Two New Sesquiterpene Lactones with the Sulfonic Acid Group from *Saussurea lappa*

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Two new sesquiterpene lactones with the unusual sulfonic acid group, 13-sulfo-dihydrosantamarine (1) and 13-sulfo-dihydroreynosin (2), have been isolated from the roots of *Saussurea lappa* C. Their structures, including the absolute configurations, were elucidated by spectroscopic methods.

Key words *Saussurea lappa* C; Compositae; sesquiterpene lactone; 13-sulfo-dihydrosantamarine; 13-sulfo-dihydroreynosin

Saussurea lappa introduced from India has been cultivated in Southwest China. It has been a traditional Chinese medicine and also an important spice since ancient time, which is now in common use in China and Japan. It possesses the function of spasmolysis, antihypertension and antibacteria.[1)] Its chemical constituents have been studied by some groups. The main chemical and bioactive constituents of this plant are sesquiterpenes and sesquiterpene lactones.[2,3)] In order to clarify the medicinal value of *Saussurea lappa* and to search for biologically active compounds, we have reinvestigated the chemical constituents of the plant. This paper deals with the isolation and structure elucidation of two new sesquiterpene lactones with the sulfonic acid group named 13-sulfo-dihydrosantamarine (**1**) and 13-sulfo-dihydroreynosin (**2**) from *Saussurea lappa* C.

Compound **1**, obtained as a white powder, has a molecule formula of $C_{15}H_{22}SO_6$ based on the negative-ion high resolution HR-SI-MS, showing a $[M-H]^-$ ion peak at *m/z* 329.1066 (Calcd for 329.1064). The IR spectrum exhibited the presence of hydroxyl (3205 cm^{-1}), γ-lactone carbonyl (1769 cm^{-1}) and sulfonic acid (1262, 1035 cm^{-1}) groups. The $[M+Na-H_2SO_3]^+$ ion peak at *m/z* 271 in the positive ESI-MS also indicated the presence of sulfonic acid group. The ^{13}C-NMR (150 MHz, C_5D_5N) data showed 15 signals of carbon and the signal at δ 177.4 was due to the lactone carbonyl, which were suggested a sesquiterpene lactone. The signals at δ 5.39 (1H, br s) in ^{1}H-NMR and δ 121.0, 132.4 in ^{13}C-NMR showed the presence of a pair of olefinic carbons. The signals at δ 3.75 (1H, m) in ^{1}H-NMR and δ 73.0 in ^{13}C-NMR suggested the presence of a methine carbon which was attached to the hydroxyl group. In addition, the ^{1}H- and ^{13}C-NMR spectra also displayed a tertiary methyl (δ_H 1.01, δ_C 10.0) and a vinyl methyl (δ_H 1.88, δ_C 22.5). Detailed comparisons of ^{1}H- and ^{13}C-NMR data for **1** with those for santamarine (**3**) suggested that **1** possessed a eudesmane skeleton and was similar to santamarine,[4)] except for the 13-*exo*-methylene position. These results were supported by the HSQC and HMBC experiments. In the HMBC experiment, the correlations between H-14 and C-1, C-5, C-9, C-10, H-15 and C-3, C-4, C-5 have been observed. The signals at δ 4.01 (1H, dd, J=6.7, 14.1 Hz) and δ 3.52 (1H, dd, J=6.7, 14.1 Hz) which were due to the protons of C-13 indicated that the sulfonic acid group was attached to C-13 (δ_C 51.0). This was also confirmed by the HMBC experiment in which the correlated peaks between H-11 and C-13, H-13 and C-7 were observed. Based on the known moiety of sesquiterpene lactone and the observation of the NOE correlation between the H-11β (δ_H 3.35) and H-6β (δ_H 4.03) in NOESY spectrum, the CH_2SO_3H-11 was proved to be α-configuration. Furthermore, in NOESY spectrum, the NOE correlations between the H-6β and H_3-14β, H-1α and H-5α, H-5α and H-7α have been observed too. On the basis of the above evidences, the structure of **1** was determined.

The molecule formula of compound **2** was $C_{15}H_{22}SO_6$ based on the negative-ion HR-SI-MS, showing a $[M-H]^-$ ion peak at *m/z* 329.1064 (Calcd for 329.1064). The IR spectrum exhibited the presence of hydroxyl (3384 cm^{-1}), γ-lactone carbonyl (1769 cm^{-1}) and sulfonic acid (1261, 1040 cm^{-1}) groups. The $[M+Na-H_2SO_3]^+$ ion peak at *m/z* 271 in the positive ESI-MS also indicated the presence of sulfonic acid group. The ^{13}C-NMR (150 MHz, C_5D_5N) data showed 15 signals of carbon and the signal at δ 179.0 was due to the lactone carbonyl, which were suggested a sesquiterpene lactone. The signals at δ 4.98 (1H, br s), 4.94 (1H, br s) in ^{1}H-NMR and δ 145.1, 109.4 in ^{13}C-NMR showed the presence of an *exo*-methylene. In addition, the ^{1}H- and ^{13}C-NMR also displayed a methine carbon (δ_H 3.56, δ_C 77.4) which was attached to the hydroxyl group and a tertiary methyl (δ_H 0.98, δ_C 12.1). By careful analysis of the ^{1}H- and ^{13}C-NMR data of **2**, we found that those data were

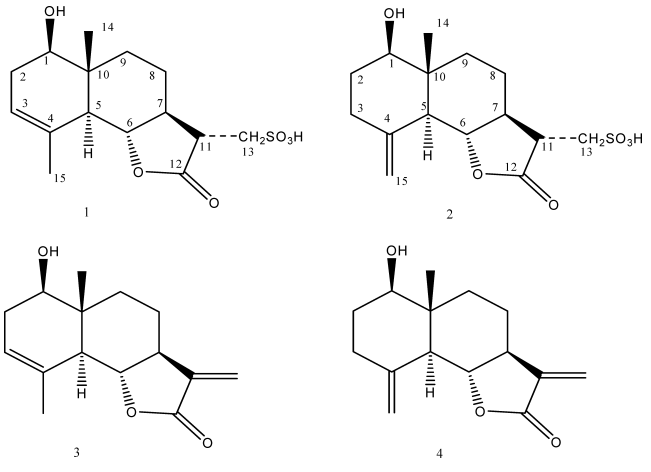

Fig. 1. The Structures of Compounds **1**—**4**

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closely similar to those of **1** except for some signals due to the olefin moiety and the structure of **2** was similar to that of reynosin (**4**),[4] except for the 13-*exo*-methylene position. Similarly, the sulfonic acid group was clarified to be linked with C-13 and the CH_2SO_3H-11 was proved to be α-configuration. Therefore, the structure of **2** was determined.

We have done some experiments with Human Leukemia cells (HL-60) and without seeing significant cytotoxic and cell growth inhibitory effect. Extensive activities evaluations are in progress.

Experimental

General Experimental Procedures Melting points were measured on Yanaco apparatus (uncorrected) and opital rotations on a P-E 241 MC polarimeter using methanol as the solvent. IR spectra were taken on a Bruker IFS-55 infrared spectrophotometer. The NMR data were recorded on Bruker AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) in C_5D_5N with TMS as internal standard. The HR-SI-MS and ESI data were obtained on the Bruker Daltonics and Agilent MSD Trap mass spectrometer respectively. Chromatography was performed with D101 macroporous resin, silica gel (200—300 mesh), Sephadex LH-20, reversed-phase MPLC (Medium Pressure Liquid Chromatography) and LPLC (Low Pressure Liquid Chromatography).

Plant Material The roots of *Saussurea lappa* were bought from the Cooperation of Traditional Chinese Medicine of Shenyang, the People's Republic of China. A voucher specimen (No. 20021101) was identified by Prof. Qi-shi Sun and deposited in the School of Traditional Chinese Medicine of Shenyang Pharmaceutical University, China.

Extraction and Isolation The roots of *Saussurea lappa* (10 kg) were extracted with 95% ethanol under reflux. The alcohol extract was concentrated and successively partitioned with petroleum ether, $CHCl_3$, AcOEt and *n*-BuOH. The *n*-BuOH-soluble fraction (289 g) was chromatographed over a D101 macroporous resin column with H_2O and 95% EtOH. The fraction (125 g) eluted with 95% EtOH was subjected to silica gel (1250 g) column chromatography eluted with a solvent system of $CHCl_3$–CH_3OH (0% CH_3OH—100% CH_3OH). The fraction eluted with $CHCl_3$–CH_3OH (10 : 2) was subjected to Sephadex LH-20 and reversed-phase MPLC and LPLC to afford to **1** (5 mg) and **2** (6 mg).

13-Sulfo-dihydrosantamarine (**1**): mp >300 °C, $[\alpha]_D^{20}$ +22.5° (c=0.002, CH_3OH); IR (KBr) cm^{-1} 3205, 1769, 1262, 1098, 1035; ^{1}H-NMR (600 MHz, C_5D_5N) δ 1.01 (3H, s, H-14), 1.88 (3H, s, H-15), 3.35 (1H, m, H-11), 3.52 (1H, dd, J=6.7, 14.1 Hz, H-13a), 4.01 (1H, dd, J=6.7, 14.1 Hz, H-13b), 3.75 (1H, m, H-1), 4.03 (1H, dd, J=10.5, 10.5 Hz, H-6), 5.31 (1H, br s, H-3); ^{13}C-NMR (150 MHz, C_5D_5N) δ 73.0 (C-1), 32.3 (C-2), 121.0 (C-3), 132.4 (C-4), 49.4 (C-5), 80.4 (C-6), 50.0 (C-7), 22.4 (C-8), 34.2 (C-9), 39.9 (C-10), 42.5 (C-11), 177.4 (C-12), 51.0 (C-13), 10.0 (C-14), 22.5 (C-15); ESI-MS (positive) *m/z*: 353 $[M+Na]^+$, 335 $[M+Na-H_2O]^+$, 317 $[M+Na-2H_2O]^+$, 271 $[M+Na-H_2SO_3]^+$.

13-Sulfo-dihydroreynosin (**2**): mp >300 °C, $[\alpha]_D^{20}$ +48.3° (c=0.002, CH_3OH); IR (KBr) cm^{-1} 3384, 1769, 1261, 1099, 1040; ^{1}H-NMR (600 MHz, C_5D_5N) δ 0.98 (3H, s, H-14), 3.41 (1H, m, H-11), 3.56 (1H, dd, J=6.7, 14.1 Hz, H-13a), 3.95 (1H, dd, J=6.7, 14.1 Hz, H-13b), 3.56 (1H, m, H-1), 4.20 (1H, dd, J=10.5, 10.5 Hz, H-6), 4.94 (1H, br s, H-15a), 4.98 (1H, br s, H-15b); ^{13}C-NMR (150 MHz, C_5D_5N) δ 77.4 (C-1), 36.9 (C-2), 34.3 (C-3), 145.1 (C-4), 50.8 (C-5), 80.0 (C-6), 52.9 (C-7), 24.1 (C-8), 32.3 (C-9), 43.5 (C-10), 44.4 (C-11), 179.0 (C-12), 51.2 (C-13), 12.1 (C-14), 109.4 (C-15); ESI-MS (positive) *m/z*: 353 $[M+Na]^+$, 335 $[M+Na-H_2O]^+$, 317 $[M+Na-2H_2O]^+$, 271 $[M+Na-H_2SO_3]^+$.

Assay of Cell Growth Inhibition The growth inhibitions of the two compounds on HL-60 cells were determined by trypan-blue exclusion. HL-60 cells were seeded at 1×10^4 cells/ml and treated with or without the different concentrations of test compounds for 3 d. Cell numbers including death cells and viable cells were measured by hemocytometer and growth inhibition was calculated to untreated cells (%).[5]

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