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Sculponins A–C, three new 6,7-*seco*-*ent*-kauranoids from *Isodon sculponeatus*

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Abstract—Three new 6,7-*seco*-*ent*-kauranoids (**1**–**3**) were isolated and structures were elucidated from *Isodon sculponeatus*. Diterpenoids **1**–**3** possessing multicyclic skeletons formed via oxygen atoms are all unprecedented among *ent*-kauranes. Compound **1** displayed significant cytotoxic activity against K562, A549, and HepG2 human tumor cell lines, with IC_{50} values of 1.4, 2.3, and 2.0 μM, respectively, equal to the positive control. Plausible pathways for the biosynthesis of **1** and **2** from one related diterpenoid were also postulated.

As part of our program to search for new diterpenoids from the genus *Isodon* (Labiatae) with promising antitumor activity,[1] *Isodon sculponeatus* (Vaniot) Kudo was selected for investigation. *I. sculponeatus*, a kind of herb, is mainly distributed in southern China.[2] Its stems and leaves have been used in traditional Chinese medicine to treat diarrhea.[3] Previous phytochemical investigations of *I. sculponeatus* collected from Yunnan and Anhui Provinces proved that it was a rich source of bioactive 6,7-*seco*-*ent*-kauranoids.[4–11] Since the metabolites of the genus *Isodon* often exhibit biodiversity attributing to their different ecological environments,[14–19] we further explored *I. sculponeatus* indigenous to Xichang Prefecture, Sichuan Province, looking forward to searching for structurally unique and bioactive *ent*-kauranoid constituents. As a result, three new 6,7-*seco*-*ent*-kauranoids, sculponins A–C (**1**–**3**) were obtained. All compounds were evaluated for cytotoxicity against K562, A549, and HepG2 human tumor cell lines, of which compound **1** showed significant cytotoxic activity, with IC_{50} values of 1.4, 2.3, and 2.0 μM, respectively, in agreement with the positive control.

Sculponin A (**1**) exhibited a quasi-molecular ion peak at *m*/*z* 381.1301 $[M+Na]^+$ in its HRESIMS, corresponding to $C_{20}H_{22}O_6$, establishing ten degrees of unsaturation.[20] Examination of the ^1H, ^{13}C, and DEPT-135 NMR spectra showed the presence of a partial structure of a carbonyl group conjugated with an exomethylene, one lactonic carbonyl group, one singlet methyl, five methylenes (one of which was oxygenated), seven methines (including four oxygenated ones), and three quaternary carbons (Tables 1 and 2). These data indicated a skeleton of 6,7-*seco*-1,7-olide-*ent*-kauranoid, partially similar to nodosin.[12,13] Twenty-two protons were all attached to corresponding carbons, indicating the absence of free hydroxyl groups. Importantly, the presence of seven rings was required to satisfy the degrees of unsaturation. Thus, there must be two more rings in **1** than in nodosin. In the HMBC spectrum, H-11 correlated to C-6, H-6 correlated to C-18 (or C-19), and H-18 (or H-19) correlated to C-20, suggesting three rings were formed by three oxygen atoms between C-6 and C-11, C-6 and C-18 (or C-19), C-18 (or C-19) and C-20, respectively (Fig. 1A). The ROESY correlations

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Table 1. ^{1}H NMR data of compounds **1**–**3** in C_5D_5N (400 MHz)[a]

No.	**1**[b]	**2**	**3**
1β	4.60 (dd, 12.0, 4.8)	5.59 (t, 8.6)	5.88 (dd, 10.6, 6.4)
2α	2.28 (m)	1.86 (2H, m)	2.68 (m)
2β	1.90 (m)		2.12 (m)
3α	1.84 (dd, 15.0, 6.5)	1.32 (d, 14.0)	1.56 (overlap)
3β	1.48 (m)	1.48 (m)	1.35 (m)
5β	2.60 (d, 3.0)	2.75 (br s)	3.00 (d, 6.4)
6	5.89 (d, 3.0)	5.78 (br s)	10.46 (d, 6.4)
9	2.01 (d, 9.0)	3.22 (d, 4.0)	3.35 (overlap)
11	4.35 (dd, 10.0, 9.0)	5.16 (m)	5.29 (m)
12α	2.14 (2H, m)		3.49 (t, 10.8)
12β		4.69 (dd, 9.2, 2.8)	2.00 (m)
13	3.08 (br s)	3.25 (m)	2.93 (br s)
14α	2.04 (dd, 12.5, 5.0)	2.36 (dd, 12.0, 3.8)	3.35 (overlap)
14β	2.57 (d, 12.5)	3.82 (d, 12.0)	1.56 (overlap)
15α			5.82 (br s)
16β		2.93 (t, 6.0)	
17a	6.11 (br s)	4.22 (d, 8.4)	5.23 (br s)
17b	5.37 (br s)	3.75 (dd, 8.4, 5.6)	5.07 (br s)
18	0.86 (3H, s)	0.92 (3H, s)	0.59 (3H, s)
19a	5.07 (s)	0.98 (3H, s)	4.20 (d, 12.0)
19b			3.65 (d, 12.0)
20a	3.83 (d, 12.0)	4.38 (d, 9.2)	6.67 (s)
20b	3.77 (d, 12.0)	4.32 (d, 9.2)	

[a] Assignments were based on HSQC, HMBC, and ROESY experiments.
[b] 500 MHz.

Table 2. ^{13}C NMR data of compounds **1**–**3** in C_5D_5N (100 MHz)[a]

Position	**1**[b]	**2**	**3**
1	78.5 d	79.2 d	68.0 d
2	25.4 t	24.1 t	32.8 t
3	29.8 t	37.5 t	39.0 t
4	42.2 s	31.5 s	33.3 s
5	46.0 d	55.9 d	63.7 d
6	99.8 d	102.2 d	205.4 d
7	170.3 s	171.7 s	174.7 s
8	57.8 s	56.6 s	53.9 s
9	39.4 d	46.4 d	44.1 d
10	34.3 s	49.7 s	45.3 s
11	66.2 d	69.5 d	66.7 d
12	36.9 t	81.8 d	40.8 t
13	36.5 d	39.8 d	42.8 d
14	33.0 t	32.8 t	32.1 t
15	198.4 s	215.3 s	78.0 d
16	146.1 s	54.1 d	157.8 s
17	118.9 t	71.8 t	104.7 t
18	22.3 q	33.0 q	23.7 q
19	105.9 d	23.4 q	69.0 t
20	65.8 t	75.0 t	96.6 d

[a] Assignments were based on HSQC and HMBC experiments.
[b] 125 MHz.

of H-6 to H-5β, H-12β, and Me-18, H-11 to H-9α, and H-19 to H-3α revealed the stereochemistry of H-1β, H-6β, H-11α, and H-19β (Fig. 1B). Consequently, compound **1** was determined to be 6,11;6,19;19,20-triepoxy-6,7-*seco*-*ent*-kaur-16-en-15-one-1β,7-olide.

Sculponin B (**2**), isolated as colorless needles, has a molecular formula $C_{20}H_{26}O_7$ from its HRESIMS, implying eight sites of unsaturation.[21] Its NMR data suggested that compound **2** was also a 6,7-*seco*-1,7-olide-*ent*-kauranoid, similar to nodosin. Comparison of its NMR data (Tables 1 and 2) with those of nodosin revealed that an exomethylene and a methylene in nodosin were replaced by an oxygenated methine, an oxygenated methylene, and a methine in **2**. In combination with the unsaturation degrees in **2**, one additional ring should be formed between the above mentioned oxygenated methine and the oxygenated methylene. The HMBC correlations (Fig. 2A) observed from H-11 to C-9, C-12, and C-13, from H-12 to C-17, from H-16 to C-12, C-13, C-15, and C-17, and from H-17 to C-12, disclosed a tetrahydrofuran ring formation among C-12, 13, 16, and 17. Thus, a planar structure of **2** was elucidated. The ROESY spectrum displayed correlations (Fig. 2B) between H-16 and H-13β, H-13β and H-12β, reflecting the α-orientation of the tetrahydrofuran ring. Since compound **2** is an *ent*-kauranoid on biogenetic grounds,[1] the configuration of C-16 was determined as *S*. Therefore, the structure, 16(*S*)-6β, 11β-dihydroxy-6,20;12,17-diepoxy-6,7-*seco*-*ent*-kaur-15-one-1β,7-olide, was assigned to **2**.

The molecular formula of diterpenoid **3** was determined to be $C_{20}H_{26}O_7$ by HRESIMS, corresponding to eight degrees of unsaturation.[22] IR absorptions at 3433, 2878, 1710, and 1636 cm^{-1} implied the existence of hydroxyl groups, aldehyde, carbonyl, and olefinic groups. The ^{1}H NMR spectrum indicated the presence of one angular methyl, one aldehyde, six oxygenated, and two olefinic protons (Table 1). The ^{13}C NMR spectrum revealed 20 carbon signals due to one methyl at δ_C 23.7, five methylenes (one of which was oxygenated at δ_C 69.0) in the aliphatic region, eight methines (including an aldehyde carbon at δ_C 205.4, a hemiketal carbon at δ_C 96.6, and three other oxygenated carbons at δ_C 78.0, 68.0, 66.7), four quaternary carbons (including a

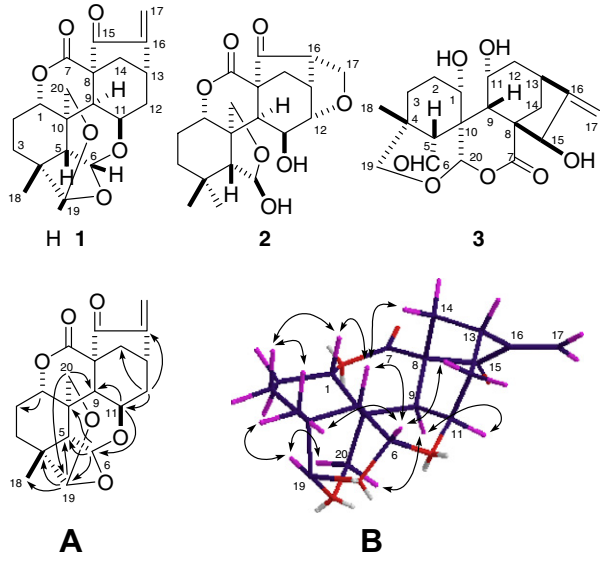


Figure 1. Key HMBC (A) and ROESY (B) correlations of **1**.

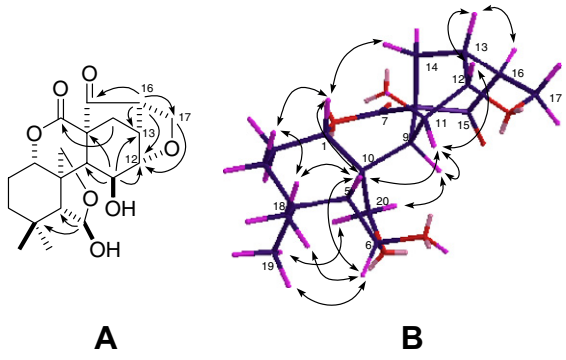


Figure 2. Key HMBC (A) and ROESY (B) correlations of **2**.

lactonic carbonyl carbon at δ_C 174.7), and one 1,1-disubstituted double bond at δ_C 157.8 and 104.7 (Table 2). The above information suggested that compound **3** is a 6,7-*seco*-7,20-olide-*ent*-kauranoid possessing one more

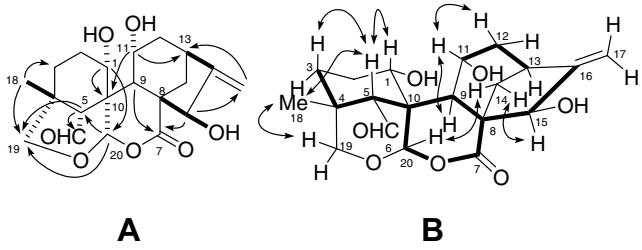
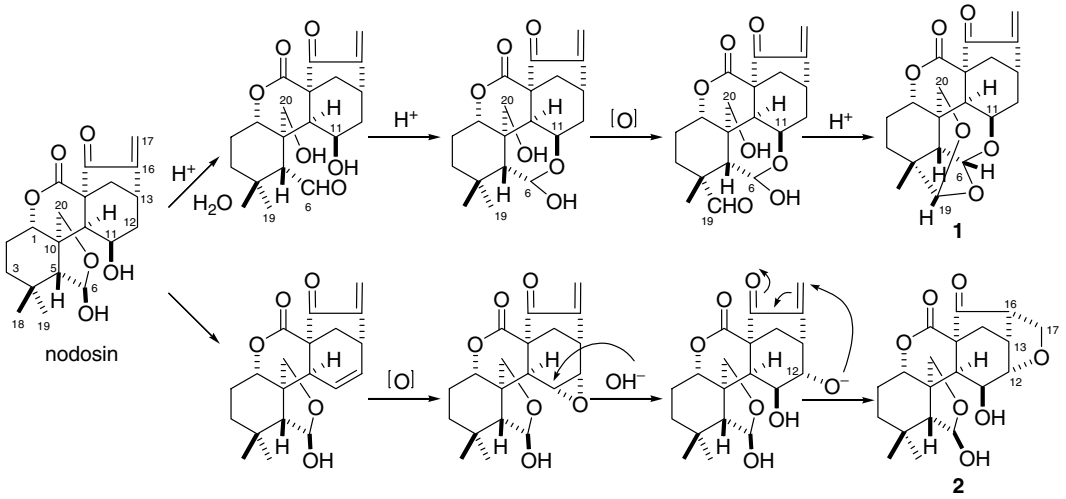


Figure 3. Key HMBC (A) and ROESY (B) correlations of **3**.

ring than classical ones. In the HMBC spectrum, H-20 correlating to C-5, C-10, and C-19, and H-5 correlating to C-3, C-6, C-10, and C-19, revealed that the aldehyde group was attached to C-6, and C-19 with C-20 was cyclized through an oxygen atom. In addition, the hydroxyls were assigned at C-1, C-11, and C-15 by the following key HMBC correlations: from H-1 to C-10 and C-20; from H-11 to C-9, C-10, C-12, and C-13; and from H-15 to C-7, C-8, C-9, C-16, and C-17 (Fig. 3A). The relative stereochemistry was determined by the ROESY spectrum, in which H-5β showed correlation to H-1β, H-3β, and Me-18, H-11β showed correlation to H-9β and H-12β, and H-15α showed correlations to H-14α, disclosing the α, α, and β-orientation of the hydroxyl groups attached to C-1, C-11, and C-15, respectively (Fig. 3B). Therefore, the structure of compound **3** was elucidated as 1α,11α,15β-trihydroxy-19,20-epoxy-6,7-*seco*-*ent*-kaur-16-en-6-al-7,20-olide.

Although the absolute stereochemistry of diterpenoids **1**–**3** was not established by spectroscopic methods, they are presumed to be *ent*-kaurane-type diterpenoids in consideration of the presence of *ent*-kaurane type diterpenoid as characteristic components in the genus *Isodon*.[1]

Plausible biosynthetic pathways for **1** and **2** from nodosin were outlined in Scheme 1. Compound **1** was formed through intramolecular nucleophilic addition and oxidation reactions after nodosin released the masked aldehyde group at C-6 under an acid condition.



Scheme 1. Hypothetical biogenetic pathways proposed for compounds **1** and **2**.

Compound **2** was likely to be converted from nodosin through oxidation and Michael addition reactions.

Compounds **1**–**3** represent a 6,11;6,19;19,20-triepoxy-6,7-*seco*-1,7-olide-*ent*-kaurane, a 6,20;12,17-diepoxy-6,7-*seco*-1,7-olide-*ent*-kaurane, and a 19,20-epoxy-6,7-*seco*-7,20-olide-*ent*-kaurane skeletons, respectively. To the best of our knowledge, the current study is the first account of such natural products in the possession of multicyclic systems containing oxygen atoms among *ent*-kauranes. Undoubtedly, three new compounds expand the functionalities of the *ent*-karanes considerably.

The cytotoxicity of compounds **1**–**3** was tested against K562 (chronic myelogenous leukemia), A549 (lung cancer), and HepG2 (hepatocellular carcinoma) human cell lines using the method described in the literature,[23] with cis-platinum as the positive control ($IC_{50} = 1.1$, 3.8, and 1.3 μM, respectively). Compound **1** showed significant activity against these cell lines, with IC_{50} values of 1.4, 2.3, and 2.0 μM, respectively, at the same level as cis-platinum. On the contrary, compounds **2** and **3** were noncytotoxic in these tested systems ($IC_{50} > 100$ μM). The above results suggest that the cyclopentanone conjugated with an exomethylene group is the active center.[24]

Acknowledgments

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20. Sculponin A (**1**): colorless needles, mp 278–280 °C; $[\alpha]_D^{13}$ −73.0 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε): 235 (3.78) nm; IR (KBr) ν_{max} 2950, 2899, 1766, 1721, 1640, 1462, 1386, 1270, 1209, 1112, 1096, 1054, 1023, 978, 932, 905 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; positive ESIMS: *m/z* 381 $[M+Na]^+$, 739 $[2M+Na]^+$; positive HRESIMS $[M+Na]^+$ *m/z* 381.1301 (calcd for $C_{20}H_{22}O_6Na$ $[M+Na]^+$, 381.1314).
21. Sculponin B (**2**): colorless needles, mp 257–259 °C; $[\alpha]_D^{13}$ −182.2 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 205 (3.04) nm; IR (KBr) ν_{max} 3516, 3438, 2944, 2884, 1747, 1704, 1458, 1353, 1340, 1320, 1285, 1256, 1224, 1166, 1093, 1070, 1050, 1019, 1004, 960, 916, 897, 878, 558 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; positive ESIMS: *m/z* 401 $[M+Na]^+$, 779 $[2M+Na]^+$; positive HRESIMS $[M+Na]^+$ *m/z* 401.1587 (calcd for $C_{20}H_{26}O_7Na$ $[M+Na]^+$, 401.1576).
22. Sculponin C (**3**): white powder; $[\alpha]_D^{13}$ +24.1 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε): 205 (3.79) nm; IR (KBr) ν_{max} 3433, 2932, 2878, 1710, 1636, 1466, 1387, 1268, 1244, 1226, 1160, 1125, 1089, 1063, 1038, 1011, 987, 964, 912, 893 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; positive ESIMS: *m/z* 401 $[M+Na]^+$, 779 $[2M+Na]^+$; positive HRESIMS $[M+Na]^+$ *m/z* 401.1589 (calcd for $C_{20}H_{26}O_7Na$ $[M+Na]^+$, 401.1576).
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