Diterpenoids from *Isodon sculponeatus*

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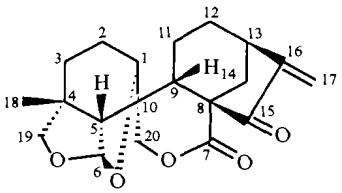
Further investigation on the leaves of *Isodon sculponeatus* afforded two new *ent*-kaurane diterpenoids, *sculponeatins J* and *K*. Their structures were elucidated on the basis of their spectral properties, as well as X-ray crystallographic analysis. The cytotoxicities of these two new compounds against human tumor cells K562 and T24 were also tested, and *sculponeatin J* showed significant inhibitory effects with IC_{50} values less than 1.0 μg/mL.

Keywords *Isodon sculponeatus*, Labiatae, *ent*-kauranoid, *sculponeatins J* and *K*, cytotoxicity

Introduction

Isodon species are rich in *ent*-kaurane diterpenoids which have been verified to be the main bioactive constituents of the plants. *Isodon sculponeatus* (Vaniot) Kudo, a perennial herb of Labiatae family, is distributed mainly over southwest China and often used as a medicinal herb to treat dysentery and beriberi in local folk.[1,2] Previous research on chemical constituents of *I. sculponeatus* had led to the isolation of several *ent*-kaurane diterpenoids.[3-7] However, in our recent re-investigation of this plant collected from Dali, Yunnan, from EtOAc extract was isolated two new *ent*-kaurane diterpenoids possessing the partial structures of 6,1:6,19-diepoxy, which were further verified the structure of *sculponeatin C*,[3] a diterpenoid isolated from the same plant before. In addition, bioactive tests for their cytotoxicities toward K562 and T24 cells indicated that compound **1** exhibited the significant inhibitory effect against both cells with IC_{50} value less than 1.0 μg/mL. In this paper, we wish to report their structure elucidations and the results of bioactive tests.



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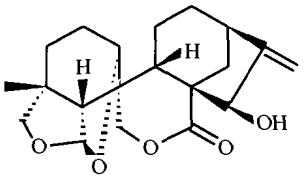

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Results and discussion

Sculponeatin J (**1**), colorless crystals, had a molecular formula of $C_{20}H_{24}O_5$ established by HREIMS (found 344.1629, calcd 344.1624), suggesting nine degrees of unsaturation. Its UV-vis (λ_{max} at 233.5 nm), IR (ν_{max} at 1718 and 1641 cm^{-1}) and NMR [^1H NMR δ: 6.08 (s, H-17a) and 5.51 (s, H-17b); ^{13}C NMR δ: 199.40 (s), 150.29 (s) and 118.72 (t)] spectral data indicated an *exo*-methylene group conjugated with a carbonyl group. It clearly revealed no hydroxyl group on the structure in IR spectrum due to no absorption at the region with wave number higher than 3000 cm^{-1}. The ^{13}C NMR

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and DEPT spectra exhibited the presence of one methyl, eight methylenes (including two oxygenated ones), five methines (including an acetal methine at δ 108.27) and six quaternary carbons (including two carbonyl groups). Considering the correlation of biosynthesis between natural products in the same plant, as well as the types of diterpenoids isolated from the *Isodon* genus, **1** was supposed to be an *ent*-kaurene diterpenoid with several epoxy rings in the structure. This assumption was approved by the results of 2D-NMR experiments.

In the ^{1}H-^{1}H COSY spectrum, three partial structures, $CHCH_2CH_2$ (C-1—C-3), CHCH (C-5 and C-6) and $CHCH_2CH_2CHCH_2$ (C-9, C-11—C-14), were clearly revealed by the cross peaks. From the HMBC spectrum, the correlations were also observed between the proton at δ 2.57 (d, H-5) with the signals of C-1, C-4, C-9, C-18, C-19 and C-20, and the proton at δ 2.43 (overlap, H-9) with the signals of C-7 and C-15, which assured **1** of an *ent*-kaurene diterpenoid. On the other hand, instead of the correlation between H-5 and C-7 or H-6 and C-7, the correlation between H-20 and C-7 (s, δ 171.12) was clearly observed in the HMBC spectrum, suggesting that **1** should possess a basic skeleton of 6,7-*seco*-7,20-olide-*ent*-kaurene. Moreover, besides an ether link between C-6 and C-19, an extra oxo-bridge should be also established in the carbons between C-6 and C-1 because of the correlation between H-6 and an oxygenated methine at δ 76.21 (d, C-1) in the HMBC spectrum. Most of correlations in HMBC spectrum of **1** were showed in Fig. 1.

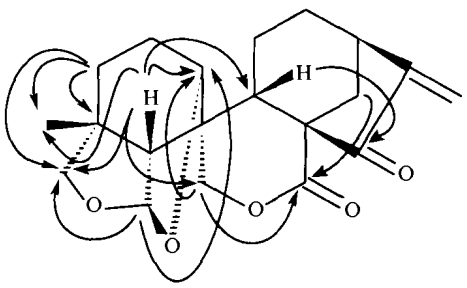

Fig. 1 Selected HMBC correlations of **1**.

The relative configurations of **1** were achieved by the analysis of the NOESY spectrum, and the key correlations were shown in Fig. 2. Thus, *sculponeatin J* (**1**) was elucidated as 6,1:6,19-diepoxy-6,7-*seco*-7,20-olide-*ent*-kaur-16-en-15-one.

Using the same method, *sculponeatin K* (**2**) was determined to be 15-hydroxy-6,1:6,19-diepoxy-6,7-*seco*-7,20-olide-*ent*-kaur-16-en which was further confirmed by X-ray crystallographic analysis (Fig. 3).

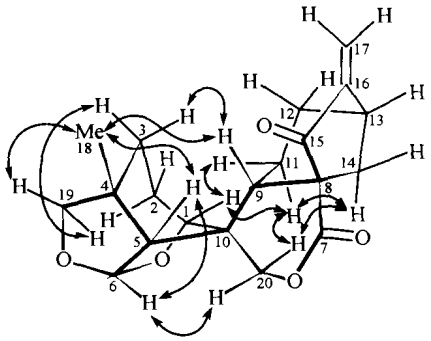

Fig. 2 Key NOESY correlations of **1**.

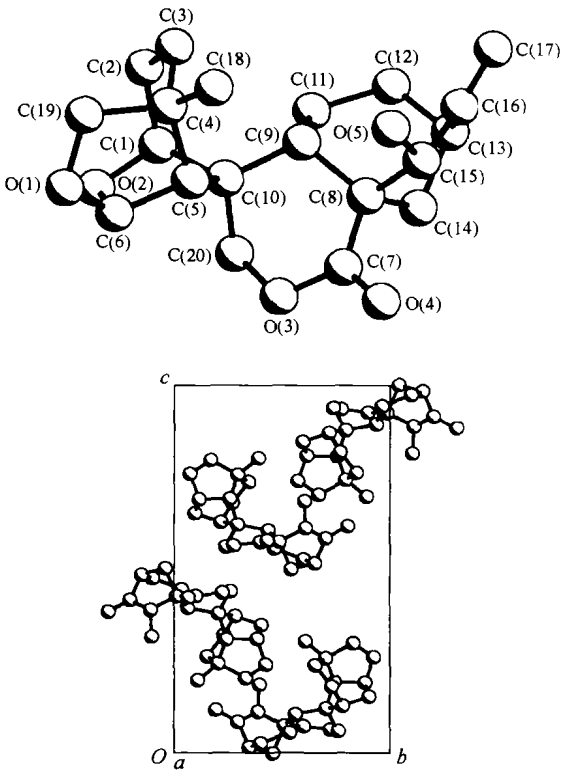

Fig. 3 Crystal structure of **2**.

According to the stereochemical structure of 6,1:6,19-diepoxy-6,7-*seco*-7,20-olide-*ent*-kauranoid, the C-19 was obviously extended to the exocyclic space because of the extra ether link between C-6 and C-1, which made the C-19 show a significant deshielding effect and appear at the downfield in ^{13}C NMR spectrum. Therefore, the C-19 in *sculponeatin C* should be revised to the carbon at δ

83.9 instead of the one at δ 72.2.[3]

As shown in Table 1, compounds **1** and **2** were studied for their cytotoxicities against human tumor cells K562 and T24, respectively, and **1** showed significant inhibitory effects to both cells with IC_{50} values less than 1.0 μg/mL, while *cis*-platinum was used as a positive reference substance, indicating the partial structure of C-15 ketone conjugated with the *exo*-methylene group to be decisive to the bioactivities of the compounds against K562 and T24.

Table 1 Cytotoxic activity of compounds **1** and **2**

Test compound	*M. W.*	IC_{50}(μg/mL)	
		K562	T24
1	344	0.849	0.642
2	346	ND[a]	ND[a]
cis-platinum		2.018	1.155

[a] ND: not determined.

Experimental

General procedure

Melting points (uncorrected) were obtained on an XRC-1 apparatus. Optical rotations were taken on a JASCO DIP-370 digital polarimeter. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV-vis spectra were obtained on a UV 210A spectrometer. MS spertra were recorded on a VG Auto Spec-3000 spectrometer. 1D- and 2D-NMR spectra were run on a Bruker AM-400 and a DRX-500 instrument with TMS as internal standard, respectively.

Plant material

The leaves of *I. sculponeatus* were collected in Dali prefecture of Yunnan province in August, 1997, and air-dried. The identity of plant material was verified by Prof. Zhong-Wen Lin, and a voucher specimen (KIB-97-08-28 Lin) is deposited in the Herbarium of Department of Taxonomy, Kunming Institute of Botany, Academia Sinica.

Extraction and isolation

The dried and powdered leaves (8.03 kg) were extracted with 70% Me_2CO and filtered. After concentration *in vacuo*, the filtrate was extracted with petroleum ether and EtOAc successively. The residue (260 g) of the EtOAc extract was applied to column chromatography over Si gel (200—300 mesh, 2.3 kg) column eluting with a $CHCl_3$-Me_2CO (1:0—0:1, *V*:*V*) gradient system to yield fractions **I**—**VIII**. Fraction **II** ($CHCl_3$-Me_2CO 10:1, *V*:*V*) was evaporated *in vacuo* to give a light yellow residue (30 g) which was dissolved with acetone (50 mL ×4) and then filtered. TLC inspection indicated that the filtrate was mainly composed of triterpenoids. The filter residue was further dissolved by $CHCl_3$ for three times, and filtered again. The filtrate was evaporated to yield a white residue (5.5 g), which was chromatographed over Si gel ($CHCl_3$-MeOH 10:1, *V*:*V*) by means of MPLC to produce **1** (142 mg). Fraction **V** ($CHCl_3$-Me_2CO 8:2, V:V) was evaporated *in vacuo* to give a pale yellow residue (22.9 g). The residue was dissolved by hot methanol repeatedly, and then filtered. The filtrate was concentrated, and then, adsorbed on 30 g silica gel and subjected to column chromatography over Si gel (200—300 mesh, 302 g) column eluting with a cyclohexane-chloroform-isopropanol (10:3:1, *V*:*V*:*V*) gradient system to yield **2** (195 mg).

Sculponeatin J (**1**) Colorless crystals ($CHCl_3$), m.p. 195.5—197.0 ℃, $[\alpha]_D^{26}$ − 110.58 (*c* 0.127, MeOH); UV-vis (MeOH) λ_{max}: 233.5 (log ε 3.98) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.08 (s, 1H, H-17a), 5.64 (d, *J* = 3.82 Hz, 1H, H-6β), 5.51 (s, 1H, H-17b), 4.34 (d, *J* = 11.92 Hz, 1H, H-20a), 4.15 (dd, *J* = 2.30, 11.92 Hz, 1H, H-20b), 3.87 (overlap, 1H, H-1β), 3.85 (d, *J* = 8.58 Hz, 1H, H-19a), 3.73 (d, *J* = 8.58 Hz, 1H, H-19b), 3.10—3.15 (m, 1H, H-13α), 2.57 (d, *J* = 3.82 Hz, 1H, H-5β), 2.43 (overlap, 1H, H-9β), 2.42 (d, *J* = 12.32 Hz, 1H, H-14α), 2.36—2.30 (m, 1H, H-12α), 2.06 (overlap, 2H, H-2α,β), 2.01 (dd, *J* = 4.88, 12.32 Hz, 1H, H-14β), 1.83—1.75 (m, 1H, H-11α), 1.59 (overlap, 1H, H-3α), 1.57 (overlap, 1H, H-11β), 1.54 (overlap, 1H, H-12β), 1.43—1.36 (m, 1H, H-3β), 1.14 (s, 3H, Me-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 76.21 (d, C-1), 29.42 (t, C-2), 30.87 (t, C-3), 41.03 (s, C-4), 51.73 (d, C-5), 108.27 (d, C-6), 171.12 (s, C-7), 54.46 (s, C-8), 36.48 (d, C-9), 51.73 (s, C-10), 19.57 (t, C-11), 29.23 (t, C-12), 35.04 (d, C-13), 31.87 (t, C-14), 199.40 (s, C-15), 150.29 (s, C-16), 118.72 (t, C-17), 27.58 (q, C-18), 84.42 (t, C-19), 69.40 (t, C-20); IR (KBr) ν: 2967, 2954, 2875, 1754,

1718, 164, 1488, 1471, 1452, 1378, 1274, 1252, 1204, 1175, 1137, 1108, 1085, 1064, 1042, 1006, 974, 932, 895, 849 cm^{-1}; EIMS (70 eV) *m/z* (%): 344 (M^+, 100), 326 (26), 316 (87), 298 (21), 287 (41), 269 (57), 257 (68), 241 (19), 229 (53), 213 (15), 201 (18), 188 (16), 173 (19), 165 (35), 145 (32), 133 (49), 119 (77), 105 (67); HREIMS calcd for $C_{20}H_{24}O_5$ 344.1624, found 344.1629.

Sculponeatin K (**2**) Colorless crystals ($CHCl_3$), m.p. 233.5—235.0 ℃, $[\alpha]_D^{26}$ − 95.40 (*c* 0.176, MeOH); UV-vis (MeOH) λ_{max}: end absorption; ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.60 (d, J = 3.70 Hz, 1H, H-6β), 5.15 (s, 1H, H-17a), 5.10 (s, 1H, H-17b), 5.07 (d, J = 2.52 Hz, 1H, H-15α), 4.39 (d, J = 11.72 Hz, 1H, H-20a), 4.10 (d, J = 11.72 Hz, 1H, H-20b), 3.85 (overlap, 1H, H-19a), 3.83 (overlap, 1H, H-1β), 3.74 (d, J = 8.52 Hz, 1H, H-19b), 2.82 (overlap, 1H, OH-15β), 2.79 (overlap, 1H, H-9β), 2.75—2.71 (m, 1H, H-13α), 2.32 (d, J = 3.70 Hz, 1H, H-5β), 2.13 (overlap, 1H, H-12α), 2.09 (overlap, 2H, H-2α, β), 2.01—1.96 (m, 1H, H-3α), 1.89 (d, J = 11.88 Hz, 1H, H-14α), 1.60—1.44 (overlap, 5H, H-12β, 3β, 14β, 11α and 11β), 1.29 (s, 3H, Me-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 76.46 (d, C-1), 29.18 (t, C-2), 30.55 (t, C-3), 40.99 (s, C-4), 51.66 (d, C-5), 108.01 (d, C-6), 175.85 (s, C-7), 51.17 (s, C-8), 30.40 (d, C-9), 52.27 (s, C-10), 18.16 (t, C-11), 31.62 (t, C-12), 36.37 (d, C-13), 31.99 (t, C-14), 78.45 (d, C-15), 155.70 (s, C-16), 108.83 (t, C-17), 28.00 (q, C-18), 83.36 (t, C-19), 69.21 (t, C-20); IR (KBr) ν: 3417, 2980—2886, 1726, 1487, 1389, 1283, 1202, 1182, 1104, 1079, 1063, 1034, 1022, 1001, 973, 862 cm^{-1}; EIMS (70 eV) *m/z* (%): 346 (M^+, 100), 328 (43), 318 (52), 298 (39), 289 (17), 282 (14), 269 (50), 253 (14), 241 (36), 227 (15), 213 (24), 199 (15), 187 (20), 180 (27), 173 (25), 165 (49), 147 (62), 135 (70), 119 (65), 107 (87); HREIMS calcd for $C_{20}H_{26}O_5$ 346.1780, found 346.1782.

X-Ray crystallographic analysis of sculponeatin K (**2**)

A colorless prismatic crystal of $C_{20}H_{26}O_5$ having approximate dimensions 0.20 mm × 0.35 mm × 0.50 mm was mounted on a glass fiber. Cell constants and an orientation matrix for data collection corresponded to a primitive monoclinic cell with dimensions: a = 0.83630(2) nm, b = 1.06890(4) nm, c = 1.92180(4) nm, V = 1.71794(10) nm^3. For Z = 2 and M_r = 346.42, the calculated density is 1.339 g/cm^3. The space group was determined to be $P2_12_12_1$. The final R-factor is 0.048 with R_w 0.064 ($w = 1/\sigma|F|^2$). All measurements were made on a MAC DIP 2030K imaging plate area detector with graphite monochromated Mo Kα radiation.

Cytotoxicity against human cells K562 and T24

The cytotoxicity assays were performed by a method of MTT, the experimental details of which have been reported previously.[8]

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