Four New Diterpenoids from *Isodon melissoides*

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Received April 1, 2003; accepted April 30, 2003

Three new 11β,16β-epoxy-*ent*-kauranoids melissoidesins I—K (1—3) and one new *ent*-abietanoid melissoidesin L (4) were obtained from the aerial parts of *Isodon melissoides* (BENTHAM) H. HARA, their structures were established on the basis of the spectral methods, especially two dimensional (2D) NMR spectroscopy.

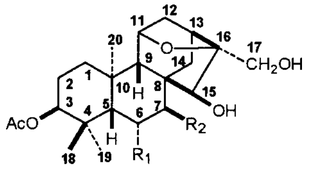
Key words *Isodon melissoides*; Labiatae; melissoidesin I; melissoidesin L; *ent*-kauranoid; *ent*-abietanoid

In the long course of our search for diterpenoids from *Isodon* species, only four 11β,16β-epoxy-*ent*-kauranoids in more than five hundred *ent*-kauranoids have been reported up to now.[1,2] Previous phytochemical researches on *Isodon melissoides* (BENTHAM) H. HARA, a perennial herb which mainly distributed in the northwest of Yunnan Provice, have revealed a series of 20-non-oxygenated *ent*-kauranoids.[3,4] Reinvestigated on this plant led to the isolation of three additional new 11β,16β-epoxy-*ent*-kauranoids (**1**—**3**) and one new *ent*-abietanoid (**4**). This paper reports the isolation and structural elucidation of these new compounds.

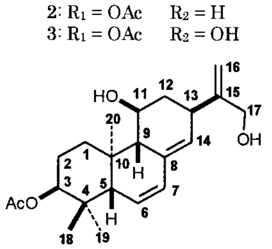
Results and Discussion

Compound **1** was obtained as colorless crystals, giving the molecular formula $C_{22}H_{34}O_6$ by positive high resolution electrospray ionization mass spectroscopy (HR-ESI-MS) (obsd 395.2451, calcd 395.2433). Besides an acetoxy group at δ_C 170.4 (s), 21.5 (q), δ_H 2.00 (3H, s), three methyls, six methylenes (including an oxygenated one), seven methines (including four oxygenated ones) and four quaternary carbons (including an oxygenated one) were observed in its NMR spectra.

Considering the diterpenoids previously isolated from this plants,[3,4] **1** was presumed to have a 20-non-oxygenated *ent*-kaurane skeleton. Analysis of the unsaturated degrees of the molecule suggested the existence of epoxy ring in **1** besides the four regular rings.



1: R_1 = OH R_2 = H
2: R_1 = OAc R_2 = H
3: R_1 = OAc R_2 = OH



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From the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 1) of **1**, H-12 [δ_H 2.31 (1H, dd, J=14.0, 3.0 Hz)] and H-14 [δ_H 2.68 (1H, d, J=12.4 Hz)] showed long-rang correlations with the oxyquaternary carbon at δ_C 87.2 (s), suggesting that this oxyquaternary carbon was attributable to C-16 and an epoxy unit was formed between C-11 and C-16. Thus, **1** is an 11β,16β-epoxy-*ent*-kaurane similar to liangshanin G.[5] Comparing ^{13}C-NMR and MS data of these two compounds, the obvious differences focused on three more oxygenated substituents (one acetoxy group and two hydroxyls) which occurred only in **1** at the C-3, C-6 and C-15 positions respectively. The results were supported by the following cross-peaks appeared in the HMBC spectrum: H-3 [δ_H 4.83 (1H, br s)] with C-5, C-18, C-19 and OAc; H-6 [δ_H 4.64 (1H, br s)] with C-8 and C-10; H-15 [δ_H 3.60 (1H, d, J=6.0 Hz)] with C-9 and C-13.

The stereochemistry of the substituents in **1** were revealed by the rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum (Fig. 2), in which the correlations of H-3 with H_3-18 and H_3-19; H-6 with H_3-18 and H_3-19; H-15 with H-7α, H-14β and H_2-17 were observed, suggesting H-3, H-6 and H-15 should be at α-, β- and α-orientations respectively. Therefore, **1** was determined to be 3β-acetoxy-6α,15β,17-tri-

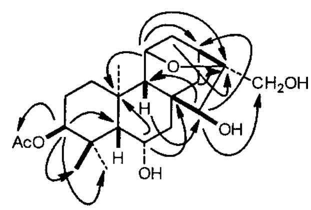

Fig. 1. Selected HMBC Correlations of **1**

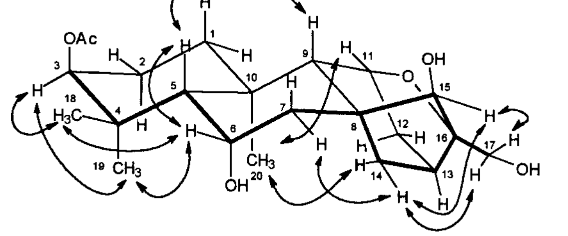

Fig. 2. The Key ROESY Correlations of **1**

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hydroxy-11β,16β-epoxy-*ent*-kaurane, named melissoidesin I.

Compound **2** gave a molecular ion peak at *m*/*z* 436, corresponding to a molecular formula $C_{24}H_{36}O_7$, as confirmed by high resolution electron impact mass spectrum (HR-EI-MS) (obsd 436.2466, calcd 436.2461). Carefully analysis of its ^{1}H, ^{13}C-NMR and two dimensional (2D) NMR spectral data implied the structure of **2** was very smilar to that of **1**. One more acetyl group in **2** resulted in the only difference between the two compounds. HMBC cross-peak of H-6 with acetyl carbonyl carbon inferred the acetyl to be attached to C-6 position. Moreover, the results of ROESY experiment suggested that the relative configurations of all substituents were same as those of **1**. Consequently, **2** was elucidated as 3β,6α-diacetoxy-15β,17-dihydroxy-11β,16β-epoxy-*ent*-kaurane, and named melissoidesin J.

Compound **3** exhibited a molecular ion peak at *m*/*z* 475.2342 $[M+Na]^+$ (calcd 475.2307) in positive HR-ESI-MS, corresponding to the molecular formula $C_{24}H_{36}O_8$. NMR and MS spectra showed **3** was another 11β,16β-epoxy-*ent*-kaurane diterpenoid structurally similar to **2**. The only difference was at C-7 position. The C-7 methylene at δ_C 39.3 (t) in **2** was hydroxylated to be an oxymethine at δ_C 76.1 (d) in **3**, as supported by the HMBC correlation between the oxymethine signal at δ_H 3.87 (1H, d, J=2.2 Hz) and C-5, C-9, C-15, and the ^{1}H–^{1}H correlation spectroscopy (COSY) correlation between this oxymethine proton and H-6. The stereochemistry of H-7 was deduced as α-orientation by the cross-peak between H-7 and H-14β in the ROESY spectrum. Thus, **3** was deduced as 3β,6α-diacetoxy-7β,15β,17-trihydroxy-11β,16β-epoxy-*ent*-kaurane, and named melissoidesin K.

Compound **4**, giving a molecular ion peak at *m*/*z* 360, was showed the molecular formula $C_{22}H_{32}O_4$ by positive HR-ESI-MS [obsd 383.2152 $(M+Na)^+$, calcd 383.2198 $(M+Na)^+$]. In addition to the signals of an acetoxy at δ_C 170.4 (s), 21.1 (q), δ_H 2.01 (3H, s) in NMR spectra, there were three methyls, five methylenes (including an exomethylene one), eight methines (including two oxygenated ones and three olefinic ones) and four quaternary carbons (including two olefinic ones), suggesting a diterpenoid skeleton of **4**. However, unlike the *ent*-kauranoids **1**—**3**, the absence of one characteristic quaternary carbon in the high field of ^{13}C-NMR spectrum of **4**, together with biogenetic view, implied that **4** was an *ent*-abietanoid resmbling adenanthin L,[6] laxiflorin O[7] and glutinosin C[8] isolated from the plants of the same genus.

A general analysis of the 2D NMR spectra of **4** indicated a terminal olefinic bond between C-15 and C-16, an acetoxy group at C-3, and OH-11 and OH-17, as occurred in adenanthin L. In addition, two double bonds were found to be formed at C-6(7) and C-8(14) positions, which were established by the correlations of H-6 [5.63 (1H, br d, J=9.9 Hz)] with C-4, C-8 and C-10, H-7 [6.24 (1H, d, J=9.9 Hz)] with C-5 and C-9, H-14 [5.71 (1H, br s)] with C-9 and C-12 in the HMBC spectrum (Fig. 3) of **4**. Furthermore, H-3 and H-11 were both determined to be α-orientated due to the ROESY correlations of H-3 with H_3-18, H_3-19 and H-11 with H-1α, H_3-20. Therefore, **4** was characterised as 3β-acetoxy-11β,17-dihydroxy-*ent*-abieta-6(7),8(14),15(16)-triene, and named melissoidesin L.

Table 1. ^{13}C-NMR Data of Compounds **1**—**4** (125.8 MHz, in C_5D_5N, δ in ppm, J in Hz)

No.	1	2	3	4
1	37.4 t	37.1 t	37.2 t	33.0 t
2	23.1 t	22.9 t	22.9 t	23.7 t
3	79.4 d	78.6 d	78.9 d	77.4 d
4	36.7 s	36.7 s	36.9 s	36.7 s
5	51.3 d	50.2 d	42.9 d	50.5 d
6	66.5 d	69.9 d	72.8 d	127.4 d
7	43.8 t	39.3 t	76.1 d	131.7 d
8	44.6 s	44.2 s	45.1 s	137.0 s
9	52.7 d	52.0 d	48.1 d	57.5 d
10	37.6 s	37.3 s	36.7 s	39.2 s
11	76.7 d	76.3 d	76.3 d	68.0 d
12	39.4 t	39.3 t	39.7 t	41.0 t
13	39.7 d	39.5 d	39.7 d	39.9 d
14	40.1 t	39.2 t	37.0 t	129.6 d
15	79.3 d	78.9 d	80.4 d	154.5 s
16	87.2 s	87.2 s	87.5 s	108.4 t
17	63.0 t	62.9 t	62.7 t	64.2 t
18	29.2 q	28.7 q	28.7 q	27.3 q
19	24.1 q	23.4 q	23.9 q	22.2 q
20	21.1 q	21.2 q	21.0 q	14.8 q
OAc	170.4 s	170.3 s	170.2 s	170.4 s
	21.5 q	170.2 s	170.2 s	21.1 q
		21.7 q	21.6 q	
		21.0 q	21.5 q	

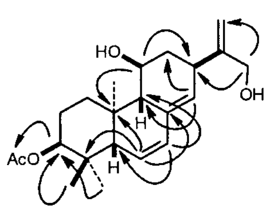

Fig. 3. Selected HMBC Correlations of **4**

Experimental

General Procedures Optical rotation was recorded on a SEPA-300 polarimetre. UV spectrum was obtained on a UV 210 A spectrometer. IR spectrum was measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. MS were recorded on a VG Auto Spec-3000 spectrometer or API QSTAR Pulsar I system Q-TOF MS instrument. 1D and 2D NMR spectra were taken on a Bruker AM-400 and DRX-500 instrument with TMS as internal standard, respectively. Silica gel for column chromatography and TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China. Fractions were monitored by TLC, and spots were visualized by heating silica gel plated sprayed with 10% H_2SO_4 in ethanol.

Plant Material The aerial parts of *I. melissoides* were collected in Heqing, northwest of Yunnan Province, People's Republic of China, in August 2002, and were identified by Professor Xi-Wen Li. The voucher specimen (KIB 02-08-10) is deposited in Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

Extract and Isolation The dried and powdered aerial plants (3.2 kg) were extracted with 95% ethanol under reflux for 5×3 h at 90 °C. The extract was concentrated *in vacuo*, and partitioned between petrol-ether and H_2O, and then between EtOAc and H_2O. The EtOAc extract (85 g) was subjected to column chromatography over silica gel and eluted with $CHCl_3$/Me_2CO (from 1 : 0 to 0 : 1) to give seven fractions. Then compounds **1** (20 mg), **2** (240 mg), **3** (26 mg) were purified from the $CHCl_3$/Me_2CO (9 : 1) fraction, and **4** (12 mg) was obtained from the $CHCl_3$/Me_2CO (7 : 3) fraction, after repeatly column chromatograph and recrystalliztion respectively.

Compound **1**: Colorless crystals, mp 124—126 °C. $[\alpha]_D^{20}$ +27.27 (c=0.11, MeOH), UV λ_{max} (MeOH) nm (log ε): no absorption. IR (KBr) ν_{max} cm^{-1}: 3435, 2955, 2921, 1722, 1651, 1456, 1266, 1087, 1029, 921. ^{1}H-NMR (C_5D_5N, 400 MHz) δ ppm: 1.04 (3H, s, Me-18), 1.41 (1H, overlap, H-14β), 1.43 (1H, overlap, H-12β), 1.44 (1H, overlap, H-7α), 1.50 (3H, s, Me-19), 1.53 (1H, br s, H-5β), 1.65 (3H, s, Me-20), 1.96 (1H, br d, J=12.0, 2.5 Hz, H-7β), 2.00 (3H, s, OAc), 2.06 (1H, m, H-1β), 2.07 (2H, m. H-

2α/β), 2.21 (1H, br d, J=11.0 Hz, H-1α), 2.25 (1H, br s, H-9β), 2.31 (1H, dd, J=14.0, 3.0 Hz, H-12α), 2.68 (1H, d, J=12.4 Hz, H-14α), 2.85 (1H, t, J=6.0 Hz, H-13α), 3.60 (1H, d, J=6.0 Hz, H-15α), 4.18 (1H, d, J=13.4 Hz, H-17a), 4.19 (1H, d, J=13.4 Hz, H-17b), 4.54 (1H, br s, H-11α), 4.64 (1H, br s, H-6β), 4.83 (1H, br s, H-3α), 5.64 (1H, br s, OH-6), 6.44 (1H, br s, OH-17); EI-MS *m/z* (%): 394 $[M]^+$ (10), 363 $[M-CH_2OH]^+$ (44), 319 (100), 301 (10), 285 (10), 273 (14), 245 (13), 227 (20), 211 (16), 166 (16), 152 (11), 131 (7), 105 (24), 91 (39), 79 (32), 43 (79); HR-ESI-MS *m/z*: 395.2461 $[M+H]^+$ (calcd 395.2433).

Compound **2**: Colorless crystals, mp 130—132 °C. $[\alpha]_D^{20}$ +38.39 (c= 0.25, MeOH). UV λ_{max} (MeOH) nm (log ε): no absorption. IR (KBr) ν_{max} cm^{-1}: 3518, 3440, 2961, 2361, 1734, 1714, 1379, 1271, 1245, 1047, 1035, 934. ^{1}H-NMR (C_5D_5N, 400 MHz) δ ppm: 0.95 (3H, s, Me-18), 1.01 (3H, s, Me-19), 1.19 (1H, dd, J=12.2, 6.0 Hz, H-14β), 1.37 (3H, s, Me-20), 1.40 (1H, overlap, H-12β), 1.41 (1H, m H-1β), 1.55 (2H, m, H-2α/β), 1.59 (1H, s, H-5β), 1.63 (1H, dd, J=12.0, 2.8 Hz, H-7β), 1.85 (1H, m, H-1α), 1.96 (3H, s, OAc), 2.07 (1H, overlap, H-12α), 2.08 (3H, s, OAc), 2.19 (1H, d, J=12.2 Hz, H-14α), 2.20 (1H, br d, J=12.0 Hz, H-7α), 2.29 (1H, br s, H-9β), 2.83 (1H, t, J=6.0 Hz, H-13α), 3.50 (1H, br s, H-15α), 4.15 (1H, d, J=13.6 Hz, H-17b), 4.17 (1H, d, J=13.6 Hz, H-17a), 4.50 (1H, br s, H-11α), 4.74 (1H, t, J=3.1 Hz, H-3α), 5.64 (1H, br s, H-6β). EI-MS *m/z*: 436 $[M]^+$ (9), 405 $[M-CH_2OH]^+$ (47), 376 $[M-AcOH]^+$ (5), 361 (13), 334 (14), 316 $[M-2AcOH]^+$ (69), 301 (100), 270 (27), 255 (85), 227 (33), 211 (52), 187 (21), 173 (28), 157 (29), 147 (34), 133 (40), 123 (44), 107 (39), 91 (41), 81 (50), 69 (46). HR-EI-MS *m/z*: 436.2466 (calcd 436.2461).

Compound **3**: Colorless crystals, mp 185—187 °C. $[\alpha]_D^{20}$ +45.45 (c= 0.09, MeOH). UV λ_{max} (MeOH) nm (log ε): no absorption. IR (KBr) ν_{max} cm^{-1}: 3438, 2941, 2877, 1731, 1651, 1645, 1376, 1243, 1033. ^{1}H-NMR (C_5D_5N, 400 MHz) δ ppm: 1.03 (3H, s, Me-18), 1.04 (3H, s, Me-19), 1.26 (1H, dd, J=12.4, 6.5 Hz, H-14β), 1.40 (3H, s, Me-20), 1.45—1.47 (2H, m, H-1α/β), 1.43 (1H, overlap, H-12β), 1.57 (1H, m, H-2α), 1.87 (1H, m , H-2β), 1.92 (3H, s, OAc), 2.00 (3H, s, OAc), 2.12 (1H, overlap, H-12α), 2.16 (1H, d, J=11.7 Hz, H-14α), 2.49 (1H, br s, H-5β), 2.68 (1H, br s, H-9β), 2.85 (1H, t, J=6.5 Hz, H-13α), 3.87 (1H, d, J=2.2 Hz, H-7α), 4.11 (1H, d, J=8.3 Hz, H-15α), 4.12 (1H, d, J=13.1 Hz, H-17a), 4.14 (1H, d, J=13.1 Hz, H-17b), 4.53 (1H, br s, H-11α), 4.77 (1H, br s, H-3α), 5.56 (1H, d, J=2.2 Hz, H-6β), 6.53 (1H, br s, OH), 6.74 (1H, s, OH), 6.95 (1H, d, J=8.3 Hz, OH-15); EI-MS *m/z*: 392 $[M-AcOH]^+$ (27), 374 (4), 359 (4), 332 $[M-2AcOH]^+$ (76), 317 (68), 299 (44), 285 (11), 271 (17), 263 (6), 253 (9), 243 (12), 225 (30), 209 (17), 185 (12), 173 (15), 157 (19), 135 (25), 121 (44), 107 (34), 91 (36), 81 (45), 55 (48), 43 (100). HR-ESI-MS *m/z*: 475.2342 $[M+Na]^+$ (calcd 475.2307).

Compound **4**: Colorless crystals, mp 90—92 °C. $[\alpha]_D^{20}$ −14.71 (c=0.03, MeOH). UV λ_{max} (MeOH) nm (log ε): 243 (3.66), 235 (3.65). IR (KBr) ν_{max} cm^{-1}: 3441, 2931, 2363, 2338, 1732, 1716, 1651, 1616, 1456, 1248, 1031. ^{1}H-NMR (C_5D_5N, 400 MHz) δ ppm: 0.88 (3H, s, Me-18), 0.94 (3H, s, Me-19), 0.96 (3H, s, Me-20), 1.77 (1H, m, H-2β), 1.93 (1H, m, H-2α), 1.97 (1H, d, J=12.0 Hz, H-12β), 2.01 (3H, s, OAc), 2.24 (1H, overlap, H-12α), 2.26 (1H, m, H-1β), 2.51 (1H, m, H-1α), 2.54 (1H, s, H-9β), 2.65 (1H, br s, H-5β), 3.31 (1H, m, H-13α), 4.17 (1H, m, H-11α), 4.47 (2H, br s, H_2-17), 4.87 (1H, t, J=2.5 Hz, H-3α), 5.12 (1H, s, H-16a), 5.50 (1H, s, H-16b), 5.63 (1H, br d, J=9.9 Hz, H-6), 5.71 (1H, br s, H-14), 6.24 (1H, br d, J=9.9 Hz, H-7). EI-MS *m/z*: 360 $[M]^+$ (7), 300 $[M-AcOH]^+$ (15), 282 (44), 267 (100), 249 (27), 225 (44), 209 (33), 197 (31), 183 (30), 173 (41), 145 (40), 131 (42), 121 (49), 105 (60), 91 (62). HR-ESI-MS *m/z*: 383.2152 $[M+Na]^+$ (calcd 383.2153).

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