Sulfonated Guaianolides from *Saussurea lappa*

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Two new guaiane-type sesquiterpene lactones with an unusual sulfonic acid group, sulfocostunolide A (1) and sulfocostunolide B (2), were isolated from the roots of *Saussurea lappa*. Their structures were elucidated on the basis of extensive spectroscopic analysis.

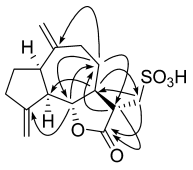
Key words *Saussurea lappa*; sesquiterpene lactone; sulfonated guaianolide; sulfocostunolide A; sulfocostunolide B

Saussurea lappa (Costus root; Chinese name: Yun Mu Xiang; Asteraceae) is a famous traditional Chinese medicine and also an important spice since ancient times, and is now in common use in China and Japan. Its roots can be used for the treatment of abdominal distension, indigestion, nausea, abdominal pain, *etc.* The main chemical and bioactive constituents of this plant are several types of sesquiterpenoids[1—6] including two sulfonated eudesmanes.[7] Further to search for biologically active compounds, we have reinvestigated the chemical constituents of this plant. This paper reports the isolation and structure elucidation of two new guaiane sesquiterpene lactones with a sulfonic acid group named sulfocostunolide A (**1**) and sulfocostunolide B (**2**) as a pair of epimers from the roots of *S. lappa*.

Compound **1**, obtained as viscid solid, has a molecular formula of $C_{15}H_{20}O_5S$ based on the negative-ion HR-ESI-MS, showing a quasi-molecular ion peak at *m/z* 311.0946 (Calcd for $C_{15}H_{19}O_5S$, 311.0953). The strong fragment ions at *m/z* 81 $[SO_3H]^-$, 80 $[SO_3]^-$ and the characteristic isotope peak at *m/z* 313 $[C_{15}H_{19}O_5S^{34}]^-$ in the negative FAB-MS indicated the existence of a sulfonic acid moiety. The IR spectrum showed absorption bands of γ-lactone carbonyl (1767 cm^{-1}), double bonds (3081, 1641 cm^{-1}) and sulfonic acid functionality (1213, 1054 cm^{-1}). The ^{13}C-NMR spectrum (CD_3OD) exhibited 15 carbon signals including two sets of terminal double bonds at δ 153.8 (s), 108.9 (t); 151.8 (s), 111.8 (t), a γ-lactone carbonyl resonance at δ 179.9 (s) and an oxygen-bearing methine carbon at δ 88.0 (d), suggestive of a skeleton of sesquiterpene lactone containing three rings. The NMR spectra were similar to those of dehydrocostus lactone (**3**),[1,4] isolated as the major sesquiterpene of this medicinal plant. Nevertheless, there were several significant differences as follows: a) the resonances of terminal double bond within lactone ring in **3** were absent and replaced by a set of signals at "δ_C 45.2 (d); δ_H 2.82 (ddd, J=11.4, 5.5, 5.1 Hz)" and "δ_C 51.1 (t); δ_H 3.11 (dd, J=14.6, 5.5 Hz), 3.35 (dd, J=14.6, 5.1 Hz)"; b) the γ-lactone carbonyl resonance was also evidently shifted downfield to the saturated zone at δ 179.9 (s), which indicated that the terminal double bond was hydrogenated then substituted by a sulfonic acid group.

Further validation was made by careful analysis of the HMBC spectrum (Fig. 2), in which the following significant correlations were observed: from H-11 to C-8, C-12; from H-13 to C-7, C-12. The correlation peaks in the ROESY spectrum (Fig. 3) between H-11 and H-8β, H-11 and H-6β, and H-6β and H-8β were clearly detectable, indicative of α orientation of the sulfomethyl group at C-11. Therefore the structure of **1** was elucidated as 13-sulfo-4(15),10(14)-guaiadien-12,6-olide, as shown in Fig. 1, named sulfocostunolide A.

Compound **2** was also obtained as viscid solid, possessing the same molecule formula $C_{15}H_{20}O_5S$ as sulfocostunolide A (**1**) based on the negative-ion HR-ESI-MS. The IR and NMR (Table 1) spectra were considerably in accordance with those of **1**, and their TLC behaviors (see Experimental) were very close but discriminable, suggesting a structurally wondrous similarity for both substances. Unexpectedly, analysis of the HMBC spectrum led to the same planar structure as sulfocostunolide A. Therefore the structure difference consequentially arose from the stereochemistry. The significant correlations in the ROESY experiment (Fig. 3): H-13 and H-8β, H-13 and H-6β, and H-6β and H-8β were observed, indicative of β orientation of the sulfomethyl group. Consequently, the structure of **2** was determined as shown in Fig. 1, named sulfocostunolide B.



1

Fig. 2. Selected HMBC Correlations of Compound **1**

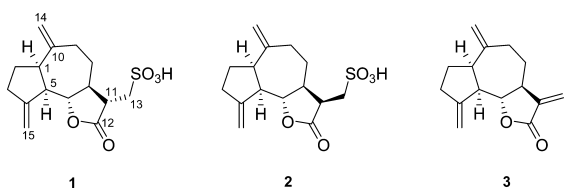


Fig. 1. Structures of Compounds **1**—**3**

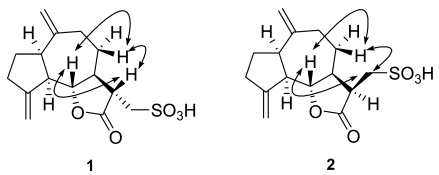


Fig. 3. Significant ROESY Correlations of Compounds **1** and **2**

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Table 1. NMR Spectral Data for Compounds **1** and **2**

No.	Sulfocostunolide A (**1**)[a]		Sulfocostunolide B (**2**)[b]	
	δ_C	δ_H	δ_C	δ_H
1	48.1 (d)	2.95 (ddd, 8.2, 4.1, 3.5)	47.6 (d)	2.73 (m)
2	31.1 (t)	1.89 (m), 1.97 (m)	30.6 (t)	1.72 (m), 1.81 (m)
3	33.5 (t)[c]	2.49 (m), 2.53 (m)	33.2 (t)	2.42 (m), 2.47 (m)
4	153.8 (s)		153.2 (s)	
5	53.1 (d)	2.87 (br dd, 9.4, 8.1)	53.2 (d)	2.68 (br dd, 9.2, 8.1)
6	88.0 (d)	4.04 (dd, 9.4, 9.4)	87.1 (d)	4.09 (dd, 9.6, 9.2)
7	47.6 (d)	2.59 (m)	44.9 (d)	2.53 (m)
8	33.6 (t)[c]	1.35 (H_β, m), 2.47 (H_α, m)	29.8 (t)	1.50 (H_β, m), 2.47 (H_α, m)
9	38.9 (t)	2.09 (ddd, 12.6, 11.7, 5.0) 2.45 (m)	38.1 (t)	1.90 (ddd, 12.1, 11.6, 4.2) 2.36 (m)
10	151.8 (s)		151.2 (s)	
11	45.2 (d)	2.82 (ddd, 11.4, 5.5, 5.1)	42.6 (d)	3.84 (m)
12	179.9 (s)		180.4 (s)	
13	51.1 (t)	3.11 (dd, 14.6, 5.5) 3.35 (dd, 14.6, 5.1)	49.1 (t)	3.53 (dd, 14.5, 4.8) 3.67 (dd, 14.5, 6.6)
14	111.8 (t)	4.75 (br s), 4.86 (br s)	112.1 (t)	4.71 (br s), 4.83 (br s)
15	108.9 (t)	5.00 (br s), 5.12 (br s)	109.6 (t)	5.07 (br s), 5.26 (br s)

a) Determined in CD_3OD (δ_H 3.30 ppm, δ_C 49.00 ppm as int. standard). *b*) Measured in $C_5D_5N : D_2O \approx 10 : 1$ (δ_H 8.71 ppm, δ_C 149.9 ppm as int. standard). *c*) Interchangeable. The unambiguous assignments were made on the basis of HSQC and HMBC experiments.

Experimental

General Experimental Procedures Optical rotations were obtained on a Horiba SEPA-300 polarimeter. IR spectra were taken on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were recorded with a Bruker DRX-500 instrument. EI-MS, FAB-MS and HR-ESI-MS were measured on Finnigan-MAT 90, VG Auto Spec-3000 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. Fractions were monitored by TLC (Qingdao Marine Chemical Inc., China) and spots visualized by heating silica gel plates immersed with vanillin-H_2SO_4 in ethanol.

Plant Material The roots of *S. lappa* were purchased from Kunming Market of Traditional Chinese Medicine and identified by Prof. Hua PENG. The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The dry roots (7.0 kg) were extracted with 95% ethanol at room temperature. The alcohol extract was concentrated to give a residue (230 g), which was subjected to silica gel column chromatography eluted with a solvent system of petroleum ether/acetone (0—100% acetone) followed by using pure MeOH. The fraction (33.5 g) eluted with pure MeOH was repeatedly subjected to silica gel column to obtain a target portion (2.3 g), which was further isolated and purified by Sephadex LH-20 column ($CHCl_3 : MeOH = 1 : 1$) to afford the new sesquiterpenes **1** (890 mg) and **2** (110 mg).

Sulfocostunolide A (**1**): Viscid solid. *Rf*=0.30 ($CHCl_3 : MeOH = 5 : 1$). $[\alpha]_D^{22}$ +24.2° (*c*=0.87, MeOH). IR (KBr) cm^{-1}: 3470, 3081, 1767, 1641, 1213, 1054, 1000, 892. ^1H- and ^{13}C-NMR data: see Table 1. FAB-MS (neg.): 311 $[M-H]^-$ (58), 81 (100), 80 (72). HR-ESI-MS (neg.): 311.0946 ($C_{15}H_{19}O_5S$, Calcd 311.0953).

Sulfocostunolide B (**2**): Viscid solid. *Rf*=0.36 ($CHCl_3 : MeOH = 5 : 1$). $[\alpha]_D^{20}$ +32.3° (*c*=0.43, $MeOH : H_2O = 5 : 1$). IR (KBr) cm^{-1}: 3441, 3084, 1748, 1641, 1218, 1187, 1147, 1055, 1002, 888. ^1H- and ^{13}C-NMR data: see Table 1. EI-MS: 230 $[M-H_2SO_3]^+$ (45), 215 (21), 201 (39), 150 (100), 131 (43), 117 (61), 105 (47), 91 (79), 79 (58). FAB-MS (neg.): 311 $[M-H]^-$ (35), 81 (66), 80 (100). HR-ESI-MS (neg.): 311.0957 ($C_{15}H_{19}O_5S$, Calcd 311.0953).

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