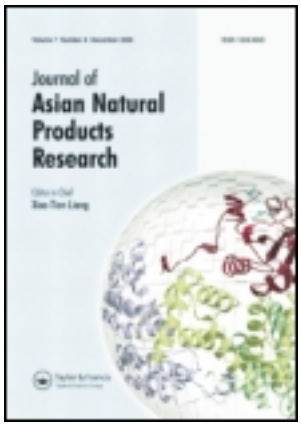

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Three new compounds from *Isodon melissoides*

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Reinvestigation of the aerial parts of *Isodon melissoides* has afforded two new *ent*-diterpenoids, melissoidesins V (**1**) and W (**2**), together with five known ones, glabcensin W (**3**), melissoidesin C (**4**), melissoidesin A (**5**), melissoidesin B (**6**) and melissoidesin D (**7**), one new ionone derivative 3α,4α-isopropyliden-β-ionol (**8**), as well as three analogues, 3-hydroxy-4-oxo-β-ionol (**9**), megastigma-7-en-3,5,6,9-tetraol (**10**) and blumenol A (**11**), and three phenolic compounds salicylic acid (**12**), syringic acid (**13**) and cirsiliol (**14**). The new structures were elucidated on the basis of spectroscopic techniques, especially the 2D-NMR spectral analysis.

Keywords: *Isodon melissoides*; Labiatae; Diterpenoids; Melissoidesin V; Melissoidesin W; Ionone derivatives; 3α,4α-Isopropyliden-β-ionol

1. Introduction

Isodon melissoides (Bentham) H. Hara (Labiatae) has been found to contain a series of 20-non-oxygenated-*ent*-kauranoids in previous phytochemical investigations [1,2]. In our research into the diversity of diterpenoids in the same plant of genus *Isodon*, we reinvestigated this plant collected in the same region, at a different season, according to the previous reports, leading to the isolation of three 11β,16β-epoxy-*ent*-kauranoids and one *ent*-abietanoid [3]. In continuation of this work, two new 20-non-oxygenated-*ent*-kauranoids (**1**, **2**), and five known ones, glabcensin W (**3**) [1], melissoidesin C (**4**) [2], melissoidesin A (**5**) [2], melissoidesin B (**6**) [2] and melissoidesin D (**7**) [2], one new ionone derivative 3α,4α-isopropyliden-β-ionol (**8**), three known analogues, *i.e.* 3-hydroxy-4-oxo-β-ionol (**9**) [4], megastigma-7-en-3,5,6,9-tetraol (**10**) [5] and blumenol A (**11**) [6], and three phenolic compounds, salicylic acid (**12**) [7], syringic acid (**13**) [8] and cirsiliol (**14**) [9], have been obtained from the aerial parts of the plant (figure 1).

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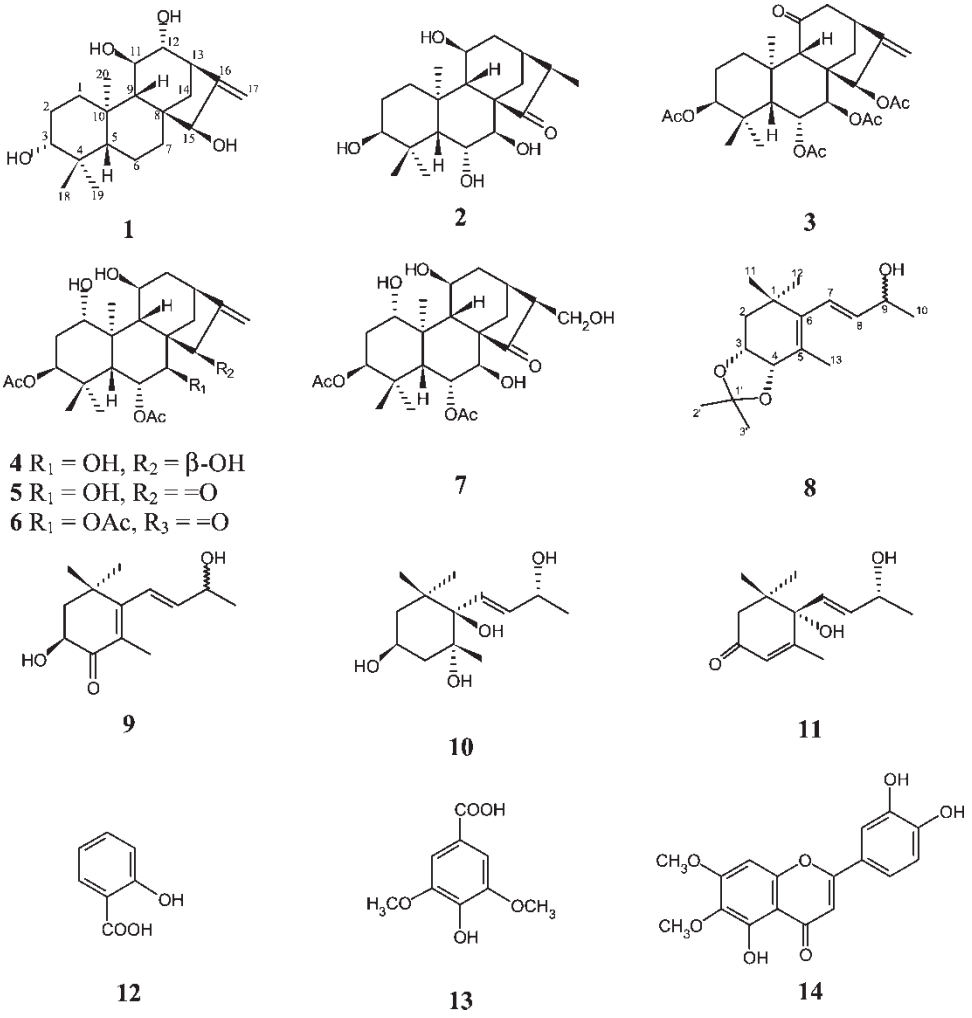

Figure 1. Compounds **1**–**14**.

2. Results and discussion

After repeated chromatographic purification on silica gel, the EtOAc-soluble portion of the 70% Me_2CO extract of *Isodon melissoides* yielded two new diterpenoids, melissoidesin V (**1**) and melissoidesin W (**2**), and one new ionone derivative, 3α,4α-isopropyliden-β-ionol (**8**), together with eleven known ones **3**–**7** and **9**–**14**.

Compound **1**, obtained as colorless needles, gave a molecular formula of $C_{20}H_{32}O_4$ at *m/z* 336.2296 by HREIMS. The NMR spectra reveal three methyls [δ_C 28.9, 17.2, 16.3 (each q), δ_H 1.06, 1.20, 1.59 (each 3H, s)], one *exo*-methylene [δ_C 156.0 (s), 107.8 (t), δ_H 5.49, 5.26 (each 1H, s)], and four oxy-methines (δ_C 83.0, 78.5, 78.2, 71.9 each d). Considering the diterpenoids previously isolated from the plant, **1** was tentatively presumed to be a 20-non-oxygenated *ent*-kauranoid with four hydroxyl groups.

In the HMBC spectrum, correlations between H_3-18 and H_3-19 with C-3 at δ_C 78.5 (d), and H-11 at δ_H 4.34 (1H, br s) with C-8, C-10 and C-13, hint that the hydroxy groups are at

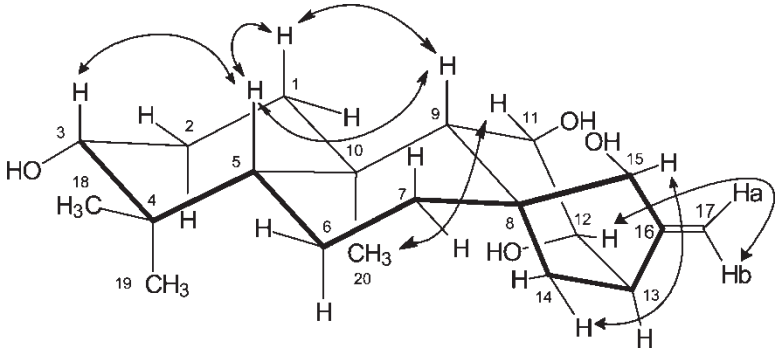

Figure 2. Key ROESY correlations of **1**.

C-3 and C-11, respectively. Moreover, the cross-peak of H-11 with a proton at δ_H 4.41 (1H, br d, $J = 3.3$ Hz) in the $^1H-^1H$ COSY spectrum suggests that there is another hydroxy group at C-12 in compound **1**, as with rabdoloxin B [10]. The signal of H-12 (δ_H 4.41) is correlated with C-9, C-14 and C-16, verifying the above deduction in the HMBC experiment. Furthermore, the last hydroxy group was assigned at C-15 based on the correlations of H-15 (δ_H 4.17 1H, d, $J = 9.9$ Hz) with C-7 and C-9 in the HMBC spectrum.

The relative configurations of the oxygenated substituents were deduced from analysis of the ROESY spectrum, which clearly shows cross-peaks of H-3 with H-5β, H-11 with Me-20, H-12 with H-17b, and H-15 with H-14β (figure 2), indicating that H-3, H-11, H-12 and H-15 possess β-, α-, β- and α-orientations, respectively. Thus, **1** was determined as 3α,11β,12α,15β-tetrahydroxy-*ent*-kaur-16-ene, named melissoidesin V.

Compound **2**, obtained as colorless crystals, displays a quasi-molecular ion peak at *m*/*z* 351.2175 $[M - H]^-$, which is consistent with a molecular formula of $C_{20}H_{31}O_5$ from its negative HR-FABMS. General analysis of its IR, UV, MS and NMR spectra leads to the conclusion that compound **2** also has an *ent*-kaurane as basic skeleton with four hydroxyl groups. Comparison of the NMR spectral data of **2** with those of melissoidesin G [1] indicates that two acetoxy groups at C-3 and C-6 in melissoidesin G have been replaced by two hydroxy groups at the same positions in **2**. In addition, the two compounds differ in the moiety at C-16. For **2**, the methyl signal at δ_H 1.55 (3H, d, $J = 6.4$ Hz) is coupled to C-13 and C-15 in the HMBC spectrum (figure 3), revealing that the *exo*-methylene at C-16 in melissoidesin G has been replaced by a methyl at C-16 in **2**. Analysis of the ROESY spectrum of **2** suggests that the relative configurations of all hydroxy groups are the same as those of melissoidesin G, and the β-orientation of Me-16, displaying a relatively high field at δ_C 11.8, is caused by a steric compression effect between Me-16 and OH-11β. Thus, **2** is 3β,6α,7β,11β-tetrahydroxy-16β-methyl-*ent*-kaur-15-one, named melissoidesin W.

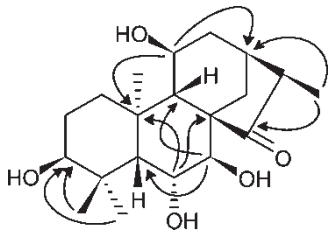

Figure 3. Selected HMBC correlations of **2**.

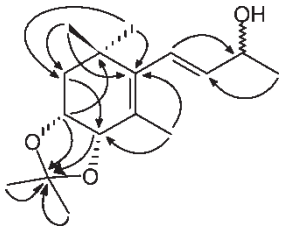

Figure 4. Selected HMBC correlations of **8**.

Compound **8** displays signals of six methyls (including one methyl doublet and five singlets), one methylene, five methines (including three oxy-methines) and four quaternary carbons (including two olefinic carbons) in its ^{13}C and DEPT spectra. Except for two methyl singlets at δ_C 26.3 and 29.4 (each q) and one quaternary carbon at δ_C 108.1 (s), suggesting an isopropylidene group, the other 13 carbons indicate a β-ionol derivative in which C-3 and C-4 are oxy-substituted, being compared with those of the aglycone of plucheoside B [11]. This was confirmed by the correlations of H-3 (δ_H 4.39 1H, m) with C-1 (δ_C 35.7 s), H_3-13 (δ_H 2.00 3H, s) with C-4 (δ_C 76.5 d) in the HMBC spectrum of **8** (figure 4). Moreover, a cross-peak of H-3 and H-4 with the quaternary carbon C-1′ (δ_C 108.1 s), and the protons of two methyl singlets correlated with the same quaternary carbon (δ_C 108.1), occurs in the HMBC, indicating that C-3 and C-4 of **8** are isopropylidenated. The stereochemistry of H-3 and H-4 were both assigned as β-orientated according to the correlations between these two protons and Me-11. Therefore, **8** is elucidated as 3α,4α-isopropyliden-β-ionol. This compound is most likely an artifact of the extraction and isolation procedure.

3. Experimental

3.1 General experimental procedures

Melting points (uncorrected) were measured on an XRC-1 apparatus. Optical rotations were taken on a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a UV 210A spectrometer. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D and 2D NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as internal standard. MS and HRMS were recorded on a VG Auto Spec-3000 spectrometer. Silica gel (200–300 mesh) for column chromatography and TLC was obtained from Qingdao Marine Chemical Factory, Qingdao, China.

3.2 Plant material

The aerial parts of *Isodon melissoides* were collected in Dali, southwest of Yunnan Province, China, in July 2002. An authentic sample was identified by Professor Xi-Wen Li, and a voucher specimen (001-02 Lin) has been deposited in the Laboratory of Phytochemistry, Kunming Institute of Botany.

3.3 Extraction and isolation

Dried, powdered aerial plants (3.2 kg) were extracted with 95% ethanol under reflux for 5 × 3 h at 90°C. The extract was then concentrated *in vacuo* and partitioned between light

petroleum (bp 60–90°C) and H_2O, and then between EtOAc and H_2O. The EtOAc extract (85 g) was then subjected to column chromatography over silica gel (200–300 mesh) and eluted with $CHCl_3$–Me_2CO (from 1:0 to 0:1) to give fractions I–VII. Fraction II (32 g) was subjected to repeated column chromatography on silica gel, eluting with light petroleum–EtOAc (4:1, 3:1) and cyclohexane–EtOAc (6:1, 5:1) to yield **3** (12 mg), **5** (72 mg). Fraction III (18 g) was purified by column chromatography over silica gel (cyclohexane–acetone, 10:1) to yield **2** (95 mg), **6** (14 mg), **7** (16 mg), **4** (21 mg), **12** (14 mg), **13** (10 mg) and **14** (11 mg). Fractions IV and V (26 g) were subjected to column chromatography on silica gel ($CHCl_3$–MeOH, 10:1, 9:1 and cyclohexane–isopropanol 15:1) to give **1** (14 mg), **8** (16 mg), **9** (11 mg), **10** (28 mg) and **11** (14 mg).

3.3.1 Melissoidesin V (1). Colorless crystals (acetone), mp 150–152°C; $[\alpha]_D^{25}$ −4.3 (*c* 0.12 MeOH); UV (MeOH) λ_{max} (log ϵ): no absorption; IR (KBr) ν_{max} (cm^{-1}): 3540, 3455, 3370, 2938, 2907, 1689, 1461, 1443, 1267, 1101, 1069, 1041, 931; EIMS (70eV) *m/z* (rel. int.): 336 $[M]^+$ (13), 318 $[M - H_2O]^+$ (65), 300 (43), 285 (33), 267 (26), 243 (15), 236 (9), 227 (12), 201 (14), 187 (25), 175 (30), 160 (40), 147 (52), 135 (94), 121 (81); HR-EIMS *m/z*: 336.2296 (calcd. for $C_{20}H_{32}O_4$, 336.2301); ^1H and ^{13}C NMR spectral data see Table I.

3.3.2 Melissoidesin W (2). Colorless crystals (acetone), mp 248–250°C; $[\alpha]_D^{25}$ −26.3 (*c* 0.07 MeOH); UV (MeOH) λ_{max} (log ϵ): 210.0 (3.30) nm; IR (KBr) ν_{max} (cm^{-1}): 3415, 2974, 2934, 2871, 2337, 1635, 1456, 1284, 1057, 1019, 993; negative FABMS *m/z*: 351 ($[M - H]^-$); negative HR-FABMS *m/z*: 351.2175 (calcd. for $C_{20}H_{31}O_5$, 351.2171); for ^1H and ^{13}C NMR spectral data see table 1.

Table 1. ^{13}C (100 MHz) and ^1H (400 MHz) NMR data of compounds **1** and **2** in C_5D_5N (δ ppm).

δ_C	*1*	*2*	δ_H	*1*	*2*
1	30.9 t	35.4 t	1α	2.17 (m)	2.43 (overlap)
2	20.3 t	26.5 t	1β	1.53 (m)	2.22 (m)
3	78.5 d	76.5 d	2α	1.64 (m)	2.23 (overlap)
4	39.6 s	38.4 s	2β	1.83 (m)	1.78 (overlap)
5	55.0 d	42.1 d	3α		3.64 (br s)
6	28.1 t	70.8 d	3β	3.43 (dd, 11.6, 4.6 Hz)	
7	39.6 t	78.5 d	5β	1.01 (dd, 11.8, 1.8 Hz)	2.58 (br s)
8	45.1 s	50.9 s	6α	1.93 (m)	
9	57.4 d	59.2 d	6β	1.61 (m)	4.59 (br s)
10	38.0 s	38.8 s	7α	1.42 (m)	4.22 (d, 3.2 Hz)
11	71.9 d	64.5 d	7β	2.04 (dd, 9.9, 6.3 Hz)	
12	78.2 d	34.1 t	9β	2.10 (br s)	2.54 (br s)
13	48.4 d	36.0 d	11α	4.34 (br s)	4.33 (d, 4.5 Hz)
14	31.0 t	37.3 t	12α		2.09 (m)
15	83.0 d	228.5 s	12β	4.41 (br d, 3.3 Hz)	2.09 (m)
16	156.0 s	50.7 d	13α	3.06 (br s)	2.41 (br s)
17	107.8 t	11.8 q	14α	2.78 (d, 11.8 Hz)	3.15 (d, 12.6 Hz)
18	28.9 q	29.7 q	14β	1.08 (overlap)	1.78 (overlap)
19	16.3 q	25.1 q	15α	4.17 (d, 9.9 Hz)	
20	17.2 q	19.9 q	16α		2.42 (overlap)
			17	5.49, 5.26 (each 1H, s)	1.55 (3H, d, 6.4 Hz)
			18	1.06 (s)	1.39 (s)
			19	1.20 (s)	1.57 (s)
			20	1.59 (s)	1.71 (s)

3.3.3 3α,4α-Isopropyliden-β-ionol (8). Colorless gum, $[\alpha]_D^{25}$ −8.7 (*c* 0.6 MeOH); UV λ_{max} (MeOH) (log ϵ): 206 (3.79) nm; ^{1}H NMR (C_5D_5N, 400 MHz) δ (ppm): 6.29 (1H, d, $J = 14.7$ Hz, H-7), 5.80 (1H, dd, $J = 14.7, 5.8$ Hz, H-8), 4.65 (1H, m, H-9), 4.41 (1H, overlap, H-4β), 4.39 (1H, m, H-3β), 2.00 (3H, s, Me-13), 1.72 (2H, br d, $J = 15.1$ Hz, H-2a/b), 1.54 (3H, s, H-2′), 1.49 (3H, d, $J = 6.4$ Hz, Me-10), 1.44 (3H, s, H-3′), 1.05 (3H, s, Me-12), 0.96 (3H, s, Me-11); ^{13}C NMR (C_5D_5N, 100 MHz) δ (ppm): 35.7 (s, C-1), 42.2 (t, C-2), 72.3 (d, C-3), 76.5 (d, C-4), 125.3 (s, C-5), 142.2 (s, C-6), 124.6 (d, C-7), 141.1 d (C-8), 68.1 (d, C-9), 25.3 (q, Me-10), 24.6 (q, Me-11), 28.7 (q, Me-12), 18.8 (q, Me-13), 108.1 (s, C-1′), 29.4† (q, C-2′), 26.3‡ (q, C-3′) (a, b could be interchanged); EIMS (70 eV) *m/z* (rel. int.): 266 $[M]^+$ (10), 248 $[M - H_2O]^+$ (13), 210 (38), 193 (11), 179 (13), 165 (21), 149 (32), 135 (58), 121 (57), 107 (100), 95 (33); HR-ESIMS (positive) *m/z*: 289.1792 $[M + Na]^+$ (calcd. for $C_{16}H_{26}O_3Na$, 289.1779).

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