New Terpenoids from *Isodon sculponeata*

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Two new *ent*-kaurane diterpene derivatives, sculponeatins N (1) and O (2), and a new A-ring contracted oleanane triterpenoid, sculponeatic acid (5), were isolated from the plant *Isodon sculponeata*. Their structures were elucidated on the basis of extensive spectroscopic analysis. This is the first report of natural occurrence of an A-ring contracted oleanane triterpenoid.

Key words *Isodon sculponeata*; *ent*-kauranoid; A-ring contracted oleanane triterpenoid

Isodon (synonym: *Rabdosia*, Labiatae family) is a rich source of bioactive *ent*-kaurane diterpene derivatives, and about 500 *ent*-kauranoids have been isolated from the genus up to now.[1)] *Isodon sculponeata* (VANIOT) HARA, a perennial herb, is mainly distributed over southwest China and often used as a medicinal herb to treat dysentery and beriberi in local people.[2)] Previous research on this plant has resulted in the isolation of several 6,7-*seco*-*ent*-kauranoids.[3—9)] As one part of our efforts to study on the chemical constituents of medicinal plants from Yunnan Province (located in the southwest China, one of the biodiversity hotspots of the world), reinvestigation of *I. sculponeata* led to the isolation of two new *ent*-kauranoids, sculponeatins N (**1**) and O (**2**), and a new A-ring contracted oleanane triterpene, sculponeatic acid (**5**), as well as three known terpenoids, sculponeatin A (**3**),[4)] sculponeatin K (**4**),[9)] and hyptadienic acid (**6**).[10)] This paper describes the isolation and structural elucidation of these new compounds.

Results and Discussion

Compound **1**, obtained as colorless needles, has the molecular formula of $C_{25}H_{40}O_4$ based on HR-ESI-MS (pos.), showing a quasi-molecular ion peak at *m*/*z* 427.2829 (Calcd for $C_{25}H_{40}O_4Na$, 427.2824). The IR spectrum showed absorption bands of hydroxyl (3572, 3499, 3463 cm^{-1}), conjugated ester carbonyl (1725 cm^{-1}), and double bond (1645 cm^{-1}) groups. The ^{13}C-NMR (distortionless enhancement by polarization transfer (DEPT)) spectrum (Table 1) exhibited 25 carbon signals, including a set of characteristic resonances at δ_C 166.7 (s), 115.6 (d), 157.6 (s), 27.4 (q), 20.3 (q) attributable to 3-methyl-2-butenoyl group, the remainder were very similar to those of *ent*-kaurane-7α,16β,17-triol.[11)] In the ^1H-NMR spectrum (Table 1), an obvious downfield shift ($\Delta \approx 0.60$ ppm) was observed for the H-17 proton signals. Comparing with those of *ent*-kaurane-7α,16β,17-triol, these indicated that the hydroxyl at C-17 was esterified by the 3-methyl-2-butenoyl group. The above deduction was further confirmed by the following HMBC correlations (Fig. 2): from δ_H 4.21, 4.25 (each d, J=11.4 Hz, H-17) to δ_C 166.7 (s, C-1′). The ROESY correlations between H-7 and H-14α, H-15α, H-17 and H-9, and H-15β as well as characteristic coupling constants of H-7 indicated that the compound possessed the same stereochemistry as *ent*-kaurane-7α,16β,17-triol, confirmed by X-ray analysis.[11)] Therefore the structure of **1** was determined as 17-(3-methyl-2-butenoyl)-*ent*-kaurane-7α,16β-diol, named sculponeatin N.

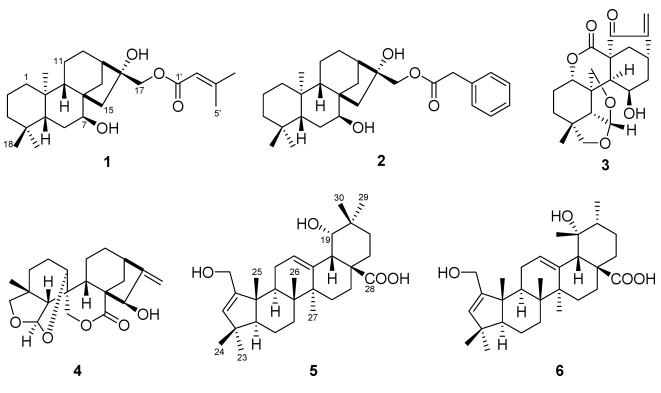

Fig. 1. Structures of Compounds **1**—**6**

Compound **2** was also obtained as colorless needles. Its molecular formula was determined to be $C_{28}H_{40}O_4$ on the basis of HR-ESI-MS (pos.), showing a quasi-molecular ion peak at *m*/*z* 463.2824 (Calcd for $C_{28}H_{40}O_4Na$, 463.2824). The NMR signals (Table 1) were very similar to those of sculponeatin N (**1**), suggesting that **2** was also an *ent*-kaurane diterpenoid. However, there was a prominent difference as follows: the signals assigned to 3-methyl-2-butenoyl moiety in **1** were not present, and there was a set of newly arisen resonances: δ_H 3.67 (2H, s), 7.25—7.33 (5H, m); δ_C 171.6 (s), 41.4 (t), 133.9 (s), 129.2 (2×d), 128.6 (2×d), 127.2 (d), which was easily determined as a phenylacetoxyl unit. In the HMBC spectrum, a significant correlation from δ_H 4.22, 4.25 (each d, J=11.7 Hz, H-17) to δ_C 171.6 (s, C-1′) was observed, indicating that the phenylacetoxyl unit was also linked at C-17 of *ent*-kaurane skeleton. Stereochemically, it was in accordance with sculponeatin N (**1**) by analysis of the ROESY spectrum (Fig. 3). Consequently, the structure of **2** was elucidated as 17-phenylacetoxyl-*ent*-kaurane-7α,16β-diol, named sculponeatin O.

Compound **5**, obtained as colorless powder, had molecular formula $C_{30}H_{46}O_4$ based on HR-ESI-MS (neg.) at *m*/*z* 469.3319 (Calcd for $C_{30}H_{45}O_4$, 469.3317) and ^{13}C-NMR (DEPT) spectrum. The IR spectrum showed absorption bands of hydroxyl (3434 cm^{-1}), carboxyl (1695 cm^{-1}), and double bond (1630 cm^{-1}) groups. The NMR signals (Table 2) were similar to those of hyptadienic acid (**6**)[10)]—an A-ring contracted ursane, and their spectral difference was due to the E-ring. Whereas the ^{13}C-NMR signals assigned to E-

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Table 1. NMR Spectroscopic Data for Compounds **1** and **2** in $CDCl_3$

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	0.80 (m, H_β), 1.74 (m, H_α)	40.0 (t)	0.79 (m, H_β), 1.73 (m, H_α)	40.0 (t)
2	1.41 (m), 1.61 (m)	18.5 (t)	1.41 (m), 1.61 (m)	18.5 (t)
3	1.18 (m, H_β), 1.39 (m, H_α)	41.9 (t)	1.17 (ddd, 13.9, 13.0, 4.5, H_β) 1.39 (m, H_α)	41.9 (t)
4		32.7 (s)		32.6 (s)
5	1.41 (m)	46.1 (d)	1.40 (m)	46.1 (d)
6	1.62—1.69 (m)	27.7 (t)	1.60—1.69 (m)	27.7 (t)
7	3.69 (dd, 2.9, 2.7, H_α)	77.1 (d)	3.67 (m, H_α)	77.0 (d)
8		48.7 (s)		48.7 (s)
9	1.37 (m)	50.9 (d)	1.34 (m)	50.8 (d)
10		39.1 (s)		39.1 (s)
11	1.44 (m), 1.59 (m)	17.7 (t)	1.37 (m), 1.57 (m)	17.6 (t)
12	1.50—1.60 (m)	26.6 (t)	1.41 (m), 1.53 (m)	26.5 (t)
13	2.08 (m)	45.9 (d)	1.96 (m)	45.8 (d)
14	1.67 (m), 1.81 (m)	35.9 (t)	1.63 (m), 1.78 (m)	35.8 (t)
15	1.64 (d, 15.6), 1.80 (d, 15.6)	49.4 (t)	1.57 (m), 1.72 (m)	49.2 (t)
16		80.0 (s)		79.9 (s)
17	4.21 (d, 11.4), 4.25 (d, 11.4)	67.4 (t)	4.22 (d, 11.7), 4.25 (d, 11.7)	68.6 (t)
18	0.84 (s)	33.3 (q)	0.84 (s)	33.3 (q)
19	0.79 (s)	21.5 (q)	0.80 (s)	21.5 (q)
20	1.01 (s)	17.5 (q)	1.00 (s)	17.5 (q)
1′		166.7 (s)		171.6 (s)
2′	5.71 (br s)	115.6 (d)	3.67 (s)	41.4 (t)
3′		157.6 (s)		133.9 (s)
4′	1.90 (br s)	27.4 (q)	7.27 (m)	129.2 (d)
5′	2.16 (br s)	20.3 (q)	7.32 (m)	128.6 (d)
6′			7.26 (m)	127.2 (d)
7′			7.32 (m)	128.6 (d)
8′			7.27 (m)	129.2 (d)

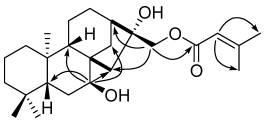

Fig. 2. Key HMBC Correlations of **1**

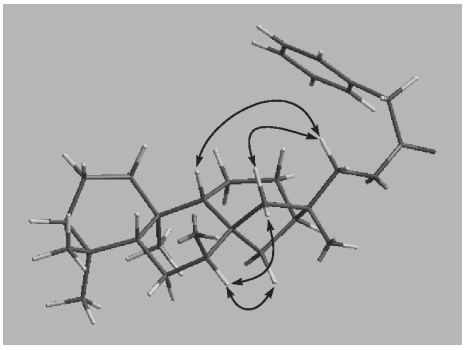

Fig. 3. Important ROESY Correlations of **2**

ring of **5** were in good accordance with those of 19α-hydroxyolean-12-en-28-oic acid analogues, for example, rubiprasin C,[12] so the structure of **5** was established as 2-hydroxymethyl-19α-hydroxy-1-norolean-2,12-dien-28-oic acid, named sculponeatic acid. The deduced structure was also confirmed by the following HMBC correlations (Table 2): from δ_H 3.58 (dd, J=5.6, 3.5 Hz, H-19) to δ_C 145.2 (s, C-13), 46.1 (s, C-17), 29.0 (q, C-29), 24.8 (q, C-30), from δ_H 4.46, 4.53 (each d, J=14.4 Hz, H-1) to δ_C 134.4 (d, C-3), 51.2 (s, C-10). A small (^3J=3.5 Hz) coupling constant between H-18 and H-19 allowed to α orientation of the hydroxyl. To the best of our knowledge, this is the first example of an A-ring contracted oleanane triterpene.

Experimental

General Experimental Procedures Melting points were measured on a PHMK 79/2289 micro-melting point apparatus and uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with a Bruker DRX-500 instrument at room temperature. EI-MS and ESI-MS (including HR-ESI-MS) were measured on Finnigan-MAT 90 and API QSTAR Pulsar i mass spectrometers, respectively. Silica gel (200—300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Medium pressure liquid chromatography (MPLC) was performed on a Büchi Sepacore System equipping pump manager C-615, pump modules C-605, and fraction collector C-660 (Büchi Labortechnik AG, Switzerland), and columns packed with Chromatorex C-18 (40—75 μm, Fuji Silysia Chemical Ltd., Japan). Fractions were monitored by TLC (Qingdao Marine Chemical Inc., China) in combination with reversed-phase HPLC (Agilent 1200, Eclipse XDB-C18 column, 5 μm, 4.6×150 mm).

Plant Material The aerial parts of *I. sculponeata* were collected in Yunnan Province, China and identified by Prof. Dr. Hua Peng. A voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The air-dried powdered aerial parts of *I. sculponeata* (2.5 kg) were extracted with 95% ethanol at room temperature. The alcohol extract was concentrated to give a residue (*ca.* 100 g), which was fractionalized by silica gel column chromatography eluted with a solvent system of petroleum ether (PE)/acetone to yield fractions A—M. Fr. J eluted by 20% acetone was separated on silica gel to obtain a sub-fraction (PE : acetone=100 : 12), which was further isolated and purified by silica gel, Sephadex LH-20 ($CHCl_3$: MeOH=1 : 1), and MPLC (MeOH/H_2O)

Table 2. NMR Spectroscopic Data for Compounds **5** and **6** in C_5D_5N

No.	**5**			**6**
	δ_H	δ_C	HMBC (selected)	δ_C
1	4.46 (d, 14.4), 4.53 (d, 14.4)	60.8 (t)	C-3, C-10	61.0 (t)
2		156.6 (s)		156.8 (s)
3	5.70 (s)	134.4 (d)	C-1, C-5, C-10	133.7 (d)
4		42.6 (s)[a)]		42.6 (s)[b)]
5	1.59 (dd, 11.3, 3.3)	63.9 (d)	C-7, C-23, C-24, C-25	63.8 (d)
6	1.44 (m)	17.7 (t)		17.8 (t)
7	1.36 (m), 1.53 (m)	34.4 (t)		34.7 (t)
8		41.6 (s)		42.1 (s)
9	2.58 (dd, 10.8, 6.7)	44.2 (d)	C-2, C-5, C-14, C-25, C-26	42.5 (d)
10		51.2 (s)		51.0 (s)
11	2.38 (ddd, 18.3, 10.8, 3.8)	27.0 (t)	C-10, C-13	27.0 (t)
	2.50 (ddd, 18.3, 6.7, 3.8)			
12	5.58 (br t, 3.8)	123.7 (d)	C-9, C-14, C-18	128.2 (d)
13		145.2 (s)		140.3 (s)
14		42.5 (s)[a)]		42.5 (s)[b)]
15	1.24 (m), 2.15 (m)	29.6 (t)		29.8 (t)
16	2.11 (m), 2.82 (m)	28.4 (t)	C-28	27.2 (t)
17		46.1 (s)		48.4 (s)
18	3.62 (br d, 3.5)	45.2 (d)	C-12, C-14, C-16, C-28	54.9 (d)
19	3.58 (dd, 5.6, 3.5)	81.1 (d)	C-13, C-17, C-21, C-29, C-30	72.8 (s)
20		35.8 (s)		43.8 (d)
21	1.13 (m), 2.13 (m)	29.1 (t)		26.5 (t)
22	2.01 (m), 2.16 (m)	33.7 (t)	C-20, C-28	38.6 (t)
23	1.04 (s)	30.1 (q)	C-3, C-5, C-24	30.2 (q)
24	0.96 (s)	21.8 (q)	C-3, C-5, C-23	21.9 (q)
25	1.15 (s)	18.9 (q)	C-2, C-5, C-9	18.9 (q)
26	1.11 (s)	19.2 (q)	C-7, C-9, C-14	19.1 (q)
27	1.66 (s)	25.8 (q)	C-8, C-13, C-15	25.5 (q)
28		181.1 (s)		180.9 (s)
29	1.17 (s)	29.0 (q)	C-19, C-21, C-30	27.3 (q)
30	1.08 (s)	24.8 (q)	C-19, C-21, C-29	16.9 (q)
1-OH	6.27 (br s)			
19-OH	6.03 (d, 5.6)			

a, *b*) Interchangeable.

to afford **1** (200 mg), **4** (14 mg), **5** (50 mg, MPLC: 50% MeOH), and **6** (248 mg). Fr. K eluted by 23% acetone was repeatedly isolated and purified by silica gel, Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1), and recrystallization to afford **2** (30 mg) and **3** (287 mg).

Sculponeatin N (**1**): Colorless needles, mp 173 °C, $[\alpha]_D^{20}$ −16.7° (c=0.15, MeOH). UV λ_{max} (MeOH): 218 nm. IR (KBr): 3572, 3499, 3463, 2925, 2866, 1725, 1645, 1220, 1139, 1077 cm^{-1}. NMR spectral data: see Table 1. ROESY correlations: H-17↔H-9β, H-7α↔H-14. EI-MS: 386 $[M-H_2O]^+$ (3), 291 (6), 286 (4), 273 (13), 255 (5), 230 (25), 83 (100). ESI-MS (pos.): 427 $[M+Na]^+$. HR-ESI-MS (pos.): 427.2829 (Calcd for $C_{25}H_{40}O_4Na$, 427.2824).

Sculponeatin O (**2**): Colorless needles, mp 125 °C, $[\alpha]_D^{20}$ −13.3° (c=0.15, MeOH). UV λ_{max} (MeOH): 252, 258, 263 nm. IR (KBr): 3574, 3395, 3285, 2926, 2870, 1706, 1637, 1460, 1369, 1305, 1265, 1140 cm^{-1}. NMR spectral data: see Table 1. ESI-MS (pos.): 463 $[M+Na]^+$. HR-ESI-MS (pos.): 463.2824 (Calcd for $C_{28}H_{40}O_4Na$, 463.2824).

Sculponeatic Acid (**5**): Colorless powder, $[\alpha]_D^{26}$ +80.0° (c=0.10, MeOH). IR (KBr): 3434, 2941, 2865, 1695, 1630, 1457, 1383 cm^{-1}. NMR spectral data: see Table 2. ESI-MS (neg.): 469 $[M-H]^-$. HR-ESI-MS (neg.): 469.3319 (Calcd for $C_{30}H_{45}O_4$, 469.3317).

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