SHORT PAPER

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Two new sesquiterpenes from the Chinese herb *Saussurea petrovii* and their antibacterial and antitumor activity†

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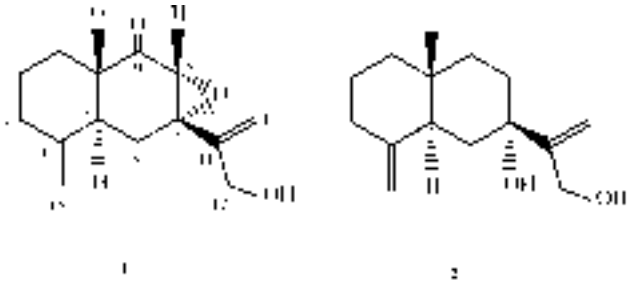
Two new sesquiterpenes petrovin A (**1**) and petrovin B (**2**) have been isolated from Chinese herb *Saussurea petrovii* and their structures established by spectroscopic methods. Compounds 1 and 2 showed significant antibacterial and antitumour activity.

Keywords: sesquiterpenes, *Saussurea petrovii*, antibacterial, antitumor

Saussurea petrovii Lipsch (Compositae) is a perennial herbaceous plant growing mainly in North China. Its rhizome has been used as a traditional Chinese medicine for treatment of rheumatism and bleeding since ancient times.[1] We present the isolation and structural elucidation of two new sesquiterpenes petrovin A (**1**) and petrovin B (**2**) isolated from the acetone extract of this herb, and their antibacterial and antitumour activity.

The chopped dry whole plant of *Saussurea petrovii* was extracted with acetone followed by carefully column chromatographic separation to give compounds **1** and **2** respectively. Compound **1** was obtained as colourless gum, [α] +12.5°(c 1.23, $CHCl_3$). HREIMS gave a molecular ion peak at *m/z* 248.1425 corresponding to the molecular formula $C_{15}H_{20}O_3$ (calcd. 248.1412). Its IR spectra showed absorption bands for hydroxyl groups (3435 cm^{-1}), double bonds (1645 cm^{-1}) and carbonyl group (1702 cm^{-1}). The ^1H NMR spectrum exhibited two sets of exomethylene proton signals at δ 5.25 and 5.23, and δ 4.92 and 4.64, respectively; three protons on oxygenated carbons at δ 3.13 (s), 4.17 (d, *J* = 13.9 Hz) and 4.23 (d, *J* = 13.9 Hz) respectively; and an angular methyl (δ 1.08, s). The ^{13}C NMR spectrum of **1** showed signals for 15 carbons, and DEPT spectra indicated the presence of one methyl, seven methylenes, two methines, and five quaternary carbons. The presence of six degree of unsaturation in compound **1** suggested that it was a eudesmane sesquiterpene derivative[2–4] with an epoxy ring, a carbonyl group and two exomethylenes (Scheme 1). The structure was further elucidated by 2D NMR experiments. The ^1H–^1H COSY and HMQC spectra confirmed correlations between $–CH_2(1)–CH_2(2)–CH_2(3)–$, $–CH(5)–CH_2(6)–$. The HMBC spectrum showed allylic couplings between $CH_2(3)–CH_2(15)$ and $CH_2(15)–CH(5)–$, and long range correlations for C-4/H-3, H-5, H-15; C-7/H-6, H-8, H-13; C-9/H-8, H-14 and C-11/H-6, H-12, H-13. The NOESY spectrum exhibited a clear correlation between H-8 and H-14 while no effect was observed between H-14 and H-5. Therefore, the structure of **1** was confirmed as 7α, 8α-epoxy-12-hydroxy-9-one-eudesm-4(15),11(13)-diene and the compound was named as petrovin A.



Scheme 1

Compound **2** was a colourless gum, $[\alpha]_D^{24}$ +23.4 (C 0.30, $CHCl_3$). Its IR spectrum showed a broad hydroxyl absorption at 3409 cm^{-1} and an absorption at 1645 cm^{-1} due to double bonds. HREIMS gave a molecular ion peak at *m/z* 236.1779 corresponding to the molecular formula $C_{15}H_{24}O_2$ (calcd. 236.1776). The 1D (^1H, ^{13}C / DEPT) and 2D (COSY, HMQC, HMBC) NMR spectra of **2** were similar to those of **1** except for the absence of the carbonyl group and the epoxide, and the appearance of a new hydroxyl carbon at δ 78.29. The location of this hydroxyl group were assigned at C-7 based on the HMBC spectrum which showed a correlation between C-7 / H-6, H-8, H-13. Therefore, the structure of **2** was assigned to be 7α,12-dihydroxy-eudesm-4(15),11(13)-diene and named as petrovin B.

Petrovin A and B exhibited significant *in vitro* cytotoxic activity against human hepatoma cells (SMMC-7721), human uterine cervix carcinoma cells (Hela) and mouse melanotic carcinoma cells (B16) (Table 1), as well as antibacterial activity

Table 1 Antitumour activity of petrovin A and petrovin B[a]

	SMMC-7721	B16	Hela
Petrovin A	108.6 ± 2.2	87.2 ± 4.3	69.9 ± 5.1
Petrovin B	126.5 ± 1.4	124.6 ± 3.4	117.4 ± 3.1
Vincristine	63.2 ± 1.8	70.7 ± 2.8	67.2 ± 2.2

[a]Activities are expressed as IC_{50} (50 % inhibitory concentration) in μg/ml.

Table 2 Antibacterial activity of petrovin A and petrovin B[a]

	B. subtilis	*E. coli*	*S. aureus*
Petrovin A	13.7	14.2	13.8
Petrovin B	8.6	13.4	14.7
Chloramphenicol	14.5	14.9	15.1

[a]Activities are expressed as the diameter of the inhibitory zone in mm.

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*

against *B. subtilis, E. coli* and *S. aureus* (Table 2). It is seen from Tables 1 and 2 that the antitumour activity of petrovin A is comparable to that of antitumour drug vincristine in case of B16 and Hela, and the antibacterial activity of both petrovin A and petrovin B is comparable to chloramphenicol.

Experimental

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The IR spectra were taken on a Nicolet 170SX IR spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM 400 NMR spectrometer with TMS as internal standard. HREIMS spectra were obtained on a VG ZAB-HS mass spectrometer.

Extraction and isolation procedure: The whole plant of *Saussurea petrovii* was collected in the suburb of Lanzhou city, Gansu, China. The chopped whole plant material (3.0 kg) was extracted repeatedly (3 times, 7 days each time) with acetone at room temperature to give a residue (80g) after evaporation. This residue was separated by silica gel (200-300 mesh) column chromatography with gradient elution of petroleum ether-acetone (30:1, 20:1, 15:1, 10:1, 5:1, 3:1, 1:1, 0:1). A gummy crude extract containing **1** and **2** was obtained from the fraction eluted with petroleum ether–acetone (10:1) and subjected to gel filtration (Sephadex, LH-20) followed by silica gel (200–300 mesh) column chromatography eluted with petroleum ether-AcOEt (8:1) to give **1** (35 mg) and **2** (25 mg).

Petrovin A (**1**): colourless gum, $[\alpha]_D^{24}$ +12.5°(c 1.23, $CHCl_3$); HREIMS: M^+ Found: 248.1425, Calcd for $C_{15}H_{20}O_3$: 248.1412; ν_{max}/cm^{-1}: 3435, 2935, 1702, 1645, 1444, 1258; δ_H (400 MHz, $CDCl_3$, TMS): 5.25 br (H-13a), 5.23 br (H-13b), 4.92 br (H-15a), 4.63 br (H-15b), 4.20 dd, *J*=13.8Hz (2H, H-12), 3.14 s (H-8), 2.49 dd, *J*=5.3, 10.6 Hz (H-5), 2.34 dd, *J*=13.0, 10.6 Hz (H-6β), 2.21 dd, *J*=13.0, 5.3Hz (H-6α), 2.25 m (H-3α), 1.92 m (H-1α), 1.89 m (H-3β), 1.69 m (H-2α), 1.47 m (H-1β), 1.45 m (H-2β), 1.09 s (3H, H-14); δ_C (100MHz, $CDCl_3$, TMS)(C-1 to C-15): δ33.67, 22.01, 36.13, 146.97, 47.99, 27.07, 66.55, 60.67, 210.32, 47.33, 146.90, 63.22, 113.44, 15.06, 109.15.

Petrovin B (**2**): colourless gum, $[\alpha]_D^{24}$ +23.4° (c 0.29, $CHCl_3$); HRMS: M^+ Found: 236.1779, Calcd for $C_{15}H_{24}O_2$: 236.1776; ν_{max} / cm^{-1}: 3409, 2926, 1078, 1025; δ_H (400 MHz, $CDCl_3$, TMS): 5.27 br (H-13a), 5.02 br (H-13b), 4.74 br (H-15a), 4.41 br (H-15b), 4.51m (2H, H-12), 2.32 m (H-3α), 2.25 d, *J*=13.6 Hz (H-5), 2.03 m (H-3β), 1.88 d, *J*=13.9 Hz (H-6α), 1.73 m (H-8α), 1.63 d, *J*=13.9 Hz (H-6β),1.58 m (H-8β), 1.57 m (H-2), 1.48 m (H-9β), 1.34 m (H-9α), 1.28 m (H-1β), 1.08 m (H-1α); δ_C (100MHz, $CDCl_3$, TMS) (C-1 to C-15): 29.69, 22.20, 36.74, 149.28, 43.59, 46.36, 78.29, 33.08, 42.03, 35.93, 152.59, 67.03, 104.72, 16.59, 106.08.

Cytotoxicity assay: The cytotoxicity of petrovin A and B was tested in three cell lines: SMMC-7721 (human hepatoma), B16 (mouse melanoma) and Hela (human carcinoma of uterine cervix). Cells were cultured at 37°C under a humidified atmosphere of 5 % CO_2 in RPMI 1640 medium supplemented with 10 % fetal calf serum and dispersed in replicate 96-well plates with 1×10^4 cells/well for 24 hours. Petrovin A or petrovin B (10-400 μmol/l) were then added. After 48-h exposure to the toxins, cell viability was determined by the methylthiazolyltetrazolium bromide (MTT) colorimetric assay[5] by measuring the absorbance at 595 nm with an ELISA reader.

Antibacterial assay: The paper-disk method[6] was used for antimicrobial tests. A 10 μg portion of petrovin A, petrovin B or chloramphenicol (used as positive control) was applied onto a paper disk, and the paper disk was air-dried. Then the disks were placed on agar plates that had been seeded with *B.subtilis*, *E.coli* and *S.aureus*, respectively, and incubated at 37°C for 24h. The antibacterial activity was determined by measuring the diameter of the inhibitory circles. Each test was performed in duplicate.

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