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ORIGINAL ARTICLE

Secondary Metabolites from *Sibiraea angustata*

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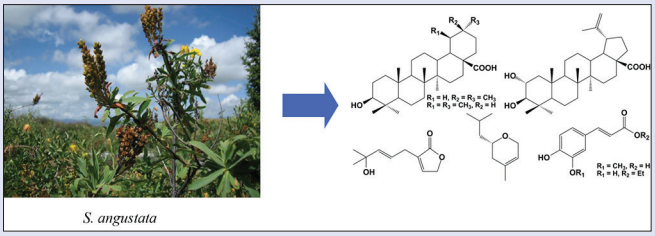
ABSTRACT

Background: *Sibiraea angustata* was used as folk medicine in Tibetan-inhabited area of Hengduan Mountains, China. **Objective:** The secondary metabolites from the leaves and twigs of *S. angustata* were studied. **Materials and Methods:** The compounds were isolated and purified using silica gel and Sephadex LH-20 column chromatography. Their structures were identified by spectra analysis. **Results:** Eight known compounds, including 2 monoterpenoids and 3 triterpenoids, were isolated and identified from *S. angustata*. **Conclusions:** Four compounds were isolated from this plant for the first time, and the ^{13}C-nuclear magnetic resonance spectra data and absolute configuration of dihydroneroloxide were given for the first time.

Key words: Electronic circular dichroism, monoterpenoids, secondary metabolites, *Sibiraea angustata*

SUMMARY

- The secondary metabolites of *Sibiraea angustata* were investigated
- Eight compounds, including 2 monoterpenoids and 3 triterpenoids, were isolated and identified
- The absolute configuration of dihydroneroloxide was given by electronic circular dichroism calculation.



Abbreviations used: NMR: Nuclear magnetic resonance; TMS: Tetramethylsilane; ECD: Electronic circular dichroism; CC: Column chromatography; TLC: Thin-layer chromatography; DFT: Density functional theory; TDDFT: Time-dependent density functional theory; PCM: Polarizable continuum model.

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INTRODUCTION

Sibiraea angustata (Rehd.) Hand.-Mazz., a shrub from family Rosaceae, was mainly distributed in bush and gravel area of Hengduan Mountains, China, at an altitude of 3000–4000 m.[1] Its leaves and twigs, called "Liucha," were used as folk medicine by Tibetans to treat indigestion and upset stomach, and weight loss of livestock was also caused by this plant.[2] Phytochemical studies on this plant revealed the presence of monoterpenoids and their glycosides, triterpenoids, phenolic acids, and fatty alcohols.[3-10] Previously, we also isolated one monoterpene lactone from *S. angustata*.[11] In the current study, eight compounds [Figure 1], named oleanolic acid (1), ursolic acid (2), sibiscolacton (3), monopalmitin (4), (5*S*)-dihydroneroloxide (5), ferulic acid (6), alphitolic acid (7), and ethyl caffeate (8), were isolated from the leaves and swigs of *S. angustata*.

MATERIALS AND METHODS

General

Nuclear magnetic resonance (NMR) spectra were recorded on Varian Unity 400/54 and Bruker AV II-600 spectrometers with tetramethylsilane as an internal standard. Electronic circular dichroism (ECD) spectra were obtained on Chirascan spectropolarimeter (Applied Photophysics). Optical rotation was measured on APVI/6W (Rudolph Research Analytical). Column chromatography (CC) was carried out using silica gel (Qingdao Marine Chemical Industry, 200–300 mesh) and Sephadex LH-20 (GE Healthcare). All the reagents and solvents used for separation and purification were analytical grade and purchased from local firms.

Plant material

The leaves and swigs of *S. angustata* were collected from Songpan County, Sichuan Province, China, in August 2008. The plant was identified by Prof. Tian-Zhi Wang, West China School of Pharmacy, Sichuan University. A voucher specimen (No. SA0808) was deposited in the Herbarium of West China School of Pharmacy, Sichuan University, Chengdu, China.

Extraction and isolation

The leaves and swigs of *S. angustata* (5 Kg) were powdered and extracted three times with 80% aq. EtOH under reflux. The solvents were evaporated in vacuo to yield ethanol extract, which was suspended in H_2O and then extracted with EtOAc. The EtOAc extract (185 g) was subjected to CC over silica gel (200–300 mesh, 2 Kg) and eluted with a gradient solvent system ($CHCl_3$-MeOH, 90:1-2:1) to give 12 fractions (Fr. 1-12). Fr. 2 (22 g) was isolated by silica gel chromatography eluted with solvent systems of cyclohexane-acetone (5:1) and preparative TLC (cyclohexane-acetone = 1.5:1) to afford mixture

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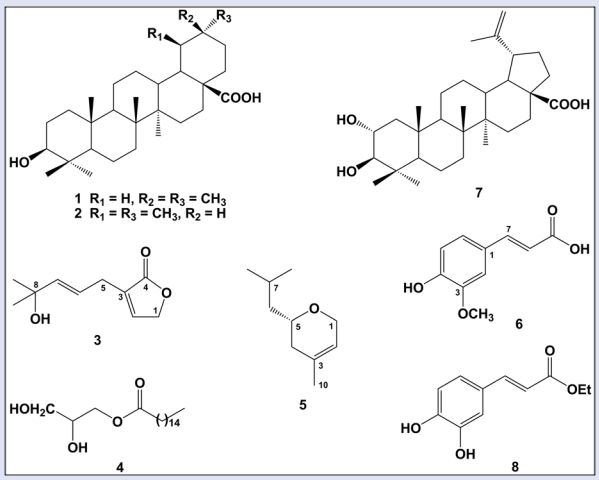

Figure 1: Structure of compounds 1-8 isolated from *Sibiraea angustata*

of compounds 1 and 2 (12 mg). Fr. 5 (4.5 g) was separated by silica gel chromatography (petroleum ether-acetone, 4.5:1) to give 10 subfractions (Fr. 5-1-5-10). Subfraction 5-8 (570 mg) was separated by silica gel chromatography (cyclohexane-acetone, 5:1; cyclohexane-EtOAc, 3:1) to give compound 3 (110 mg). Fr. 6 (2.6 g) was separated by silica gel chromatography (petroleum ether-acetone, 6:1-2:1) to give 8 subfractions (Fr. 6-1-6-8). Subfraction 6-3 (290 mg) was separated by silica gel chromatography (cyclohexane-EtOAc, 4:1; cyclohexane-acetone, 5:1) and purified on Sephadex LH-20 ($CHCl_3$-MeOH, 2:1) to give compound 4 (12 mg). Fr. 7 (3 g) was isolated by silica gel chromatography (petroleum ether-acetone, 5.5:1-3:1 and cyclohexane-acetone, 4:1) and preparative TLC (cyclohexane-EtOAc, 1:1), then purified on Sephadex LH-20 ($CHCl_3$-MeOH, 2:1) to give compound 5 (5 mg). Fr. 8 (2.6 g) yielded crystals (petroleum ether), and then, crystals were purified by Sephadex LH-20 ($CHCl_3$-MeOH, 2:1) to give compound 6 (9 mg). The residues from Fr. 8 (2 g) was separated by silica gel chromatography (petroleum ether-acetone, 5:1–2:1) to obtain 5 subfractions. Subfracton 8-2 (100 mg) yielded crystals that were recrystallized from MeOH to give compound 7 (18 mg); Subfraction 8-4/8-5 (250 mg) was separated by silica gel chromatography (cyclohexane-EtOAc, 3:1); and purified on Sephadex LH-20 ($CHCl_3$-MeOH, 2:1) to get compound 8 (17 mg).

Computational section

The theoretical ECD spectra calculation of compound 5 was performed using Gaussian Program by Yunnan Electronic Computing Center. The possible geometries were previously optimized by density functional theory (DFT) method at the B3 LYP/6-31G (d, p) level.[12] Excitation energies and rotational strengths were calculated using time-dependent DFT at the B3 LYP/6-31 G (d, p) level in methanol with polarizable continuum model.[13] The calculated ECD spectrum was generated using SpecDis in the end.[14]

RESULTS AND DISCUSSION

Mixture of compounds 1 and 2: White amorphous powder. NMR spectra data for compound 1: ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 5.24 (1H, br s, H-12), 3.13 (1H, m, H-3), 1.15, 1.14, 0.98, 0.89, 0.84, 0.80, 0.78 (each 3H, s, 7×CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): 183.2 (C-28), 143.4 (C-13), 122.3 (C-12), 78.3 (C-3), 55.0 (C-5), 47.5 (C-9), 46.2 (C-17), 45.5 (C19), 41.3 (C-14), 40.5 (C-18), 39.5 (C-8), 38.6 (C-1), 38.6 (C-4), 37.0 (C-10), 33.6 (C-21), 33.0 (C-29), 32.5 (C-7), 32.4 (C-22), 30.5 (C-20), 28.0 (C-23), 27.8 (C-15), 27.3 (C-2), 26.0 (C-27), 23.6 (C-30), 23.3 (C-16), 22.7 (C-11), 18.2 (C-6), 17.2 (C-26), 15.5 (C-24), and 15.2 (C-25). NMR spectra data for compound 2: ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 5.23 (1H, br s, H-12), 3.14 (1H, m, H-3), 1.12, 0.98, 0.84, 0.82, 0.74 (each 3H, s, 5×CH_3), 0.91 (3H, d, J = 6.4 Hz, CH_3), 0.83 (3H, d, J = 6.2 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): 180.2 (C-28), 137.8 (C-13), 125.0 (C-12), 78.3 (C-3), 55.0 (C-5), 52.9 (C-18), 48.1 (C-9), 47.0 (C-17), 41.3 (C-14), 39.3 (C-8), 38.9 (C-4), 38.6 (C-19), 38.4 (C-1), 38.4 (C-20), 37.5 (C-10), 36.6 (C-22), 33.0 (C-7), 30.5 (C-21), 27.8 (C-2), 27.6 (C-15), 27.5 (C-23), 23.2 (C-27), 23.0 (C-11), 20.8 (C-30), 18.0 (C-6), 17.9 (C-16), 16.7 (C-26), 16.4 (C-29), 15.5 (C-24), and 15.0 (C-25). The mixture of compounds 1 and 2 was compared with standard samples (oleanolic acid and ursolic acid) using special TLC method.[15] The plate with sample dots was fumigated by iodine steam first, and then developed by mixture solvents of cyclohexane-acetone-EtOAc-formic acid (8:2:1:0.2). After spray sulfuric acid-EtOH (10%) and heated, the plate can show the clearly separated sample dots of oleanolic acid and ursolic acid. The TLC comparison, together with the comparison of NMR data with those reported in literature,[16] confirmed that the mixture were oleanolic acid and ursolic acid.

Compound 3: Colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.14 (1H, m, H-2), 5.73 (1H, m, H-7), 5.68 (1H, m, H-6), 4.76 (2H, m, H-1), 2.97 (2H, m, H-5), 1.28 (6H, s, CH_3-9 and CH_3-10). ^{13}C NMR (100 MHz, $CDCl_3$): d_C 174.1 (C-4), 145.3 (C-2), 141.2 (C-7), 132.8 (C-3), 121.2 (C-6), 70.4 (C-8), 70.3 (C-1), 29.6 (C-9), 29.6 (C-10), and 28.0 (C-5). Compared NMR data of 3 with those reported in literature,[7] compound 3 was identified as sibiscolacton.

Compound 4: Colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 4.20 (1H, dd, J = 11.6, 4.5 Hz, H_a-1), 4.15 (1H, dd, J = 11.6, 6.1 Hz, H_b-1), 3.93 (1H, m, H-2), 3.70 (1H, dd, J = 11.4, 3.9 Hz, H_a-3), 3.60 (1H, dd, J = 11.4, 5.8 Hz, H_b-3), 2.35 (2H, t, J = 7.6 Hz, H-2'), 1.63 (2H, m, H-3'), 1.28 (24H, m, H-4'~H-15'), and 0.88 (3H, t, J = 6.8 Hz, H-16'). NMR data comparison of 4 with those reported in literature,[17] together with the comparison between 4 and standard in three different developing solvents (cyclohexane-acetone, 5:4; cyclohexane-EtOAc, 1:1.5; and $CHCl_3$-acetone, 4:1), confirmed that compound 4 was monopalmitin.

Compound 5: Colorless gum. $[\alpha]_D^{25}$ + 8.3 (c 0.001, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 5.51 (1H, t, J = 6.5 Hz, H-2), 4.19 (2H, d, J = 6.8 Hz, H-1), 3.81 (1H, m, H-5), 2.20 (1H, dd, J = 13.5, 3.1 Hz, H_a-4), 2.06 (1H, dd, J = 13.5, 9.2 Hz, H_b-4), 1.80 (1H, m, H-7), 1.72 (3H, s, CH_3-10), 1.42 (1H, ddd, J = 13.9, 8.7, 5.5 Hz, H_a-6), 1.22 (1H, ddd, J = 13.3, 8.3, 4.1 Hz, H_b-6), 0.92 and 0.92 (each 3H, d, J = 6.4 Hz, CH_3-8 and CH_3-9). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 136.2 (C-3), 126.5 (C-2), 66.6 (C-5), 58.9 (C-1), 48.0 (C-4), 46.2 (C-6), 24.4 (C-7), 23.1 and 21.8 (C-8 and C-9), 16.1 (C-10). Compared NMR data with those reported in literature,[18-20] 5 was identified to be dihydroneroloxide, but no absolute configuration was given in these literatures. To assign the absolute configuration, the ECD data of two possible isomers were calculated and compared with experimental spectrum. The results showed that the theoretical ECD data for (5*S*)-isomer was in good agreement with the experimental spectrum [Figure 2]. Thus, the structure of 5 was established as (5*S*)-dihydroneroloxide.

Compound 6: Colorless needle crystal. Melting point 169°C–172°C. ^{1}H NMR (600 MHz, $CDCl_3$ + CD_3OD): δ_H 7.56 (1H, d, J = 15.8 Hz, H-7), 7.08 (1H, d, J = 1.8 Hz, H-2), 7.02 (1H, dd, J = 8.2, 1.6 Hz, H-6), 6.88 (1H, d, J = 8.2 Hz, H-5), 6.27 (1H, d, J = 15.7 Hz, H-8), and 3.90 (3H, s, OCH_3). NMR data comparison of 6 with those reported in literature,[21] together with the comparison between 5 and standard in three different developing solvents (cyclohexane-acetone, 2:1; cyclohexane-EtOAc, 1:1.5; and $CHCl_3$-acetone, 4:1), confirmed that compound 6 was ferulic acid.

Compound 7: White amorphous powder. Melting point 277°C–279°C. ^{1}H NMR (600 MHz, $CDCl_3$ + CD_3OD): δ_H 4.72 (1H, s, H_a-29), 4.59 (1H, s, H_b-29), 3.63 (1H, dt, J = 10.9, 4.3 Hz, H-2), 3.01 (1H,

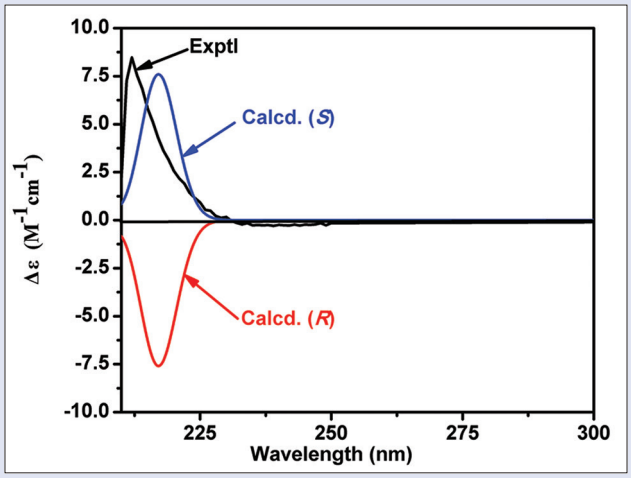

Figure 2: Experimental and calculated electronic circular dichroism spectra of compound 5

m, H-19), 2.92 (1H, d, J = 9.6 Hz, H-3), 2.26 (1H, m, H-13), 1.69, 1.00, 0.99, 0.95, 0.90, 0.78 (each 3H, s, 6×CH_3); ^{13}C NMR (150 MHz, $CDCl_3$ + CD_3OD): 179.6 (C-28), 151.2 (C-20), 109.9 (C-29), 83.9 (C-3), 69.2 (C-2), 56.8 (C-17), 56.1 (C-5), 51.2 (C-9), 49.8 (C-18), 47.7 (C-1), 47.4 (C-19), 43.0 (C-14), 41.3 (C-8), 39.9 (C-4), 38.9 (C-10), 38.9 (C-13), 37.6 (C-22), 34.8 (C-7), 32.8 (C-16), 31.1 (C-21), 30.2 (C-15), 28.8 (C-23), 26.1 (C-12), 21.6 (C-11), 19.5 (C-30), 18.9 (C-6), 17.6 (C-25), 16.9 (C-24), 16.3 (C-26), and 14.9 (C-27). Compared NMR data of 7 with those reported in literature,[22,23] compound 7 was identified as alphitolic acid.

Compound 8: White amorphous powder. Melting point 145°C–147°C. ^{1}H NMR (600 MHz, $CDCl_3$ + CD_3OD): δ_H 7.55 (1H, d, J = 15.9 Hz, H-7), 7.05 (1H, d, J = 1.7 Hz, H-2), 6.93 (1H, dd, J = 8.1, 1.8 Hz, H-6), 6.81 (1H, d, J = 8.1 Hz, H-5), 6.23 (1H, d, J = 15.9 Hz, H-8), 4.23 (2H, q, J = 7.1 Hz,-OCH_2CH$_3$), and 1.33 (3H, t, J = 7.1 Hz,-OCH$_2$CH_3). NMR data comparison of 8 with those reported in literature,[24] together with the comparison between 5 and standard in three different developing solvents (cyclohexane-acetone, 1.5:1; cyclohexane-EtOAc, 6:5; and $CHCl_3$-acetone, 4:1), confirmed that compound 8 was ethyl caffeate.

CONCLUSIONS

The leaves and swigs of *S. angustata* were popularly used by Tibetans of Hengduan Mountains for their benefit of health, and such traditional application awakened our research interest. Phytochemical research on medicinal plant will be helpful in clarifying its active ingredients. Our investigation has led to the isolation of 8 known compounds, including 2 monoterpenoids and 3 triterpenoids, and 4 of them were isolated from this plant for the first time. Previous studies indicated that monoterpenoids and their glycosides were the characteristic components of this plant, and some of them showed hypolipidemic and anti-obesity activities,[6,8,9] which may be responsible for its pharmacological effects. In this study, we also isolated two monoterpenoids, among which one was newly isolated, and such results further enriched the chemical constituents of *S. angustata*.

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Conflicts of interest

There are no conflicts of interest.

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