (HMBC) spectrum of **1**, in which the correlations between an oxygenated methine proton (δ_H 4.22, H-7) with C-5, 6, 9, and 15; H-3 (δ_H 4.71) with C-18, 19, and the acetoxy carbonyl carbon (δ_C 170.2); and H-11 (δ_H 4.28) with C-8, 9, 10, and 13, determined the locations of 7-OH, 3-OAc, and 11-OH, respectively, along with the cross-peaks arising from H-7 and 11 with two hydroxy protons (δ_H 6.86, 6.40) in the ^{1}H–^{1}H correlation spectroscopy (COSY) experiment. Therefore, compound **1** was established to be 7β,11β-dihydroxy-3β-acetoxy-*ent*-kaur-16-en-6,15-dione by the nuclear Overhauser effect (NOE) (Fig. 1) between H-3 with Me-19, H-11 with Me-20, H-7 with H-14α (δ_H 2.13, d, J=12.0 Hz) in the rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum of **1**, and called xindongnin H. All the NMR data of **1** were unambiguously assigned based on the 2D-NMR spectra including ^{1}H–^{1}H COSY, ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), HMBC, and ROESY.

Compounds **2** and **3** have the same molecular formula $C_{20}H_{28}O_5$ that was determined by their HR-MS, and presented almost the same ^{13}C-NMR spectra except for some small differences of the signals of C-5, 6, 7, 8, 9, 14, and 15 that stand around C-7. They were both suggested to be 3,7-deacetal xindongnin A (**6**) by comparison of their ^{13}C-NMR data with those of **1** and **6**. This deduction was confirmed by the HMBC spectra of **2** and **3**. Finally, they were elucidated



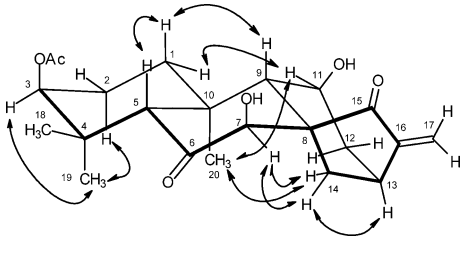


Fig. 1. Key NOE Correlations of Compound **1**

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to be a pair of 7-epimers, 3β,7β,11β-trihydroxy-*ent*-kaur-16-en-6,15-dione (**2**) and 3β,7α,11β-trihydroxy-*ent*-kaur-16-en-6,15-dione (**3**), respectively, by the NOEs of H-7/H-14α in 2 and H-7/H-9β in **3**. They were named xindongnins I and J.

A general analysis of the NMR data of compound **4** indicated that it resembled **2** very much except for C-16 and 17. The exo-methylene group at C-16 in **2** was replaced by a methine (δ_C 56.8, d) and an oxygenated methylene group (δ_C 71.0, t) in **4**. The related HMBCs confirmed this, and indicated a methoxyl group on the oxygenated methylene carbon. And the significant upfield shift of C-12 (δ_C 33.8, t), caused by the steric compression effect between H-12β and H_2-17, suggested the presence of H-16α, which was confirmed by the NOEs of H_2-17/H-12β and H-16α/H-13α.[3,6] The *R* configuration was deduced for C-16 by the possible biogenetic pathway from the known *ent*-kauranoid xindongnin A (**6**) to **4** (Fig. 2). Thus, compound **4** was assigned as 16(*R*)-3β,7β,11β-trihydroxy-17-methoxy-*ent*-kaura-6,15-dione. Similarly, compound **5** was established as 16(*R*)-7β,11β-dihydroxy-3β-acetoxy-17-methoxy-*ent*-kaura-6,15-dione. The structures of **4** and **5** well matched all their HR-MS and NMR data including kinds of 2D NMR spectra. And all their NMR data were clearly assigned. We called them xindongnins K and L.

The known compounds **6**—**10** were determined to be xindongnins A and B (**6**, **7**), melissoidesins G (**8**), dawoensin A (**9**), and glabcensin V (**10**) by comparison of their spectral data with literature values. They all are 20-non-oxygenated *ent*-kauranoids, and their major constituents were the same as those isolated previously from the plant materials collected in the northwestern Dabie Mountains. Thus, we could deduce that the chemistries of *I. rubescens* var. *rubescens* throughout the Dabie Mountains are almost the same, and that 20-non-oxygenated *ent*-kauranoids are the major constituents of this species.

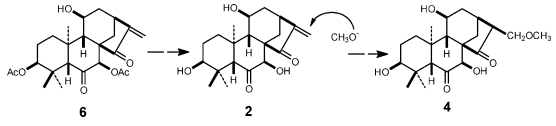

Fig. 2. Proposed Biogenetic Pathway from **6** to **4**

Table 1. ^{13}C-NMR Data of Compounds **1**—**5** (125.8 MHz, in C_5D_5N)

C	1	2	3	4	5
1	34.2 t	34.0 t	33.1 t	33.8 t	34.5 t
2	22.7 t	26.1 t	25.8 t	26.0 t	23.1 t
3	77.4 d	74.7 d	74.6 d	74.6 d	77.9 d
4	35.7 s	37.0 s	37.4 s	36.9 s	36.2 s
5	52.8 d	52.0 d	57.0 d	52.0 d	53.3 d
6	210.6 s	211.8 s	213.7 s	211.8 s	211.1 s
7	82.6 d	83.1 d	77.3 d	82.8 d	82.9 d
8	54.3 s	54.6 s	61.7 s	56.8 s	55.2 s
9	59.5 d	59.9 d	63.0 d	58.8 d	58.9 d
10	45.0 s	45.4 s	46.2 s	45.2 s	45.3 s
11	64.7 d	64.8 d	64.6 d	63.8 d	64.2 d
12	40.9 t	41.2 t	42.0 t	33.8 t	34.1 t
13	37.6 d	37.7 d	37.2 d	33.0 d	33.4 d
14	33.7 t	33.9 t	27.8 t	34.4 t	34.7 t
15	210.3 s	210.7 s	205.1 s	220.6 s	220.8 s
16	151.1 s	151.2 s	150.7 s	56.8 d	57.3 d
17	113.4 t	113.3 t	112.4 t	71.0 t	71.5 t
18	26.9 q	28.1 q	28.6 q	27.9 q	27.3 q
19	22.3 q	23.1 q	22.9 q	23.0 q	22.7 q
20	18.6 q	19.0 q	19.1 q	19.0 q	19.1 q
OAc	170.2 s				170.6 s
	20.9 q				21.4 q
OMe				58.4 q	58.9 q

Experimental

General Procedures Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV absorptions were obtained on a Shimadzu UV-2401PC UV-VIS recording spectrophotometer. IR spectra were determined on a Bio-Rad FtS-135 spectrophotometer with KBr pellets. MS were recorded on a VGAuto Spec-3000 spectrometer. 1D- and 2D-NMR spectra were run on Brucker AM-400 and DRX-50 instruments with tetramethylsilane (TMS) as an internal standard.

Plant Material The leaves of *I. rubescens* var. *rubescens* were collected in Shangcheng Prefecture of Hennan Province in August 2001, and air-dried. The identity of the plant material was verified by Prof. Zhong-Wen Lin, and a voucher specimen (KIB-09-2001-Lin) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Science.

Extraction and Isolation The dried and powdered leaves (1.0 kg) were extracted with 70% Me_2CO and filtered. The filtrate was concentrated and partitioned successively between petroleum ether and water, then EtOAc and water. The EtOAc extract (41 g) was applied to column chromatography over silica gel (100—200 mesh, 500 g) column eluting with a system of $CHCl_3$–Me_2CO (10 : 0, 9 : 1, 8 : 2, 7 : 3, 6 : 4, and 5 : 5). The $CHCl_3$–Me_2CO (9 : 1) fraction was further chromatographed repeatedly over silica gel to afford **10** (1.0 g), **8** (1.1 g), **6** (1.4 g), **9** (0.4 g), and **7** (1.8 g). Compound **1** (23 mg) was isolated from the $CHCl_3$–Me_2CO (8 : 2) fraction in the same way. The $CHCl_3$–Me_2CO (7 : 3) fraction was similarly chromatographed on silica gel to yield compounds **4** (7.2 mg), **5** (6.5 mg), **2** (13.0 mg), and **3** (5.3 mg).

Compound **1**: White amorphous powder. $[\alpha]_D^{25}$ −11.6° (*c*=0.30, MeOH). UV λ_{max} (MeOH) nm (log ε): 242 (3.64). IR (KBr) ν_{max} cm^{-1}: 3456, 2939, 1706, 1645, 1374, 1247, 1046. ^{1}H-NMR (pyridine-d_5, 500.13 MHz) δ: 6.86 (1H, s, OH-7β), 6.40 (1H, s, OH-11β), 5.99 (1H, s, H-17a), 5.30 (1H, s, H-17b), 4.71 (1H, br s, H-3α), 4.28 (1H, d, *J*=3.5 Hz, H-11α), 4.22 (1H, s, H-7α), 4.16 (1H, s, H-5β), 2.99 (1H, br s, H-13α), 2.98 (1H, s, H-9β), 2.18 (1H, br d, *J*=15.0 Hz, H-12β), 2.13 (1H, d, *J*=12.0 Hz, H-14α), 2.10 (1H, overlap, H-12α), 1.92 (3H, s, OAc), 1.86 (1H, m, H-2α), 1.70 (2H, overlap, H_2-1), 1.61 (1H, m, H-2β), 1.54 (1H, dd, *J*=4.0, 12.0 Hz, H-14β), 1.40 (3H, s, Me-19), 1.07 (3H, s, Me-18), 1.05 (3H, s, Me-20). ^{13}C-NMR (pyridine-d_5, 125.8 MHz) data: see Table 1. EI-MS: *m*/*z* (%): 390 $[M]^+$ (33), 360 (17), 330 (12), 312 (16), 284 (23), 149 (50), 123 (90). HR-EI-MS *m*/*z*: 390.2030 (Calcd for $C_{22}H_{30}O_6$, 390.2042).

Compound **2**: White amorphous powder. $[\alpha]_D^{25}$ −51.6° (*c*=0.19, MeOH). UV λ_{max} (MeOH) nm (log ε): 242 (3.75). IR (KBr) ν_{max} cm^{-1}: 3434, 2937, 1716, 1706, 1645, 1388, 1069, 1042. ^{1}H-NMR (pyridine-d_5, 500.13 MHz) δ: 6.79 (1H, s, OH-7β), 6.30 (1H, s, OH-11β), 6.09 (1H, s, OH-3β), 5.98 (1H, s, H-17a), 5.30 (1H, s, H-17b), 4.56 (1H, s, H-5β), 4.35 (1H, d, *J*=3.5 Hz, H-11α), 4.23 (1H, s, H-7α), 3.52 (1H, br s, H-3α), 3.09 (1H, s, H-9β), 3.00 (1H, br s, H-13α), 2.28 (1H, m, H-1β), 2.21 (1H, br d, *J*=14.8 Hz, H-12β), 2.20 (1H, d, *J*=12.0 Hz, H-14α), 2.14 (1H, m, H-12α), 2.06 (1H, m, H-2α), 1.75 (1H, overlap, H-1α), 1.76 (1H, overlap, H-2β), 1.57 (1H, dd, *J*=4.0, 12.0 Hz, H-14β), 1.48 (3H, s, Me-19), 1.36 (3H, s, Me-18), 1.15 (3H, s, Me-20). ^{13}C-NMR (pyridine-d_5, 125.8 MHz) data: see Table 1. EI-MS *m*/*z* (%): 348 $[M]^+$ (51), 330 (16), 312 (21), 297 (27), 191 (57), 180 (85), 167 (52), 123 (66). HR-EI-MS *m*/*z*: 348.1937, (Calcd for $C_{20}H_{28}O_5$, 348.1937).

Compound **3**: White amorphous powder. $[\alpha]_D^{22}$ −54.1° (*c*=0.22, MeOH). UV λ_{max} (MeOH) nm (log ε): 243.8 (3.64). IR (KBr) ν_{max} cm^{-1}: 3440, 2933, 1717, 1645, 1390, 1056. ^{1}H-NMR (pyridine-d_5, 500.13 MHz) δ: 6.04 (1H, s, H-17a), 5.34 (1H, s, H-7β), 5.26 (1H, s, H-17b), 4.40 (1H, d, *J*=4.0 Hz, H-11α), 3.48 (1H, br s, H-3α), 3.37 (1H, s, H-5β), 3.06 (1H, br s, H-13α), 2.69 (1H, s, H-9β), 2.49 (1H, dd, *J*=4.0, 11.0 Hz, H-14β), 2.30 (1H, br d, *J*=14.8 Hz, H-12β), 2.23 (1H, m, H-1β), 2.12 (1H, m, H-12α), 2.05 (1H, m, H-2α), 2.00 (1H, d, *J*=11.0 Hz, H-14α), 1.75 (1H, overlap, H-1α), 1.73 (1H, overlap, H-2β), 1.49 (3H, s, Me-19), 1.25 (3H, s, Me-18), 1.11 (3H, s, Me-20); ^{13}C-NMR (pyridine-d_5, 125.8 MHz) data: see Table 1. EI-MS *m*/*z* (%): 348 $[M]^+$ (51), 330 (16), 312 (21), 297 (27), 191 (57), 180 (85), 167 (52), 123 (66). HR-EI-MS *m*/*z*: 348.1937 (Calcd. for $C_{20}H_{28}O_5$, 348.1937).

Compound **4**: White amorphous powder. $[\alpha]_D^{24}$ −30.7° (*c*=0.29, MeOH). UV λ_{max} (MeOH) nm (log ε): 204.2 (3.34). IR (KBr) ν_{max} cm^{-1}: 3459, 2933,

1729, 1704, 1386, 1097, 1069, 1047. ^{1}H-NMR (pyridine-d_5, 500.13 MHz) δ: 6.64 (1H, s, OH-7β), 6.43 (1H, s, OH-11β), 6.07 (1H, s, OH-3β), 4.52 (1H, s, H-5β), 4.34 (1H, dd, J=8.0, 10.0 Hz, H-17a), 4.24 (1H, d, J=4.0 Hz, H-11α), 4.22 (1H, s, H-7β), 4.06 (1H, dd, J=4.0, 10.0 Hz, H-17b), 3.50 (1H, br s, H-3α), 3.25 (3H, s, OMe), 2.99 (1H, m, H-16α), 2.91 (1H, s, H-9β), 2.72 (1H, m, H-13α), 2.26 (1H, br d, J=15.0 Hz, H-12β), 2.24 (1H, overlap, H-1β), 2.21 (1H, d, J=12.5 Hz, H-14α), 2.03 (1H, m, H-2α), 1.96 (1H, m, H-12α), 1.74 (1H, overlap, H-1α), 1.72 (1H, overlap, H-2β), 1.60 (1H, dd, J=4.0, 12.5 Hz, H-14β), 1.46 (3H, s, Me-19), 1.35 (3H, s, Me-18), 1.12 (3H, s, Me-20). ^{13}C-NMR (pyridine-d_5, 125.8 MHz) data: see Table 1. EI-MS: m/z (%): 380 $[M]^+$ (9), 362 (4), 348 (26), 330 (14), 312 (16), 297 (24), 191 (57), 180 (86), 167 (71), 123 (97), 83 (100). HR-EI-MS m/z: 3380.2198 (Calcd for $C_{21}H_{32}O_6$, 380.2199).

Compound **5**: White amorphous powder. $[\alpha]_D^{25}$ −11.8° (c=0.21, MeOH). UV λ_{max} (MeOH) nm (log ε): 204.4 (3.34). IR (KBr) ν_{max} cm^{-1}: 3444, 2935, 1716, 1634, 1375, 1252. ^{1}H-NMR (pyridine-d_5, 500.13 MHz) δ: 6.73 (1H, s, OH-7β), 6.47 (1H, s, OH-11β), 4.68 (1H, br s, H-3α), 4.34 (1H, dd, J=8.0, 10.0 Hz, H-17a), 4.21 (1H, s, H-7β), 4.18 (1H, d, J=4.0 Hz, H-11α), 4.10 (1H, s, H-5β), 4.06 (1H, dd, J=4.0, 10.0 Hz, H-17b), 3.26 (3H, s, OMe), 2.99 (1H, m, H-16α), 2.80 (1H, s, H-9β), 2.72 (1H, m, H-13α), 2.24 (1H, br d, J=15.0 Hz, H-12β), 2.16 (1H, d, J=12.5 Hz, H-14α), 1.94 (1H, m, H-12α), 1.91 (2H, overlap, H-1α and H-2α), 1.66 (1H, overlap, H-1β), 1.63 (1H, overlap, H-2β), 1.59 (1H, dd, J=4.0, 12.5 Hz, H-14β), 1.39 (3H, s, Me-19), 1.06 (3H, s, Me-18), 1.03 (3H, s, Me-20). ^{13}C-NMR (pyridine-d_5, 125.8 MHz) data: see Table 1. Positive FAB-MS m/z: 423 $[M+H]^+$. Positive HR-FAB-MS m/z: 423.2392 (Calcd for $C_{23}H_{35}O_7$, 423.2383).

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