CHEMICAL CONSTITUENTS OF *Saussurea superba*

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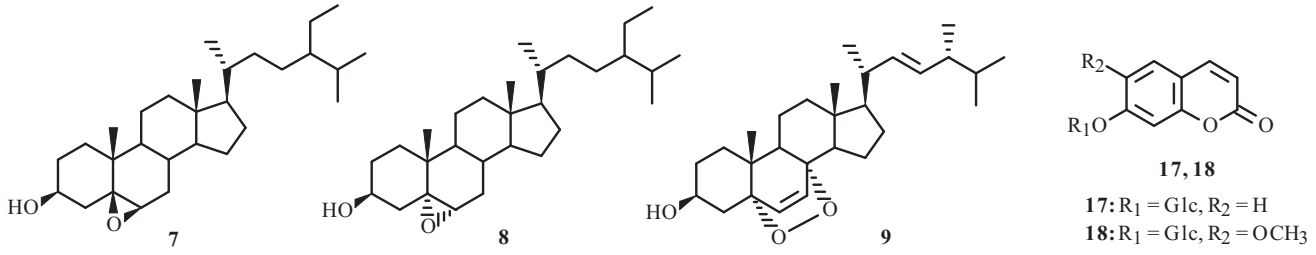
Saussurea superba Anthony belongs to the Compositae family and is one of the major medicinal plants in China. Its roots have long been used as folk medicine to treat influenza, pharyngeal swelling, measles, urticaria, and food poisoning [1]. The present research deals with the isolation and elucidation of compounds **1–20** from the whole plant of *S. superba.*

The compounds isolated were identified as follows: stigmast-1,4-dien-3-one (**1**) [2], stigmast-4-en-3-one (**2**) [3], 6β-hydroxystigmast-4-en-3-one (**3**) [3], β-sitosterol (**4**) [3], sitosterol-5-en-3β,7α-diol (**5**) [3], 3β-hydroxystigmast-5-en-7-one (**6**) [3, 4], 5,6β-epoxy-5β-sitostan-3β-ol (**7**) [5], 5,6α-epoxy-5α-sitostan-3β-ol (**8**) [12], 5α,8α-epidioxy-24(*S*)-methylcholesterol-6,22-dien-3β-ol (**9**) [6], lupeol (**10**) [7, 8], α-amyrin (**11**) [9], 3β-hydroxy-urs-12-en-11-one (**12**) [10], luteolin (**13**) [11], luteolin-3′-*O*-β-D-glucopyranoside (**14**) [12], umbelliferone (**15**) [13], scopoletin (**16**) [14], skimmin (**17**) [13, 15], scopolin (**18**) [13, 15], 2-hydroxy-4-acetoanisole (**19**) [16], and phenylacetic acid (**20**) [17]. These compounds were characterized by spectral data and comparison with those reported in previous work.

Plant Materials. The whole plant of *Saussurea superba* was collected in Huzhu county, Qinghai province, P. R. China, in August 2002. It was identified by Dr. Huan-Yang Qi. The voucher specimens (2002002) were deposited in the Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, P. R. China.

Extract, Isolation, and Purification. The dried plant material (2.0 kg) was powdered and extracted with 95% of industrial ethanol at 60°C for 24 h and then evaporated. The residue (120 g) was mixed with 500 mL water and extracted with petroleum ether, EtOAc, and *n*-butanol, respectively, to yield three parts (A, B, and C). Part A (30 g) was chromatographed on a silica gel column, eluting with petroleum ether (60–90°C)–acetone (v/v = 30:1, 20:1, 15:1, 10:1, 5:1, 3:1, 1:1, each about 3.0 L), to give four fractions (A1–A4) after concentration at reduced pressure. Fraction A1 was applied to a silica gel column, eluting with petroleum ether–EtOAc (v/v, from 30:1 to 25:1), to yield compounds **19** (600 mg), **4** (168 mg), and **10** (20 mg). Fraction A2 was eluted on a silica gel column with $CHCl_3$–acetone (40:1) to give compounds **1** (7 mg) and **3** (5 mg) after purified by pre-TLC. Compounds **2** (27 mg) and **11** (4 mg) were subsequently crystallized from fraction A3, eluting with $CHCl_3$–acetone (20:1). Fraction A4 was eluted on a silica gel column with $CHCl_3$–EtOAc (15:1) to afford a crude mixture of **5**, **9**, and **6** (10 mg) and **12** (3 mg). The mixture was processed by pre-TLC to give crystals **5** (2 mg) and **9** (7 mg). Part B (20 g) was eluted on a silica gel column with petroleum ether–EtOAc (6:1) and petroleum ether–acetone (v/v, from 10:1 to 7:1) to afford compounds **13** (14 mg), **15** (1 g), and **16** (14 mg), and then by pre-TLC (petroleum ether–EtOAc, 8:1) to give white solids **20** (120 mg), **7** (2 mg), and **8** (2 mg). Part C (54 g) was applied to a silica gel column, eluting with a $CHCl_3$–CH_3OH gradient to yield **14** (272 mg), **17** (19 mg), and **18** (7 g), respectively.



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The ^{1}H NMR and ^{13}C NMR spectra of the compounds in $CDCl_3$ or DMSO-d_6 were measured on a Varian INOVA-400/300 FT-NMR spectrometer with TMS as internal standard. Mass spectra were carried out on a Bruker APEX II. Melting points were measured on an X-4 Digital Display micro-melting apparatus and are uncorrected. Silica gel (200–300 mesh, Marine Chemical Factory, Qingdao, China) was used for column chromatography.

5,6β-Epoxy-5β-sitostan-3β-ol (7). White solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.96 (1H, m, H-3), 3.38 (1H, d, J = 2.3, H-6), 0.61 (3H, s, H-18), 1.22 (3H, s, H-19), 1.01 (3H, d, J = 6.7, H-21), 0.89 (3H, d, J = 6.5, H-26), 0.85 (3H, d, J = 6.5, H-27), 1.04 (3H, t, J = 7.2, H-29). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, δ, ppm): 37.21 (C-1), 31.03 (C-2), 69.44 (C-3), 42.26 (C-4), 62.91 (C-5), 63.72 (C-6), 32.59 (C-7), 29.74 (C-8), 51.04 (C-9), 34.82 (C-10), 21.97 (C-11), 39.79 (C-12), 42.21 (C-13), 53.81 (C-14), 24.17 (C-15), 28.15 (C-16), 55.59 (C-17), 11.89 (C-18), 15.35 (C-19), 36.05 (C-20), 18.71 (C-21), 33.85 (C-22), 25.98 (C-23), 44.37 (C-24), 29.08 (C-25), 13.91 (C-26), 19.80 (C-27), 23.02 (C-28), 12.11 (C-29).

5,6α-Epoxy-5α-sitostan-3β-ol (8). White solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 4.20 (1H, m, H-3), 2.93 (1H, d, J = 4.0, H-6), 0.69 (3H, s, H-18), 1.21 (3H, s, H-19), 0.81 (3H, d, J = 6.4, H-21), 0.83 (3H, d, J = 6.6, H-26), 0.92 (3H, d, J = 6.6, H-27), 0.84 (3H, t, J = 7.2, H-29). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 32.36 (C-1), 31.01 (C-2), 67.33 (C-3), 39.81 (C-4), 72.66 (C-5), 59.04 (C-6), 28.73 (C-7), 29.84 (C-8), 51.78 (C-9), 35.09 (C-10), 20.59 (C-11), 39.62 (C-12), 42.29 (C-13), 56.16 (C-14), 24.02 (C-15), 28.07 (C-16), 55.75 (C-17), 19.03 (C-18), 15.89 (C-19), 36.09 (C-20), 18.66 (C-21), 34.10 (C-22), 26.01 (C-23), 45.85 (C-24), 29.07 (C-25), 18.98 (C-26), 19.79 (C-27), 22.99 (C-28), 18.67 (C-29).

5α,8α-Epidioxy-24(*S*)-methylcholesterol-6,22-dien-3β-ol (9). Colorless needle crystal. ESI-MS: $[M]^+$ 428. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.94 (1H, m, H-3), 6.50 (1H, d, J = 8.4, H-6), 6.25 (1H, d, J = 8.4, H-7), 0.87 (3H, s, H-18), 0.81 (3H, s, H-19), 0.89 (3H, d, J = 6.5, H-21), 5.22 (1H, d, J = 9.0, H-22), 5.14 (1H, d, J = 9.0, H-23), 0.97 (3H, d, J = 6.7, H-26), 0.80 (3H, d, J = 6.7, H-27), 0.84 (3H, d, J = 6.6, H-28). The ^{13}C NMR spectrum was identical to that in the literature [6].

Lupeol (10). White needles, mp 208–210°C. The ^{1}H NMR and ^{13}C NMR spectra were identical to those in the literature.

α-Amyrin (11). Colorless needle crystals, mp 184–185°C. The ^{1}H NMR and ^{13}C NMR spectra were identical to those in the literature.

Skimmin (17). Yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.27 (1H, d, J = 9.6, H-3), 7.93 (1H, d, J = 9.6, H-4), 7.59 (1H, d, J = 9.0, H-5), 6.98 (1H, d, J = 9.0, H-6), 7.00 (1H, s, H-8). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 161.27 (C-2), 113.92 (C-3), 144.99 (C-4), 130.11 (C-5), 113.64 (C-6), 160.66 (C-7), 103.71 (C-8), 155.44 (C-9), 114.35 (C-10), 100.47 (C-1′), 76.76 (C-2′), 73.61 (C-3′), 70.18 (C-4′), 77.45 (C-5′), 61.20 (C-6′).

Scopolin (18). Yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.31 (1H, d, J = 9.6, H-3), 7.94 (1H, d, J = 9.6, H-4), 7.26 (1H, s, H-5), 7.14 (1H, s, H-8), 3.80 (3H, s, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 56.07 (OCH_3), 160.65 (C-2), 112.33 (C-3), 144.31 (C-4), 109.67 (C-5), 146.04 (C-6), 149.91 (C-7), 103.03 (C-8), 148.96 (C-9), 113.35 (C-10), 99.65 (C-1′), 76.80 (C-2′), 73.13 (C-3′), 69.69 (C-4′), 77.17 (C-5′), 60.73 (C-6′).

2-Hydroxy-4-acetoanisole (19). White crystals, mp 47–50°C. TLC and IR spectra were identical to those of an authentic sample.

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