Full Papers

Diterpenoids from *Isodon melissoides*

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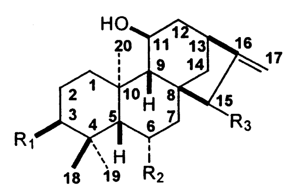
Nine new diterpenoids, melissoidesins M−U (**1**−**9**), along with five known analogues, melissoidesin F (**10**), xindongnin B (**11**), melissoidesin G (**12**), melissoidesin E (**13**), and dawoensin A (**14**), were isolated from the aerial parts of *Isodon melissoides*. The structures of new compounds were elucidated by analysis of spectral evidence including extensive 2D NMR data. Compounds **2**, **3**, **7**, **11**, **12**, and **14** showed cytotoxicity against the human tumor BGC-823 cell line with IC_{50} values less than 10 μg/mL, respectively.

A series of 20-nonoxygenated diterpenoids have been previously reported from *Isodon melissoides* (Bentham) H. Hara (Labiatae) by our research group.[1−3] In the course of our search for diterpenoids in this plant from different regions or gathered in different seasons, we reinvestigated another collection of *I. Melissoides*. Nine new 20-nonoxygenated diterpenoids, melissoidesins M−U (**1**−**9**), were obtained from the EtOAc extract. The compounds were tested for their inhibitory activity against the BGC-823 tumor cell line. This paper reports the isolation and structural elucidation of **1**−**9** and the bioassay results.

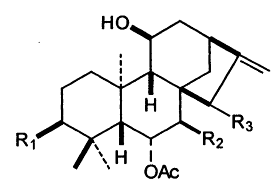


1 R_1 = OH R_2 = OAc R_3 = β-OH

2 R_1 = OH R_2 = OAc R_3 = =O

3 R_1 = OAc R_2 = OAc R_3 = =O

10 R_1 = OAc R_2 = OAc R_3 = β-OH



4 R_1 = OH R_2 = OH R_3 = β-OH

5 R_1 = OH R_2 = OAc R_3 = β-OH

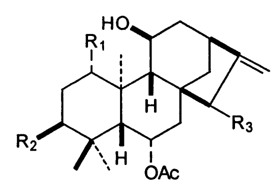
6 R_1 = OAc R_2 = OAc R_3 = β-OH

11 R_1 = OH R_2 = OH R_3 = =O

12 R_1 = OAc R_2 = OH R_3 = =O

13 R_1 = OAc R_2 = OH R_3 = β-OH

14 R_1 = OAc R_2 = OAc R_3 = =O



7 R_1 = OH R_2 = OAc R_3 = =O

8 R_1 = OH R_2 = OAc R_3 = β-OH

9 R_1 = OAc R_2 = OAc R_3 = β-OH

Results and Discussion

After repeated column chromatographic purification on silica gel, the EtOAc-soluble portion of the 70% aqueous acetone extract of aerial parts of *I. melissoides* afforded melissoidesins M−U (**1**−**9**) and five known diterpenoids, melissoidesin F (**10**),[2] xindongnin B (**11**),[4,5]melissoidesin G (**12**),[2] melissoidesin E (**13**),[2] and dawoensin A (**14**).[6]

Melissoidesin M (**1**), obtained as colorless needles, was shown to possess a molecular formula of $C_{22}H_{34}O_5$ from the positive HRESIMS molecular ion peak observed at *m*/*z* 379.2487 and analysis of its ^{1}H and ^{13}C NMR spectral data. The NMR spectra revealed the presence of three methyls, one *exo*-methylene, four oxy-methines, and one acetoxyl group. Considering the structural type of the diterpenoids previously isolated from this plant,[1,2] **1** was tentatively assigned as a 20-nonoxygenated *ent*-kaurene diterpenoid similar to melissoidesin F (**10**).[2] Comparison of the NMR data between **1** and **10** confirmed the above deduction and indicated that compound **1** was identical to **10** except for the substituent at C-3. The acetoxyl group at C-3 of **10** was replaced by a hydroxy group in **1**, which was proved by the HMBC correlations of Me-18 and Me-19 (δ 1.12, 1.25, each 3H, s) with C-3 and H-6 (δ 5.73 1H, br s) with the acetoxy carbonyl carbon, H-11 (δ 4.30 1H, br s) with C-8 and C-13, and H-15 (δ 3.98 1H, d, J = 10.0 Hz) with C-9, C-14, and C-16. According to the cross-peaks in the HMBC spectrum of **1**, the acetoxy group was placed at C-6, and three hydroxy groups were placed at C-3, C-11, and C-15, respectively.

The relative configurations of the substituents were revealed by analysis of the ROESY spectrum, in which the cross-peaks of H-3 with Me-18 and Me-19, H-6 with Me-18, H-11 with H-1α and Me-20, and H-15 with H-7α and H-14β were clearly observed, suggesting that the substituents at C-3, C-6, C-11, and C-15 possess β-, α-, β-, and

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β-orientation, respectively. Thus, **1** was determined to be 3β,11β,15β-trihydroxy-6α-acetoxy-*ent*-kaur-16-ene.

Melissoidesin N (**2**) had a quasi-molecular formula $C_{22}H_{33}O_5$ deduced by positive HRESIMS (*m/z* 377.2383). Comparison of the spectral data of **2** and **1** revealed that the two compounds were quite similar except for the moiety at C-15. The carbonyl group conjugated with the *exo*-methylene in **2** was present instead of a hydroxy group at C-15 in **1**. Absorption at 239 nm in the UV spectrum confirmed the carbonyl group. ROESY correlations of **2** indicated that the other substituents had the same orientations as those in **1**. Therefore, **2** was 3β,11β-dihydroxy-6α-acetoxy-*ent*-kaur-16-en-15-one.

Melissoidesin O (**3**) gave a molecular formula of $C_{24}H_{34}O_6$ by HREIMS (*m/z* 418.2342). NMR spectra of **3** indicated that **3** was very similar to **2** except for one more acetyl group in **3**. The acetyl group was placed at C-3 as established by the HMBC spectrum. The substituents at C-3, C-6, and C-11 had β-, α-, and β-orientation, respectively, according to the ROESY cross-peaks. Hence, **3** was established as 11β-hydroxy-3β,6α-diacetoxy-*ent*-kaur-16-en-15-one.

Compounds **1**−**3** are all *ent*-kaurenoids lacking any substituent at C-7 while possessing a substituent at C-6. However, compound **4** possessed five oxy-substituents according to analysis of the ^{13}C NMR spectrum. Further comparison of IR, UV, and 1D and 2D NMR data of **4** with those of melissoidesin E (**13**)[2] indicated one less acetyl group in **4**. The hydroxy group at C-3 in **4** was apparent instead of an acetoxy group according to the HMBC correlations of H-3 (δ 3.61 1H, br s) with Me-18 and Me-19 (δ 29.5 and 24.3) in **4**. Moreover, the cross-peaks in the ROESY spectrum suggested that the substituents at C-3, C-6, C-7, C-11, and C-15 possess β-, α-, β-, β-, and β-orientation, respectively. Therefore, melissoidesin P (**4**) was elucidated as 3β,7β,11β,15β-tetrahydroxy-6α-acetoxy-*ent*-kaur-16-ene.

Spectral data of melissoidesin Q (**5**) were very similar to that of **4**. One more acetyl group in **5** was the only difference between the two compounds. The HMBC cross-peak of H-7 (δ 5.35, 1H, d, J = 3.3 Hz) with the acetyl carbonyl carbon inferred the acetyl to be attached to C-7. The stereochemistry of H-7 was deduced by the cross-peak between H-7 and H-14β (δ 1.14, 1H, overlap) in the ROESY spectrum. Thus, **5** was deduced as 3β,11β,15β-trihydroxy-6α,7β-diacetoxy-*ent*-kaur-16-ene.

In the same way, melissoidesin R (**6**) had one more acetyl group (at C-3) than compound **5**. The stereochemistry of substituents in **6** was the same as in **5** from a ROESY experiment. Therefore, **6** was 11β,15β-dihydroxy-3β,6α,7β-triacetoxy-*ent*-kaur-16-ene.

Melissoidesin S (**7**), colorless needles, had the molecular formula $C_{24}H_{34}O_7$ by HREIMS *m/z* 434.2314 and possessed one more acetyl group than inflexanin B.[7,8] The cross-peak H-6 (δ 5.70 1H, t, J = 2.0 Hz) with an acetoxy carbonyl carbon was observed in the HMBC spectrum, which confirmed the extra acetyl group at C-6 in **7**. The ROESY experiment verified the substituents had the same relative configurations as those of inflexanin B. Thus, melissoidesin **7** was established as 1α,11β-dihydroxy-3β,6α-diacetoxy-*ent*-kaur-16-en-15-one.

Melissoidesin T (**8**) was assigned the molecular formula $C_{24}H_{36}O_7$, as deduced by its HREIMS. A general analysis of all spectra led to the conclusion that the structure of **8** was very similar to that of **7**. The only difference between **7** and **8** was a hydroxy group, rather than a carbonyl group, at C-15 in **8**, and the correlation of H-15 with H-14β in the ROESY spectrum of **8** suggested that 15-OH was β-orientated. Therefore, **8** was elucidated as 1α,11β,15β-trihydroxy-3β,6α-diacetoxy-*ent*-kaur-16-ene.

Inspection of MS and NMR data of melissoidesin U (**9**) indicated similarity to compound **8**. The only difference between **8** and **9** was that an acetoxy group, rather than a hydroxy group, was located at the 1α position in **9**, which was confirmed by the cross-peak of H-1 (δ 5.48 1H, dd, J = 14.2, 5.4 Hz) with a carbonyl carbon in the HMBC experiment. Thus, melissoidesin U (**9**) was determined to be 11β,15β-dihydroxy-1α,3β,6α-triacetoxy-*ent*-kaur-16-ene.

All of the isolated diterpenoids were tested for their ability to inhibit BGC-823 human tumor cells, using a previously described method,[9] with VCR (vincristine) as a positive control. Compound **2** demonstrated strong inhibitory activity against BGC-823 cells with an IC_{50} value of 0.036 μg/mL. Compounds **3**, **7**, **11**, **12**, and **14** showed moderate cytotoxicity with IC_{50} = 7.83, 7.71, 9.45, 6.62, and 3.54 μg/mL (VCR: IC_{50} = 0.066 μg/mL), respectively, while compounds **1**, **4**, **5**, **6**, **8**, **9**, and **10** were noncytotoxic.

Experimental Section

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, People's Republic of China.

Plant Material. The aerial parts of *Isodon melissoides* were collected in Dali, southwest of Yunnan Province, People's Republic of China, in July 2002. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 02-08-10) has been deposited in the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation. The dried and powdered aerial plants (3.2 kg) were extracted with 95% EtOH 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 (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 petrol-ether−EtOAc (4:1, 3:1) and cyclohexane−EtOAc (6:1, 5:1) to afford **1** (25 mg), **2** (18 mg), **3** (40 mg), **4** (50 mg), **6** (18 mg), **10** (69 mg), and **12** (21 mg). Fraction III (18 g) was purified by column chromatography over silica gel (cyclohexane−acetone, 10:1) to yield **5** (390 mg), **7** (1.0 g), **11** (82 mg), and **13** (21 mg). Fraction IV/V (26 g) was subjected to column chromatography on silica gel ($CHCl_3$−MeOH, 10:1, 9:1) and cyclohexane−2-propanol (15:1) to gave **8** (12 mg), **9** (230 mg), and **14** (10 mg).

Melissoidesin M (1): colorless needles (acetone); mp 210−214 °C; $[\alpha]_D^{20}$ +9.6° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (4.70) nm; IR (KBr) ν_{max} 3430, 2933, 2866, 1735, 1651, 1634, 1627, 1617, 1244, 1178, 1119, 1049, 1004, 924, 899 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 5.73 (1H, br s, H-6β), 5.53 (1H, d, J = 10.0 Hz, OH-15β), 5.36 (1H, s, H-17a), 5.18 (1H, s, H-17b), 4.30 (1H, br s, H-11α), 3.98 (1H, d, J = 10.0 Hz, H-15α), 3.57 (1H, br s, H-3α), 2.68 (1H, m, H-13α), 2.32 (1H, d, J = 12.1 Hz, H-14α), 2.25 (1H, br s, H-9β), 2.17 (1H, dd, J = 14.5, 2.96 Hz, H-1β), 2.11 (2H, overlap, H_2-12), 2.10 (3H, s, OAc), 2.09 (1H, overlap, H-7β), 2.08 (1H, s, H-5β), 1.81 (1H, overlap, H-1α), 1.80 (2H, overlap, H_2-2), 1.77 (1H, overlap, H-7α), 1.42 (3H, s, Me-20), 1.25 (3H, s, Me-19), 1.12 (3H, s, Me-18), 1.08 (1H, overlap, H-14β); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EIMS *m/z* 378 [M]$^+$ (1), 360 (4), 318 (1), 300 (31),

Table 1. ^{13}C NMR Data for Compounds **1**−**9** (C_5D_5N, 100.6 MHz, δ in ppm)

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	36.0 t	35.3 t	35.5 t	35.9 t	36.0 t	34.6 t	77.1 d	77.7 d	82.9 d
2	26.4 t	26.1 t	22.8 t	26.5 t	26.4 t	23.1 t	33.9 t	34.3 t	30.0 t
3	75.9 d	75.8 d	78.5 d	75.9 d	75.8 d	78.4 d	79.4 d	79.4 d	78.6 d
4	38.0 s	38.8 s	38.7 s	38.4 s	37.8 s	37.1 s	37.5 s	37.5 s	37.7 s
5	48.2 d	48.1 d	49.5 d	41.2 d	42.7 d	43.8 d	48.4 d	48.5 d	48.6 d
6	71.2 d	70.1 d	69.2 d	74.3 d	71.6 d	70.7 d	70.0 d	71.1 d	70.5 d
7	43.0 t	41.6 t	38.5 t	77.6 d	77.0 d	76.6 d	38.9 t	43.2 t	42.8 s
8	43.6 s	49.0 s	48.8 s	46.3 s	46.6 s	46.4 s	51.8 s	44.0 s	43.7 s
9	56.2 d	64.1 d	63.8 d	52.3 d	52.5 d	52.2 d	64.7 d	56.8 d	55.1 d
10	38.8 s	39.0 s	37.4 s	37.9 s	38.4 s	37.6 s	44.9 s	43.9 s	42.8 s
11	65.5 d	65.1 d	65.0 d	65.1 d	65.0 d	64.9 d	67.0 d	66.7 d	66.6 d
12	42.7 t	38.7 t	41.3 t	43.2 t	42.7 t	42.6 t	41.5 t	42.7 t	43.2 t
13	40.5 d	38.1 d	38.0 d	40.5 d	40.1 d	40.0 d	38.5 d	40.5 d	40.1 d
14	37.0 t	37.9 t	37.8 t	34.7 t	34.8 t	36.3 t	38.8 t	37.8 t	38.0 t
15	83.0 d	208.4 s	208.3 s	82.5 d	81.0 d	81.0 d	208.6 s	83.4 d	83.0 d
16	158.5 s	151.1 s	151.0 s	156.8 s	158.5 s	158.3 s	151.7 s	158.8 s	158.3 s
17	105.6 t	111.1 t	111.3 t	106.2 t	105.7 t	105.8 t	110.4 t	105.2 t	105.7 t
18	29.5 q	29.4 q	28.1 q	29.5 q	29.6 q	28.1 q	28.0 q	28.1 q	28.0 q
19	23.8 q	23.7 q	23.1 q	24.3 q	24.1 q	23.4 q	23.2 q	23.3 q	23.3 q
20	19.0 q	19.2 q	18.9 q	19.4 q	19.4 q	18.9 q	14.9 q	14.6 q	14.8 q
OAc	170.2 s	170.1 s	170.1 s	170.2 s	170.4 s	170.2 s	170.1 s	170.3 s	170.4 s
	21.7 q	21.6 q	170.0 s	21.5 q	169.9 s	170.2 s	170.2 s	170.2 s	170.2 s
			21.6 q		21.4 q	169.8 s	21.6 q	21.7 q	170.0 s
			20.9 q		21.4 q	21.4 q	20.9 q	20.9 q	21.7 q
						21.3 q			21.4 q
						20.9 q			20.9 q

282 (24), 267 (100), 249 (18), 239 (18), 225 916), 213 (18), 197 (16), 185 (14), 171 (16), 152 (33); positive HRESIMS *m/z* 379.2487 [M + H]$^+$ (calcd for $C_{22}H_{35}O_5$, 379.2484).

Melissoidesin N (2): colorless crystals (acetone); mp 248−250 °C; $[\alpha]_D^{20}$ −68.18° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (4.18) nm; IR (KBr) ν_{max} 3460, 2940, 2879, 1735, 1717, 1650, 1645, 1627, 1542, 1473, 1457, 1436, 1390, 1373, 1322, 1243, 1167, 1167, 1072, 1047, 965 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.00 (1H, s, H-17a), 5.78 (1H, t, J = 3.0 Hz, H-6β), 5.25 (1H, s, H-17b), 4.37 (1H, br s, H-11α), 3.53 (1H, br s, H-3α), 3.04 (1H, m, H-13α), 2.71 (1H, d, J = 12.0 Hz, H-14α), 2.50 (1H, dd, J = 15.0, 3.0 Hz, H-7β), 2.23 (1H, br s, H-9β), 2.11 (3H, s, OAc), 2.10 (1H, overlap, H-1β), 2.09 (1H, s, H-5β), 2.08 (2H, overlap, H_2-12), 1.78 (1H, dd, J = 15.0, 3.0 Hz, H-7α), 1.73 (1H, overlap, H-1α), 1.72 (2H, overlap, H_2-2), 1.48 (3H, s, Me-20), 1.34 (1H, dd, J = 12.0, 3.2 Hz, H-14β), 1.23 (3H, s, Me-19), 1.10 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EI MS *m/z* 376 [M]$^+$ (6), 334 (1), 316 [M − AcOH]$^+$ (7), 298 (13), 283 (100), 265 (21), 237 (16), 229 (21), 211 (12), 195 (13), 171 (12), 152 (51); positive HRESIMS *m/z* 377.2383 [M + H]$^+$ (calcd for $C_{22}H_{33}O_5$, 377.2327).

Melissoidesin O (3): colorless crystals (acetone); mp 226−228 °C; $[\alpha]_D^{20}$ −51.52° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (3.83), 201 (3.53) nm; IR (KBr) ν_{max} 3473, 2930, 2869, 1726, 1649, 1439, 1396, 1374, 1260, 1251, 1196, 1045, 1027, 988 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 5.99 (1H, s, H-17a), 5.65 (1H, t, J = 3.0 Hz, H-6β), 5.25 (1H, s, H-17b), 4.71 (H, br s, H-3α), 4.28 (1H, br s, H-11α), 3.02 (1H, m, H-13α), 2.62 (1H, d, J = 12.1 Hz, H-14α), 2.45 (1H, dd, J = 15.0, 3.0 Hz, H-7β), 2.26 (1H, m H-12β), 2.17 (1H, m, H-12α), 2.09 (3H, s, OAc), 1.98 (1H, d, J = 3.0 Hz, H-9β), 1.92 (3H, s, OAc), 1.88 (1H, m, H-2α), 1.73 (1H, dd, J = 15.0, 3.0 Hz, H-7α), 1.68 (1H, br s, H-5β), 1.63 (1H, dd, J = 3.3, 2.5 Hz, H-2β), 1.56 (1H, m, H-1β), 1.42 (1H, m, H-1α), 1.36 (3H, s, Me-20), 1.33 (1H, dd, J = 12.1, 3.0 Hz, H-14β), 1.03 (3H, s, Me-19), 1.00 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EIMS *m/z* 418 [M]$^+$ (3), 358 [M − AcOH]$^+$ (4), 314 (11), 298 [M − 2AcOH]$^+$ (32), 283 (100), 265 (17), 255 (8), 229 (13), 211 (5), 194 (14), 151 (26), 134 (20), 69 (16); HREIMS *m/z* 418.2342 (calcd for $C_{24}H_{34}O_6$, 418.2355).

Melissoidesin P (4): colorless crystals; mp 232−234 °C; $[ga]_D^{20}$ −8.42° (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.47) nm; IR (KBr) ν_{max} 3483, 3389, 2938, 2927, 2871, 1733, 1466, 1449, 1438, 1245, 1082, 1038, 902 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.33 (1H, br s, OH), 6.12 (1H, d, J = 6.4 Hz, OH-15β), 5.97 (1H, s, OH), 5.74 (1H, t, J = 3.5 Hz, H-6β), 5.36 (1H, d, J = 1.0 Hz, H-17a), 5.20 (1H, d, J = 1.0 Hz, H-17b), 4.56 (1H, d, J = 6.4 Hz, H-15α), 4.24 (1H, m, H-11α), 3.83 (1H, d, J = 3.5 Hz, H-7α), 3.61 (1H, m, H-3α), 2.94 (1H, d, J = 3.5 Hz, H-5β), 2.67 (1H, m, H-13α), 2.62 (1H, br s, H-9β), 2.26 (1H, d, J = 12.3 Hz, H-14α), 2.25 (1H, overlap, H-1β), 2.17 (1H, br d, J = 10.2 Hz, H-2α), 2.16 (1H, overlap, H-2β), 2.13 (3H, s, OAc), 1.79 (1H, br d, J = 10.3 Hz, H-1α), 1.41 (3H, s, Me-20), 1.31 (3H, s, Me-19), 1.12 (3H, s, Me-18), 1.09 (1H, br d, J = 12.3 Hz, H-14β); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EIMS *m/z* 394 [M]$^+$ (19), 376 [M − H_2O]$^+$ (1), 358 (12), 334 [M − AcOH]$^+$ (10), 325 (5), 316 (79), 298 (57), 283 (100), 265 (60), 255 (41), 237 (20), 229 (26), 211 (26), 187 (31), 177 (37), 157 (30), 145 (33), 135 (50), 107 (60), 91 (67), 81 (65), 69 (57), 55 (78); HREIMS *m/z* 394.2344 (calcd for $C_{22}H_{34}O_6$, 394.2355).

Melissoidesin Q (5): colorless crystals (acetone); mp 115−118 °C; $[\alpha]_D^{20}$ −52.17° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (4.66) nm; IR (KBr) ν_{max} 3438, 2934, 2873, 2364, 2339, 1743, 1652, 1634, 1372, 1245, 1056, 1040, 984 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.87 (1H, d, J = 3.0 Hz, OH-11β), 6.04 (1H, d, J = 3.5 Hz, OH-3β), 5.80 (1H, d, J = 10.8 Hz, OH-15β), 5.57 (1H, t, J = 3.3 Hz, H-6β), 5.35 (1H, d, J = 3.3 Hz, H-7α), 5.30 (1H, s, H-17a), 5.12 (1H, s, H-17b), 4.57 (1H, d, J = 10.8 Hz, H-15α), 4.31 (1H, m, H-11α), 3.61 (1H, m, H-3α), 2.69 (1H,d, J = 3.3 Hz, H-5β), 2.66 (1H, br s, H-9β), 2.62 (1H, m, H-13α), 2.31 (1H, d, J = 12.1 Hz, H-14α), 2.18 (1H, br d, J = 14.0 Hz, H-1β), 2.14 (3H, s, OAc), 2.11 (2H, m, H_2-12), 2.01 (3H, s, OAc), 1.78 (1H, m, H-1α), 1.75 (2H, m, H_2-2), 1.41 (3H, s, Me-20), 1.31 (3H, s, Me-19), 1.14 (1H, overlap, H-14β), 1.11 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EIMS *m/z* 436 [M]$^+$ (1), 376 [M − AcOH]$^+$ (1), 358 (5), 325 (6), 316 [M − 2AcOH]$^+$ (58), 298 (51), 283 (65), 265 (55), 255 (16), 237 (16), 229 (22), 211 (25), 187 (23), 173 (28), 147 (13), 133 (21), 119 (26), 105 (33), 95 (34), 81 (38), 55 (63), 43 (100); positive HRESIMS *m/z* 437.2529 [M + H]$^+$ (calcd for $C_{24}H_{37}O_7$, 437.2539).

Melissoidesin R (6): colorless crystals (acetone); mp 234−235 °C; $[\alpha]_D^{20}$ −20.62° (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (4.62) nm; IR (KBr) ν_{max} 3429, 2942, 2364, 2339, 1742, 1651, 1632, 1372, 1241, 1223, 1054, 1035 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.92 (1H, d, J = 3.3 Hz, OH-11β), 5.72 (1H, d, J = 10.7 Hz, OH-15β), 5.45 (1H, dd, J = 3.4, 2.3 Hz, H-6β), 5.32 (1H, d, J = 3.4 Hz, H-7α), 5.30 (1H, s, H-17a), 5.12 (1H, s, H-17b), 4.80 (1H, t, J = 2.7 Hz, H-3α), 4.56 (1H, d, J =

10.7 Hz, H-15α), 4.23 (1H, m, H-11α), 2.61 (1H, m, H-13α), 2.54 (1H, br s, H-9β), 2.33 (1H, d, J = 2.3 Hz, H-5β), 2.25 (1H, d, J = 12.2 Hz, H-14α), 2.17 (3H, s, OAc), 2.12 (3H, s, OAc), 2.08 (2H, overlap, H_2-12), 2.00 (3H, s, OAc), 1.82 (1H, overlap, H-1β), 1.73 (2H, m, H_2-2), 1.67 (1H, br d, J = 13.8 Hz, H-1α), 1.32 (3H, s, Me-20), 1.24 (1H, overlap, H-14β), 1.02 (3H, s, Me-19), 1.00 (3H, s, Me-18); EI MS m/z 478 $[M]^+$ (2), 436 (4), 418 $[M - AcOH]^+$ (5), 400 (19), 376 (21), 358 $[M - 2AcOH]^+$ (14), 340 (9), 325 (12), 298 $[M - 3AcOH]^+$ (21), 283 (95), 265 (100), 237 (34), 211 (27), 187 (16), 173 (25), 145 (15); positive HRESIMS m/z 479.2660 $[M + H]^+$ (calcd for $C_{26}H_{39}O_6$, 479.2644).

Melissoidesin S (7): colorless crystals (acetone); mp 238−240 °C; $[\alpha]_D^{20}$ −53.4° (c 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 249 (3.79) nm; IR (KBr) ν_{max} 3537, 3448, 2943, 1736, 1710, 1644, 1474, 1372, 1245, 1166, 1037, 999, 941 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 6.27 (1H, d, J = 5.4 Hz, OH-1α), 6.06 (1H, m, H-11α), 6.00 (1H, s, H-17a), 5.79 (1H, s, OH-11β), 5.70 (1H, t, J = 2.0 Hz, H-6β), 5.24 (1H, s, H-17a), 4.84 (1H, br s, H-3α), 4.08 (1H, m, H-1β), 3.04 (1H, m, H-13α), 2.78 (1H, d, J = 12.0 Hz, H-14α), 2.47 (1H, dd, J = 11.8, 2.0 Hz, H-7β), 2.41 (1H, br s, H-9β), 2.31 (1H, m, H-2β), 2.27 (2H, m, H_2-12), 2.14 (3H, s, OAc), 2.09 (1H, m, H-2α), 1.89 (3H, s, OAc), 1.79 (1H, dd, J = 11.8, 2.0 Hz, H-7α), 1.74 (3H, s, Me-20), 1.72 (1H, s, H-5β), 1.39 (1H, br d, J = 12.0 Hz, H-14β), 1.09 (3H, s, Me-19), 0.98 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EIMS m/z 434 $[M]^+$ (1), 374 $[M - AcOH]^+$ (9), 356 $[M - AcOH - H_2O]^+$ (7), 314 $[M - 2AcOH]^+$ (35), 296 $[M - 2AcOH - H_2O]^+$ (58), 281 (26), 270 (100), 253 (15), 245 (20), 227 (13), 193 (28), 171 (28), 150 (29); HREIMS m/z 434.2314 (calcd for $C_{24}H_{34}O_7$, 434.2305).

Melissoidesin T (8): colorless crystals (acetone); mp 246−248 °C; $[\alpha]_D^{20}$ +19.9° (c 0.23, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (3.47) nm; IR (KBr) ν_{max} 3513, 3414, 2981, 2932, 2873, 1734, 1437, 1374, 1246, 1180, 1117, 1093, 1052, 1035, 997, 978, 947, cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 6.55 (1H, br s, OH), 6.41 (1H, br s, OH), 6.12 (1H, m, H-11α), 6.04 (1H, d, J = 10.4 Hz, OH-15β), 5.69 (1H, t, J = 2.3 Hz, H-6β), 5.39 (1H, s, H-17a), 5.19 (1H, s, H-17b), 4.90 (1H, t, J = 2.9 Hz, H-3α), 4.30 (1H, br d, J = 10.8 Hz, H-1β), 4.03 (1H, d, J = 10.4 Hz, H-15α), 2.68 (1H, m, H-13α), 2.54 (1H, br s, H-9β), 2.40 (1H, overlap, H-14α), 2.38 (1H, m, H-2β), 2.17 (1H, overlap, H-7β), 2.16 (1H, overlap, H-2α), 2.16 (3H, s, OAc), 2.15 (2H, overlap, H_2-12), 1.87 (1H, dd, J = 12.0, 2.3 Hz, H-7α), 1.88 (3H, s, OAc), 1.74 (1H, br s, H-5β), 1.71 (3H, s, Me-20), 1.12 (3H, s, Me-19), 1.11 (1H, overlap, H-14β), 1.01 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EIMS m/z 436 $[M]^+$ (1), 418$[M - H_2O]^+$ (4), 358 $[M - H_2O - AcOH]^+$ (16), 340 (3), 316 $[M - 2AcOH]^+$ (8), 298 (75), 280 (66), 265 (38), 254 (100), 241 (31), 229 (47), 211 (20), 197 (32), 183 (43); HREIMS m/z 436.2465 (calcd for $C_{24}H_{36}O_7$, 436.2461).

Melissoidesin U (9): colorless crystals; mp 96−98 °C; $[\alpha]_D^{20}$ +20.1° (c 0.50, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.60) nm; IR (KBr) ν_{max} 3441, 2933, 2876, 2364, 2339, 1737, 1652, 1372, 1236, 1199, 1180, 1054, 1039, 1003 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 6.13(1H, br s, OH-11β), 6.01 (1H, d, J = 11.7 Hz, OH-15β), 5.61 (1H, m, H-6β), 5.48 (1H, dd, J = 14.2, 5.4 Hz, H-1β), 5.31 (1H, s, H-17a), 5.31 (1H, s, H-17b), 4.97 (1H, m, H-11α), 4.85 (1H, t, J = 3.5 Hz, H-3α), 3.98 (1H, d, J = 11.7 Hz, H-15α), 2.63 (1H, m, H-13α), 2.45 (1H, br s, H-9β) 2.24 (1H, d, J = 15.1 Hz, H-14α), 2.23 (2H, m, H_2-12), 2.15 (3H, s, OAc), 2.11 (1H, overlap, H-7β), 2.03 (3H, s, OAc), 1.93 (3H, s, OAc), 1.83 (2H, m, H_2-2), 1.77 (1H, overlap, H-7α), 1.78 (1H, s, H-5β), 1.64 (3H, s, Me-20), 1.10 (3H, s, Me-19), 1.05 (3H, overlap, H-14β), 0.96 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; EI MS m/z 478 $[M]^+$ (1), 418 $[M - AcOH]^+$ (2), 400 (3), 358 $[M - 2AcOH]^+$ (6), 340 (9), 298 (29), 280 (100), 265 (75), 254 (21), 247 (50), 237 (37), 207 (33), 195 (30), 183 (28), 145 (30), 138 (45), 121 (44); positive HRESIMS m/z 479.2629 $[M + H]^+$ (calcd for $C_{26}H_{39}O_8$, 479.2644).

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