6,7-*seco*-*ent*-Kaurane Diterpenoids from *Isodon sculponeatus* with Cytotoxic Activity

by **Xian Li**[a])[b]), **Jian-Xin Pu**[a]), **Zhi-Ying Weng**[a])[b]), **Yu Zhao**[a]), **Yong Zhao**[a]), **Wei-Lie Xiao**[a]), and **Han-Dong Sun***[a])

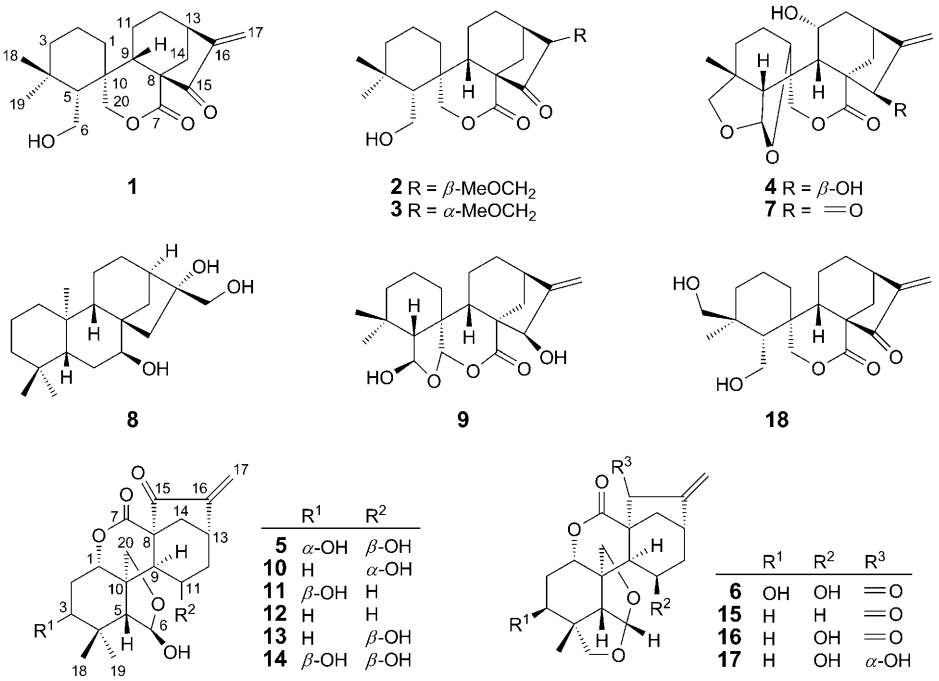
[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China
(phone: +86-871-5223251; fax: +86-871-5216343; e-mail: hdsun@mail.kib.ac.cn)
[b]) School of Pharmacy, Kunming Medical College; Kunming 650031, P. R. China

Six new 6,7-*seco*-*ent*-kaurane diterpenoids, sculponeatins N–S (**1**–**6**, resp.), together with eleven known analogues, **7**–**17**, were isolated from the aerial parts of *Isodon sculponeatus*. The structures of compounds **1**–**6** were elucidated by spectroscopic methods including extensive 1D- and 2D-NMR experiments, as well as HR-ESI-MS analysis. All diterpenoids obtained were assayed for their cytotoxic activity against K562 and HepG2 human tumor cell lines. Among them, compound **1** showed the most significant cytotoxicity with the IC_{50} values of 0.21 and 0.29 μM, respectively. The structure–activity relationships are discussed.

Introduction. – Enmein, the active *ent*-kaurane diterpenoid, was first isolated from the Japanese folk medicine '*enmeiso*' [1]. Later, *ca.* 800 new diterpenoids have been isolated and characterized from more than 80 *Isodon* species, indicating that *ent*-kauranoids are the major secondary metabolites from *Isodon* plants. Most of these diterpenoids have been assayed and shown to exhibit antitumor, antibacterial, and anti-inflammatory activities with very low toxicity [2].

Isodon sculponeatus (Vaniot) Kudo, a perennial herb, is distributed mainly in the southwest of China. Its stems and leaves have been used as treatment of dysentery and as anti-inflammatory agent. Our previous phytochemical investigations on this plant led to the isolation of a series of *ent*-kauranoids, which exhibited significant cytotoxic activity against the K562 cell line. Especially, sculponeatin C (**7**) with its complex ring system showed a marked increase of antitumor activity according to the *in vivo* tests of bioactivity [3–9]. Recently, pharmaceutical studies demonstrated that several *ent*-kaurane diterpenoids have potent anti-angiogenic activity and inhibit NF-κB transcription [10]. With the aim to further systematically access to the chemical and biological diversity of this plant, the present study on *I. sculponeatus*, which was collected in Lijiang County of Yunnan Province, China, afforded six new 6,7-*seco*-*ent*-kaurane diterpenoids, sculponeatins N–S (**1**–**6**, resp.), along with eleven known analogues, *i.e.*, sculponeatin C (**7**) [3], diol **8** [11], macrocalyxoformin E (**9**) [12], epinodosin (**10**) [13], enmein (**11**) [1], isodocarpin (**12**) [14], nodosin (**13**) [15], longirabdolide C (**14**) [16], macrocalyxoformin B (**15**) [12], and sculponeatins A and B (**16** and **17**, resp.) [3]. Here, we describe the isolation and structure elucidation of six

new diterpenoids, sculponeatins N–S (**1**–**6**, resp.), and their cytotoxic activities against K562 and HepG2 human tumor cell lines.



Results and Discussion. – Sculponeatin N (**1**), a white amorphous powder, has the molecular formula of $C_{20}H_{28}O_4$ as deduced from the HR-ESI mass spectrum (*m/z* 355.1894 ([M+Na]$^+$)), implying seven degrees of unsaturation. The ^{1}H-NMR spectrum showed signals for two tertiary Me groups (δ(H) 1.02 (*s*, Me(18)) and 0.84 (*s*, Me(19))). The downfield shift of the signal of olefinic quaternary C-atom (δ(C) 151.4 (C(16))) and the upfield shift of that of the olefinic CH_2 (δ(C) 117.5 (C(17))), coupled with the C=O C-atom signal (δ(C) 203.3 (C(15))) in the ^{13}C-NMR spectrum (*Table 1*), along with the characteristic absorption bands (UV (MeOH): 230 (4.01) nm. IR (KBr): 1701 and 1643 cm^{-1}) in the UV and IR spectra, indicated the presence of an α,β-unsaturated C=O group in **1**. The above data, corroborated with the 20 C-atom signals in the NMR spectra, suggested that **1** was an *ent*-kaurane diterpenoid.

In addition, on the basis of the characteristic signals of three CH groups (δ(C) 51.6 (C(5)), 45.0 (C(9)), and 35.5 (C(13))), three quaternary C-atoms (δ(C) 58.3 (C(8)), 42.3 (C(10)), and 34.1 (C(4))), and two oxygenated CH_2 C-atoms (δ(C) 70.6 (C(20)) and 58.4 (C(6))), together with the consideration on the structures of diterpenoids previously isolated from this genus, we assumed that **1** should be a 6,7-*seco*-*ent*-kaurane-7,20-olide compound, similar to isolongirabdiol [17]. Careful analysis of the NMR spectral data of **1** and comparison with those of isolongirabdiol revealed great similarities, except for the moiety at C(18). Observation of a signal of a Me C-atom and the absence of that of a OCH_2 C-atom in the NMR spectra in **1** indicated a Me group at C(18) for **1** instead of a CH_2OH group in the same position in isolongirabdiol. The

Table 1. *^{13}C-NMR Data of Compounds* **1**–**6** *and* **18** (100 MHz, (D_5)pyridine, δ in ppm)[a])

Position	**1**	**2**	**3**	**4**	**5**	**6**	**18**
1	27.7 (*t*)	27.5 (*t*)	27.8 (*t*)	78.2 (*d*)	77.2 (*d*)	75.6 (*d*)	27.9 (*t*)
2	18.1 (*t*)	18.1 (*t*)	18.1 (*t*)	30.1 (*t*)	33.1 (*t*)	31.6 (*t*)	17.6 (*t*)
3	42.6 (*t*)	42.7 (*t*)	42.7 (*t*)	30.6 (*t*)	73.1 (*d*)	68.4 (*d*)	36.7 (*t*)
4	34.1 (*s*)	34.1 (*s*)	34.1 (*s*)	41.5 (*s*)	38.1 (*s*)	47.9 (*s*)	38.9 (*s*)
5	51.6 (*d*)	51.3 (*d*)	51.7 (*d*)	52.8 (*d*)	55.6 (*d*)	52.7 (*d*)	44.8 (*d*)
6	58.4 (*t*)	58.5 (*t*)	58.6 (*t*)	107.8 (*d*)	102.1 (*d*)	112.0 (*d*)	58.0 (*t*)
7	171.5 (*s*)	171.4 (*s*)	171.3 (*s*)	176.0 (*s*)	172.0 (*s*)	171.9 (*s*)	171.3 (*s*)
8	58.3 (*s*)	58.8 (*s*)	58.9 (*s*)	51.4 (*s*)	56.6 (*s*)	56.4 (*s*)	58.2 (*s*)
9	45.0 (*d*)	44.1 (*d*)	44.4 (*d*)	35.8 (*d*)	48.6 (*d*)	46.3 (*d*)	58.2 (*d*)
10	42.3 (*s*)	41.7 (*s*)	42.1 (*s*)	51.6 (*s*)	50.5 (*s*)	50.2 (*s*)	42.1 (*s*)
11	17.6 (*t*)	17.1 (*t*)	17.7 (*t*)	65.8 (*d*)	66.6 (*t*)	65.5 (*d*)	17.5 (*t*)
12	30.0 (*t*)	20.1 (*t*)	29.9 (*t*)	45.4 (*t*)	41.4 (*t*)	41.0 (*t*)	29.9 (*t*)
13	35.5 (*d*)	30.9 (*d*)	32.0 (*d*)	37.5 (*d*)	35.5 (*d*)	35.2 (*d*)	35.4 (*d*)
14	29.8 (*t*)	32.2 (*t*)	30.3 (*t*)	33.1 (*t*)	34.2 (*t*)	34.2 (*t*)	29.8 (*t*)
15	203.3 (*s*)	214.7 (*s*)	214.4 (*s*)	80.4 (*d*)	201.1 (*s*)	200.8 (*s*)	203.2 (*s*)
16	151.4 (*s*)	54.8 (*d*)	57.5 (*d*)	160.0 (*s*)	151.1 (*s*)	150.8 (*s*)	151.3 (*s*)
17	117.5 (*t*)	69.2 (*t*)	72.1 (*t*)	108.0 (*t*)	117.6 (*t*)	118.2 (*t*)	117.4 (*t*)
18	34.3 (*q*)	34.3 (*q*)	34.3 (*q*)	29.1 (*q*)	29.8 (*q*)	26.4 (*q*)	71.7 (*t*)
19	23.6 (*q*)	23.6 (*q*)	23.6 (*q*)	83.0 (*t*)	16.2 (*q*)	78.2 (*t*)	19.9 (*q*)
20	70.6 (*t*)	71.1 (*t*)	70.5 (*t*)	72.4 (*t*)	74.2 (*t*)	73.1 (*t*)	71.1 (*t*)
MeO		58.5 (*q*)	58.7 (*q*)				

[a]) The assignments were based on DEPT, HMQC, and HMBC experiments.

HMBC correlations of Me(18) with C(3), C(5), and C(19) further supported the above assignments. The relative configuration of **1** was determined by a ROESY experiment, in which correlations from H–C(5) to Me(18) confirmed the β-orientation of H–C(5). Therefore, structure of compound **1** was elucidated as 6-hydroxy-15-oxo-6,7-*seco-ent*-kaur-16-en-7,20-olide, the absolute configuration being assumed for chemotaxonomic reasons.

Sculponeatin O (**2**) was isolated as white amorphous powder, whose molecular formula was inferred as $C_{21}H_{32}O_5$ by HR-ESI-MS (*m/z* 387.2151 ($[M+Na]^+$)) and NMR data, implying six degrees of unsaturation. Its UV and IR spectra did not show α,β-unsaturated C=O group absorption. The ^{1}H- and ^{13}C-NMR data of **2** were very similar to those of **1** except for the signals corresponding to the *D*-ring atoms. One CH (δ(C) 54.8 (*d*), δ(H) 2.97–2.99 (*m*, 1 H), attributable to C(16)/H_α–C(16)) and a $MeOCH_2$ group (δ(H) 3.15 (MeO), 3.66 (*dd*, J = 10.0, 4.7, 1 H), and 3.61–3.63 (*m*, 1 H) (CH_2(17))) in **2** replaced the *exo*-CH_2 group in **1**. The ROESY correlations (*Fig. 1*) from H–C(16) to H_α–C(13), along with the upfield shift of δ(C) 20.1 (C(12)) in **2** compared with that of δ(C) 30.0 (C(12)) in **1**, due to the δ-*syn*-axial effect between the MeO–C(17) and H_β–C(12), confirmed the β-orientation of the $MeOCH_2$ group at C(16) in **2**. Thus, compound **2** was determined to be (16*R*)-6-hydroxy-16β-(methoxymethyl)-15-oxo-6,7-*seco-ent*-kauran-7,20-olide.

Sculponeatin P (**3**) had the molecular formula $C_{21}H_{32}O_5$ as deduced from HR-ESI spectrum (*m/z* 387.2145 ($[M+Na]^+$)). The UV, IR, and NMR data indicated that **3** was

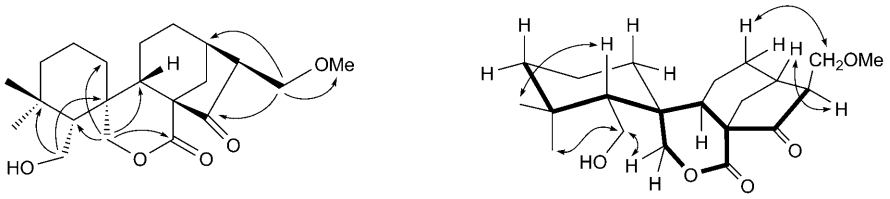


Fig. 1. *Selected HMBC* (H→C) *and ROESY* (H↔H) *correlations of sculponeatin O* (**2**)

remarkably similar to **2** except for the configuration of the substituent at C(16). The configuration of the $MeOCH_2$ group at C(16) in **3** was deduced to be α on the basis of the ROESY cross-peaks from H−C(16) to H_β−C(12). Therefore, **3** was identified as (16*S*)-6-hydroxy-16α-(methoxymethyl)-15-oxo-6,7-*seco-ent*-kauran-7,20-olide.

Sculponeatin Q (**4**), isolated as a white amorphous powder, had the molecular formula $C_{20}H_{26}O_6$ deduced from the positive-ion HR-ESI mass spectrum (*m*/*z* 385.1632 ($[M+Na]^+$)). On the basis of the characteristic signals of a lactone C=O C-atom for C(7) (δ(C) 176.0) and a noticeable acetal for C(6)/H−C(6) (δ(C) 107.8 (*d*) and δ(H) 5.89 (*d*, J=3.4)), compound **4** was inferred to be a 6,1α:6,19-diepoxy-6,7-*seco-ent*-kaurane diterpenoid, similar to sculponeatin C (**7**), a known analog also isolated from this plant. Comparison of the spectroscopic data of **4** with those of **7** revealed that they were closely similar except that the substituent at C(15) in **4** was a OH group, which was confirmed by the HMBC results. Further 2D-NMR experiments identified **4** as 6,1α:6,19-diepoxy-11α,15β-dihydroxy-6,7-*seco-ent*-kaur-16-en-7,20-olide.

Sculponeatin R (**5**) was assigned the molecular formula $C_{20}H_{26}O_7$, as deduced from the positive-ion HR-ESI mass spectrum (*m*/*z* 401.1571 ($[M+Na]^+$)). Its NMR spectroscopic data suggested **5** to be an 6,20-epoxy-15-oxo-6,7-*seco-ent*-kaur-16-en-1α,7-olide diterpenoid, with a lactone C=O group, a hemiketal CH C-atom, and a noticeable oxygenated CH_2 group (δ(C) 74.2 (*t*), δ(H) 4.62 and 4.45 (*d*, J=9.0, each 1 H) attributable to C(20)/CH_2(20)). The MS and NMR data indicated that **5** was very similar to longirabdolide C (**14**), except for the α-orientation of the HO group at C(3) in **5**. This was supported by the cross-peaks of H_β−C(3) with H_β−C(5) and Me(18) in its ROESY spectrum. It was further confirmed by the abnormal upfield shift of δ(C) 16.2 (Me(19)) in **5** compared with that of **14** δ(C) 23.2 (Me(19)) due to the γ-*gauche* steric-compression effect between HO_α−C(3) and Me(19). The other substituents had the same orientations as those of **14** on the basis of the ROESY results. Thus,

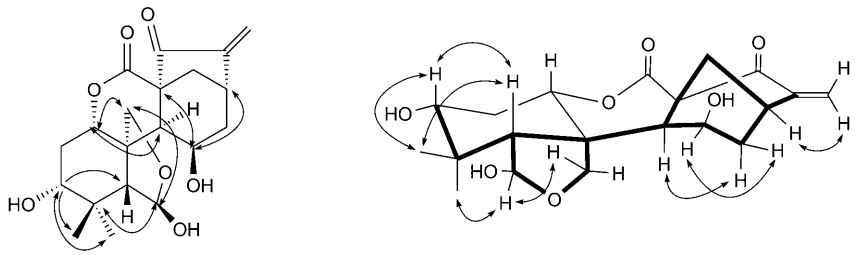


Fig. 2. *Selected HMBC* (H→C) *and ROESY* (H↔H) *correlations of sculponeatin R* (**5**)

compound **5** was determined as 6,20-epoxy-3α,6β,11β-trihydroxy-15-oxo-6,7-*seco-ent*-kaur-16-en-1α,7-olide.

Sculponeatin S (**6**), a white amorphous powder, was recognized as a 6,20:6,19-diepoxy-6,7-*seco-ent*-kauran-1α,7-olid by the analysis of its 1D-NMR spectra, with the molecular formula $C_{20}H_{24}O_7$ determined by the HR-ESI-MS data (*m/z* 399.1428 ([M+Na]$^+$)). By comparison of the ^{13}C-NMR data of **6** with those of sculponeatin A (**16**), a known 6,20:6,19-diepoxy-6,7-*seco-ent*-kauran-1α,7-olide obtained at the same time, and the only observed difference was that **6** had one additional OH group. The OH group was at C(3) in **6** on the basis of the HMBC correlations. The OH group at C(3) was β-oriented, unambiguously revealed by the significant upfield shift of δ(C) 26.4 (Me(18)) in **6** compared with that of δ(C) 30.0 (Me(18)) in **16** due to the γ-*gauche* steric-compression effect between the HO−C(3) and Me(18). Further 2D-NMR analysis confirmed that the configurations of remaining substituents in **6** were the same as those in **16**. Thus, **6** was determined as 6,20:6,19-diepoxy-3β,11β-dihydroxy-15-oxo-6,7-*seco-ent*-kaur-16-en-1α,7-olide.

The cytotoxicities of compounds were assayed against K562 and HepG2 human tumor cell lines using the method described in [18], with cisplatinum as the positive control (IC_{50} 1.33 and 0.38 μM, resp.; *Table 2*). Sculponeatin N (**1**) was the most active against the two human tumor cells mentioned above, with IC_{50} values of 0.21 and 0.29 μM, respectively. Due to the lack of an α,β-unsaturated ketone group, sculponeatin P (**3**), sculponeatin Q (**4**), diol **8**, macrocalyxoformin E (**9**), and sculponeatin B (**17**) were not cytotoxic against two tumor cell lines (IC_{50} > 100 μM). However, the cytotoxicity of sculponeatin O (**2**; IC_{50} values 0.34 and 0.39 μM, resp.) was observed, though an α,β-unsaturated ketone group was absent in **2**. These results suggest that an α,β-unsaturated ketone group is the active center, but the substituents in the molecule also influence the cytotoxicity.

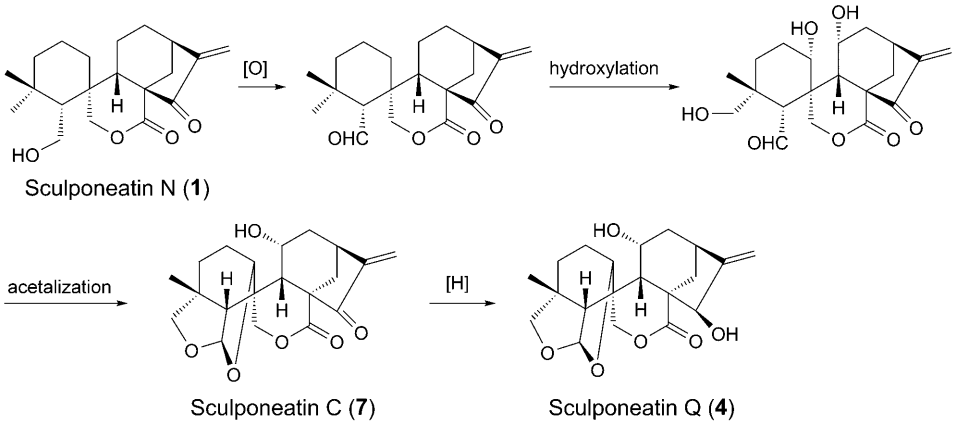
Table 2. *Cytotoxicity Data of Compounds* **1**–**17** (IC_{50} [μM])

Compound	K562	HepG2	Compound	K562	HepG2
1	0.21 ± 0.03	0.29 ± 0.03	**10**	0.32 ± 0.04	0.45 ± 0.05
2	0.34 ± 0.04	0.39 ± 0.05	**11**	0.59 ± 0.06	22.09 ± 0.89
3	> 100	> 100	**12**	1.02 ± 0.08	2.43 ± 0.21
4	> 100	> 100	**13**	0.58 ± 0.06	2.55 ± 0.23
5	2.63 ± 0.21	20.46 ± 0.65	**14**	3.98 ± 0.20	> 100
6	> 100	> 100	**15**	0.89 ± 0.06	20.89 ± 0.65
7	0.29 ± 0.04	0.33 ± 0.03	**16**	0.23 ± 0.03	0.31 ± 0.04
8	> 100	> 100	**17**	> 100	> 100
9	> 100	> 100	Cisplatin	1.33 ± 0.10	0.38 ± 0.03

Conclusions. – *ent*-Kauranoids are the major metabolites isolated from *Isodon* (Labiatae) plants. Most of these diterpenoids show antitumor, anti-inflammatory, antibacterial, and anti-angiogenic activities. In this phytochemical investigation of the aerial parts of *I. sculponeatus*, we found that diterpenes isolated from this plant are classified into two groups (*C(20)*-non-oxygenated *ent*-kauranes and 6,7-*seco-ent*-kauranes) including two subgroups, enmein type and spiro-lactone type of 6,7-*seco-ent*-

kaurane [19]. Especially, sculponeatin R and C (**4** and **7**, resp.) contained a complex ring system with two additional tetrahydrofuran rings formed by the condensation of an CHO group at C(5) with HO–C(1) and HO–C(19). A plausible biogenetic pathway, in which sculponeatin Q (**4**) is biosynthesized from sculponeatin N (**1**) (*Scheme*), is proposed. The possibility that **4** was artifact produced during extraction and purification can be excluded, because the isolation conditions were very mild, and did not involve temperatures above 60° or of the use of acids or bases. Moreover, significant cytotoxic activities (*Table 2*) were observed for spiro-lactone group, *i.e.*, sculponeatin N (**1**), sculponeatin O (**2**), and sculponeatin C (**7**).

Scheme. *Proposed Biogenesis of Sculponeatin Q* (**4**)



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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh). TLC: SiO_2 GF_{254}. Semiprep. reversed-phase (RP) HPLC: *Agilent 1100* liquid chromatograph with a *Zorbax SB-C_{18}* column. Optical rotations: *SEPA-300* polarimeter. UV Spectra: *Shimadzu 210A* double-beam spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *BIO-RAD FTS-135* spectrometer with KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* and *DRX-500* spectrometer with (D_5)pyridine as solvent; δ in ppm rel. to Me_4Si as internal standard, J in Hz. MS: *VG Auto Spec-3000* magnetic-sector instrument; m/z (rel.%).

Plant Material. The aerial parts of *I. sculponeatus* were collected in Lijiang County of Yunnan Province, P. R. China, in August 2005, and identified by Prof. *Xi-Wen Li*. A voucher specimen (KIB 05081918) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered aerial parts of *I. sculponeata* (2.5 kg) were extracted with 70% acetone (3 × 10 l, 24 h) at r.t. and filtered. The filtrate was evaporated to give a residue, which was suspended in H_2O (2.5 l) and then extracted with AcOEt (3 × 3 l). The AcOEt extract

(60.0 g) was decolorized on MCI gel and eluted with 90% aq. MeOH to yield a yellowish gum (46.0 g). The gum was subjected to CC (SiO_2 (200–300 mesh); $CHCl_3$/acetone 1:0→0:1 gradient system), to afford *Frs. A–E*. *Fr. A* (5.5 g) provided **16** (11.0 mg) after CC (SiO_2; $CHCl_3$/MeOH 40:1) and recrystallization from MeOH. *Fr. B* (4.0 g) was separated by *RP-18* (40–90% gradient system) and repeated CC (SiO_2; petroleum ether/AcOEt 3:2) to afford **13** (10.0 mg), **11** (6.0 mg), **12** (4.0 mg), and **14** (6.0 mg). *Fr. C* (6.0 g) was chromatographed on *RP-18* eluted with MeOH/H_2O (30–100%) gradient system to obtain three main *Frs.* C_1–C_3. *Fr.* C_1 (2.5 g) provided **5** (5.0 mg) and **15** (8.0 mg) after CC (SiO_2; petroleum ether/AcOEt 3:2), followed by semiprep. HPLC (40% MeOH/H_2O). Compounds **6** (5.0 mg) and **10** (9.0 mg) were obtained from *Fr.* C_3 (2.0 g) by repeated CC (SiO_2; petroleum ether/i-PrOH 15:1), followed by **4** (8.0 mg) and **7** (8.0 mg) from semiprep. HPLC (45% MeOH/H_2O). *Fr. D* (8.0 g) was passed through *RP-18* to yield two main *Frs.* D_1–D_2, eluted with 40–90% gradient system. *Fr.* D_1 was subjected to CC (SiO_2; $CHCl_3$/i-PrOH 30:1–10:1) to yield **1** (25.0 mg), **9** (11.0 mg), and **17** (6.0 mg); the remainder subjected to semiprep. HPLC (47% MeOH/H_2O) afforded **2** (6.0 mg) and **3** (4.0 mg). *Fr.* D_2 was purified by CC (SiO_2; $CHCl_3$/MeOH 20:1), followed by semiprep. HPLC (42% MeOH/H_2O), to give **8** (8.0 mg).

Sculponeatin N (=*6-Hydroxy-15-oxo-6,7*-seco-ent-*kaur-16-en-7,20-olide*; *(1*R*,2*R*,4a′*S*,7′*R*,9a′*S*)-Tetrahydro-2-(hydroxymethyl)-3,3-dimethyl-8′-methylidenespiro[cyclohexane-1,4′-[2]oxa[7,9*a*]methanocyclohepta[*c*]pyran]-1′,9′(4a′*H*)-dione*; **1**). White amorphous powder. $[\alpha]^{22.5}_{D} = +51.7$ ($c = 1.02$, pyridine). UV (MeOH): 230 (4.01). IR: 3475, 2940, 1730, 1701, 1643, 1391, 1264. ^{1}H-NMR (400 MHz): 5.92 (br. *s*, H_a–C(17)); 5.29 (br. *s*, H_b–C(17)); 4.82 (*d*, *J* = 11.6, H_a–C(20)); 4.78 (*d*, *J* = 11.6, H_b–C(20)); 3.83–3.85 (*m*, H–C(6)); 3.10 (*dd*, *J* = 12.0, 4.5, H_β–C(9)); 2.90 (*dd*, *J* = 9.2, 4.3, H_α–C(13)); 2.52 (*dd*, *J* = 12.3, 4.3, H_β–C(14)); 2.21 (*d*, *J* = 12.3, H_α–C(14)); 2.07–2.09 (*m*, H_α–C(12)); 1.77–1.79 (*m*, H_β–C(11)); 1.61–1.65 (overlapped, H_β–C(5), H_α–C(1)); 1.55–1.57 (overlapped, H_α–C(11), H_β–C(2)); 1.37–1.39 (*m*, H_β–C(12)); 1.30–1.34 (overlapped, H_β–C(3), H_α–C(2)); 1.24–1.26 (*m*, H_β–C(1)); 1.14–1.16 (*m*, H_α–C(3)); 1.02 (*s*, Me(18)); 0.84 (*s*, Me(19)). ^{13}C-NMR: *Table 1*. HR-ESI-MS (pos.): 355.1894 ($[M+Na]^+$, $C_{20}H_{28}NaO_4^+$; calc. 355.1885).

Sculponeatin O (=*(16*R*)-6-Hydroxy-16β-(methoxymethyl)-15-oxo-6,7*-seco-ent-*kauran-7,20-olide*; *(1*R*,2*R*,4a′*S*,7′*R*,8′*S*,9a′*S*)-Tetrahydro-2-(hydroxymethyl)-8′-(methoxymethyl)-3,3-dimethylspiro[cyclohexane-1,4′-[2]oxa[7,9*a*]methanocyclohepta[*c*]pyran]-1′,9′(4a′*H*)-dione*; **2**). White amorphous powder. $[\alpha]^{22.5}_{D} = +40.4$ ($c = 0.85$, pyridine). UV (MeOH): 203 (3.47). IR: 3463, 2937, 1742, 1713, 1390, 1250, 1114, 1031. ^{1}H-NMR (500 MHz): 4.81 (*d*, *J* = 12.0, H_a–C(20)); 4.79 (*d*, *J* = 12.0, H_b–C(20)); 3.88–3.90 (*m*, H–C(6)); 3.66 (*dd*, *J* = 10.0, 4.7, H_a–C(17)); 3.61–3.63 (*m*, H_b–C(17)); 3.15 (*s*, MeO); 3.02 (*dd*, *J* = 12.4, 5.0, H_β–C(9)); 2.97–2.99 (*m*, H_α–C(16)); 2.69–2.71 (*m*, H_α–C(13)); 2.51 (*dd*, *J* = 12.4, 3.8, H_β–C(14)); 2.25 (*d*, *J* = 12.4, H_α–C(14)); 1.73 (overlapped, H_α–C(12), H_β–C(11)); 1.65 (br. *s*, H_β–C(5)); 1.62–1.64 (*m*, H_α–C(1)); 1.54–1.58 (overlapped, H_β–C(12), H_α–C(11)); 1.47–1.49 (*m*, H_β–C(2)); 1.35–1.37 (overlapped, H_β–C(3), H_α–C(2)); 1.24–1.26 (*m*, H_β–C(1)); 1.14–1.16 (*m*, H_α–C(3)); 1.03 (*s*, Me(18)); 0.86 (*s*, Me(19)). ^{13}C-NMR: *Table 1*. FAB-MS (pos.): 365 ($[M+H]^+$). HR-ESI-MS (pos.): 387.2151 ($[M+Na]^+$, $C_{21}H_{32}NaO_5^+$; calc. 387.2147).

Sculponeatin P (=*(16*S*)-6-Hydroxy-16α-(methoxymethyl)-15-oxo-6,7*-seco-ent-*kauran-7,20-olide*; *(1*R*,2*R*,4a′*S*,7′*R*,8′*R*,9a′*S*)-Tetrahydro-2-(hydroxymethyl)-8′-(methoxymethyl)-3,3-dimethylspiro[cyclohexane-1,4′-[2]oxa[7,9*a*]methanocyclohepta[*c*]pyran]-1′,9′(4a′*H*)-dione*; **3**). White amorphous powder. $[\alpha]^{22.5}_{D} = +101.0$ ($c = 0.80$, pyridine). UV (MeOH): 203 (3.40). IR: 3508, 2935, 1743, 1696, 1392, 1110, 1028. ^{1}H-NMR (500 MHz): 4.79 (*d*, *J* = 11.5, H_a–C(20)); 4.74 (*d*, *J* = 11.5, H_b–C(20)); 3.86–3.88 (*m*, H–C(6)); 3.52–3.54 (*m*, H_a–C(17)); 3.46 (*dd*, *J* = 9.4, 4.0, H_b–C(17)); 3.15 (*s*, MeO); 3.08 (*dd*, *J* = 12.5, 4.5, H_β–C(9)); 2.79 (*dd*, *J* = 12.5, 4.5, H_β–C(14)); 2.49–2.51 (*m*, H_α–C(13)); 2.45–2.47 (*m*, H_β–C(16)); 2.14 (*d*, *J* = 12.5, H_α–C(14)); 2.07–2.09 (*m*, H_α–C(12)); 1.77–1.79 (*m*, H_β–C(11)); 1.67 (br. *s*, H_β–C(5)); 1.64–1.66 (*m*, H_α–C(1)); 1.54–1.56 (overlapped, H_α–C(11), H_β–C(2)); 1.32–1.36 (overlapped, H_β–C(12), H_β–C(3), H_α–C(2)); 1.20–1.22 (*m*, H_β–C(1)); 1.17 (*m*, H_α–C(3)); 1.04 (*s*, Me(18)); 0.85 (*s*, Me(19)). ^{13}C-NMR: *Table 1*. FAB-MS (pos.): 365 ($[M+H]^+$). HR-ESI-MS (pos.): 387.2145 ($[M+Na]^+$, $C_{21}H_{32}NaO_5^+$; calc. 387.2147).

Sculponeatin Q (=*6,1α:6,19-Diepoxy-1α,15β-dihydroxy-6,7*-seco-ent-*kaur-16-en-7,20-olide*; *(1*R*,3a*R*,4a′*S*,5′*R*,7*S*,7′*S*,7a*R*,9′*R*,9a′*S*)-Dodecahydro-5′,9′-dihydroxy-3a-methyl-8′-methylidene-3*H*-spiro[1,6-epoxy[2]benzofuran-7,4′-[7,9*a*]methanocyclohepta[*c*]pyran]-1′-one*; **4**). White amorphous powder.

$[\alpha]_D^{25.7} = -170.6$ ($c = 0.59$, pyridine). UV (MeOH): 200 (3.74). IR: 3515, 2904, 1743, 1640, 1020, 944. ^{1}H-NMR (500 MHz): 5.89 (*d*, $J = 3.4$, H–C(6)); 5.72–5.74 (*m*, H_α–C(15)); 5.55 (*d*, $J = 11.3$, H_a–C(20)); 5.48 (br. *s*, H_a–C(17)); 5.21 (br. *s*, H_b–C(17)); 4.74 (*d*, $J = 5.2$, H_β–C(1)); 4.44–4.46 (*m*, H_β–C(11)); 4.36 (*d*, $J = 11.3$, H_b–C(20)); 3.95 (*d*, $J = 8.4$, H_a–C(19)); 3.76 (*d*, $J = 8.4$, H_b–C(19)); 3.15 (br. *s*, H_β–C(9)); 3.11 (*d*, $J = 11.2$, H_β–C(14)); 2.87–2.89 (*m*, H_α–C(13)); 2.66 (*d*, $J = 3.4$, H_β–C(5)); 2.44–2.46 (*m*, H_β–C(12)); 2.17–2.19 (*m*, H_β–C(2)); 2.09–2.11 (*m*, H_α–C(2)); 1.92–1.94 (*m*, H_α–C(12)); 1.75 (*dd*, $J =$ 11.2, 6.0, H_α–C(14)); 1.60–1.62 (*m*, H_β–C(3)); 1.50 (*s*, Me(18)); 1.35 (*dd*, $J = 14.0$, 8.0, H_α–C(3)). ^{13}C-NMR: *Table 1*. FAB-MS (pos.): 363 ($[M+H]^+$). HR-ESI-MS (pos.): 385.1632 ($[M+Na]^+$, $C_{20}H_{26}NaO_6^+$; calc. 385.1627).

Sculponeatin R (*=6,20-Epoxy-3α,6β,11β-trihydroxy-15-oxo-6,7-*seco-ent-*kaur-16-en-1α,7-olide*; *(2*R*,3a*S*,5a*S*,8*S*,10*R*,10a*S*,10b*S*,13*R*,13a*R*)-Decahydro-2,10,13-trihydroxy-1,1-dimethyl-7-methylidene-5*H*-5a,8-methano-11*H*-cyclohepta[c]furo[3,4-*e*][1]benzopyran-5,6(7*H*)-dione*; **5**). White amorphous powder. $[\alpha]_D^{22.9} = -85.7$ ($c = 0.72$, pyridine). UV (MeOH): 226 (3.73). IR: 3428, 2938, 1747, 1703, 1642, 1441, 1248, 1032. ^{1}H-NMR (400 MHz): 6.06 (*dd*, $J = 11.9$, 5.9, H_β–C(1)); 6.00 (br. *s*, H_α–C(6)); 5.95 (br. *s*, H_a–C(17)); 5.28 (br. *s*, H_b–C(17)); 5.19–5.21 (*m*, H_α–C(11)); 4.62 (*d*, $J = 9.0$, H_a–C(20)); 4.45 (*d*, $J =$ 9.0, H_b–C(20)); 3.99–4.01 (*m*, H_β–C(3)); 3.70 (br. *d*, $J = 11.0$, H_β–C(14)); 3.09–3.13 (overlapped, H_β–C(13), H_α–C(9)); 2.83 (br. *s*, H_β–C(5)); 2.46–2.48 (overlapped, H_α–C(12), H_β–C(2)); 2.38 (br. *d*, $J = 11.9$, H_α–C(2)); 2.20 (*dd*, $J = 11.1$, 4.0, H_α–C(14)); 1.88 (*dd*, $J = 12.0$, 5.0, H_β–C(12)); 1.34 (*s*, Me(18)); 1.30 (*s*, Me(19)). ^{13}C-NMR: *Table 1*. FAB-MS (pos.): 379 ($[M+H]^+$). HR-ESI-MS (pos.): 401.1571 ($[M+Na]^+$, $C_{20}H_{26}NaO_7^+$; calc. 401.1576).

Sculponeatin S (*=6,20:6,19-Diepoxy-3β,11β-dihydroxy-15-oxo-6,7-*seco-ent-*kaur-16-en-1α,7-olide*; *(2a*R*,3*S*,4a*S*,6a*S*,9*S*,11*R*,11a*S*,11b*S*,13a*S*,13b*R*)-Decahydro-3,11-dihydroxy-2a-methyl-8-methylidene-6*H*-6a,9-methano-2*H*,12*H*-1,5,13-trioxacyclohepta[*a*]pentaleno[1,6-*hi*]naphthalene-6,7(8*H*)-dione*; **6**). White amorphous powder. $[\alpha]_D^{22.7} = -117.4$ ($c = 1.10$, pyridine). UV (MeOH): 230 (3.79). IR: 3444, 2940, 1749, 1708, 1642, 1271, 1046. ^{1}H-NMR (400 MHz): 6.34 (*dd*, $J = 11.5$, 5.8, H_β–C(1)); 6.20 (*d*, $J = 5.2$, H_β–C(6)); 6.02 (br. *s*, H_a–C(17)); 5.35 (br. *s*, H_b–C(17)); 4.55–4.57 (*m*, H_α–C(11)); 4.37 (*d*, $J = 9.5$, H_a–C(20)); 4.29 (*d*, $J = 9.5$, H_b–C(20)); 4.06–4.08 (*m*, H_α–C(3)); 4.05 (br. *s*, H_a–C(19)); 3.64 (br. *s*, H_b–C(19)); 3.62 (br. *s*, H_β–C(14)); 3.07–3.09 (*m*, H_β–C(13)); 3.03 (*d*, $J = 5.2$, H_β–C(5)); 2.40–2.42 (*m*, H_β–C(12)); 2.35 (*m*, H–C(2)); 2.24 (br. *s*, H_α–C(9)); 2.20 (*dd*, $J = 11.2$, 4.4, H_α–C(14)); 1.68 (*dd*, $J = 11.0$, 3.5, H_α–C(12)); 1.37 (*s*, Me(18)). ^{13}C-NMR: *Table 1*. FAB-MS (pos.): 377 ($[M+H]^+$). HR-ESI-MS (pos.): 399.1428 ($[M+Na]^+$, $C_{20}H_{24}NaO_7^+$; calc. 399.1419).

Cytotoxicity Bioassay. Cytotoxicity of compounds against suspended tumor cells was determined by the trypan blue exclusion method, and against adherent cells by sulforhodamine B (SRB) assay. Cells were plated in a 96-well plate 24 h before treatment and continuously exposed to different concentrations (100, 10, 1, and 0.1 μM) of compounds for 72 h. After compound treatments, cells were counted (suspended cells), or fixed and stained with SRB (adherent cells) as described in [18]. The assays were performed in triplicate, on separate occasions. The IC_{50} values were calculated by the *Logit* method. The percentage of inhibition was calculated according to the equation inhibition [%] $= [(OD_0 - OD_1)/OD_0] \times 100$, where OD_0 is the optical density of control, OD_1 is that of the sample.

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