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CHEMICAL CONSTITUENTS OF XYLEM OF *Sophora japonica* ROOTS

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Sophora japonica (Leguminosae), a deciduous tree species, is widely distributed in various areas including China, Korea, Japan, and other countries [1]. The buds, flowers, barks, and leaves of *S. japonica* have long been used in traditional medicines for treating bleeding hemorrhoids, hematuria, hematemesis, hemorrhinia, uterine or intestinal hemorrhage, leukorrhea, conjunctivitis, pyoderma, arteriosclerosis, hypertension, and dizziness [1, 2]. Previous phytochemical investigations of the leaves, fruits, and barks of *S. japonica* led to the isolation of steroids, phospholipids, flavonoids, alkaloids, triterpenes, and other phenolic compounds [3–8]. Some of these chemical constituents have exhibited significant biological properties such as anti-inflammatory [9], anti-tumor [10, 11], and anti-oxidant activities [12]. However, to date, no study has been carried out to investigate the secondary metabolites from the xylem of *S. japonica* roots.

As part of our ongoing research to find natural biological compounds from *S. japonica*, the petroleum ether soluble extract from the xylem of *S. japonica* roots was investigated, which resulted in the isolation of six compounds. The structures of the isolated constituents were identified as *n*-heptanol [13] (**1**), betulin (**2**) [14], betulin acid (**3**) [14], β-amyrin (**4**) [15], oleanolic acid (**5**) [16], and oleanolic acid 3-*O*-β-D-glucopyranoside (**6**) [17] by NMR and MS, as well as by comparison of their spectroscopic data with the literature values. It is worth noting that compounds **1**, **3**, **4**, **5**, and **6** were isolated for the first time from *S. japonica*.

The xylem of *S. japonica* roots was obtained from the campus forest in Tianjin University of Science and Technology, P. R. China, in October 2013. The plant materials were authenticated by Dr. Wang Dan from Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry, P.R. China. A voucher specimen (No. 20131006) has been deposited in Tianjin Key Laboratory of Pulp and Paper, Tianjin University of Science and Technology, P. R. China. The xylem of *S. japonica* roots (5080.05 g) was air-dried, finely powdered, then extracted four times (each for 0.5 h) with 95% ethanol (v/v) by an ultrasound-assisted method. The extract was concentrated under reduced pressure to give a crude residue (467.21 g) and then suspended in distilled water. By fractionation with petroleum ether, ethyl acetate, and *n*-butanol, 73.80 g of petroleum ether soluble extract, 121.36 g of ethyl acetate soluble extract, and 78.65 g of *n*-butanol soluble extract were obtained after lyophilization.

A portion of the petroleum ether soluble extract (65.02 g) was fractionated on a silica gel (mesh 100–200) column and eluted with hexane–EtOAc–MeOH (1:0:0→10:1:0→5:1:1→0:0:1) to give 10 major fractions (Frs. A_1–A_{10}) on the basis of TLC analysis. Fraction A_2 was subjected to silica gel (mesh 200–300) chromatography and eluted with hexane–CH_2Cl_2 (19:1) to afford compound **1** (41.0 mg). Fractions A_4, A_5, and A_7 were then further chromatographed over silica gel columns, eluting with hexane–CH_2Cl_2 (4:1), hexane–CH_2Cl_2 (9:1), and hexane–CH_2Cl_2 (1:1) to yield compounds **2** (20.8 mg), **3** (35.2 mg), and **4** (40.9 mg), respectively. Fraction A_8 was also subjected to silica gel column chromatography with petroleum ether–acetone (10:1→2:1) serving as mobile solvent to give four subfractions labeled Subfrs. B_1–B_4. Compound **5** (45.3 mg) was crystallized

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from Subfr. B_2. Fraction A_{10} was also purified repeatedly on a Sephadex LH-20 column using MeOH–H_2O (4:1 and 1:1) as eluting solvent to give three subfractions (Subfrs. B_5–B_7). Subfraction B_6 was further purified by preparative HPLC to obtain the yellowish compound **6** (41.0 mg).

***n*-Heptanol (1)**. $C_7H_{16}O$, white oil. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.63 (1H, d, J = 48.4, 1-OH), 3.88 (2H, m, H-1), 1.67 (2H, d, J = 9.9, H-2), 1.55 (2H, dd, J = 17.9, 8.8, H-3), 1.26 (6H, m, H-4, 5, 6), 0.88 (3H, t, J = 6.5, H-7). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 63.10 (C-1), 32.83 (C-2), 31.94 (C-5), 29.85 (C-4), 25.76 (C-3), 22.70 (C-6), 14.12 (C-7). ESI-MS *m/z* 117 $[M + H]^+$.

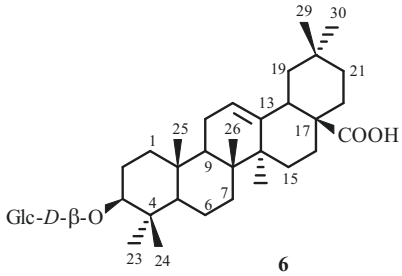
Betulin (2). $C_{30}H_{50}O_2$, white powder. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.68 (1H, d, H-29b), 4.55 (1H, d, H-29a), 3.79 (1H, d, J = 10.8, H-28b), 3.33 (1H, d, J = 10.8, H-28a), 3.18 (1H, dd, J = 5.3, H-3), 2.36 (1H, d, H-19), 1.66 (3H, s, H-30), 0.98 (3H, s, H-26), 0.96 (3H, s, H-23), 0.74 (3H, s, H-24), 0.81 (3H, s, H-25), 1.00 (3H, s, H-27). ESI-MS *m/z* 443 $[M + H]^+$.

Betulinic Acid (3). $C_{30}H_{48}O_3$, white powder. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.74 (1H, d, H-29b), 4.55 (1H, d, H-29a), 3.18 (1H, dd, J = 4.8, H-3), 3.00 (1H, m, J = 10.0, 5.4, H-19), 2.36 (1H, d, H-13), 2.18 (1H, d, J = 12.2, 3.6, H-21), 1.68 (3H, s, H-30), 0.97 (3H, s, H-26), 0.96 (3H, s, H-27), 0.93 (3H, s, H-23), 0.82 (3H, s, H-25), 0.75 (3H, s, H-24). ESI-MS *m/z* 457 $[M + H]^+$.

***β*-Amyrin (4)**. $C_{30}H_{50}O$, white powder. ^1H NMR (400 MHz, C_5D_5N, δ, ppm, J/Hz): 5.47 (1H, dd, J = 21.3, 3.9, H-12), 4.98 (1H, s, 3-OH), 3.85 (1H, dd, J = 22.3, 17.5, H-3), 1.15 (3H, s, CH_3-27), 0.96 (9H, s, CH_3-23, 24, 28), 0.90 (6H, s, CH_3-25, 26), 0.71 (6H, s, CH_3-29, 30). ESI-MS *m/z* 427 $[M + H]^+$.

Oleanolic Acid (5). $C_{30}H_{48}O_3$, white powder. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.30 (1H, s, H-12), 3.22 (1H, d, J = 7.1, H-3), 2.82 (1H, d, J = 11.1, H-18), 0.91 (3H, s, H-23), 0.78 (3H, s, CH_3-24), 0.79 (3H, s, CH_3-25), 0.90 (3H, s, CH_3-26), 1.15 (3H, s, CH_3-27), 0.92 (3H, s, CH_3-29), 1.00 (3H, s, CH_3-30). ESI-MS *m/z* 455 $[M - H]^-$.

Oleanolic Acid 3-*O*-*β*-D-Glucopyranoside (6). $C_{36}H_{58}O_8$, white powder. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.30 (1H, s, H-12), 4.59 (1H, s, H-1′), 4.42 (1H, dd, J = 12.0, 3.0, H-6′), 4.29 (t, J = 9.5, H-3′), 4.07 (t, J = 9.5, H-2′), 3.98–3.94 (m, H-4′, 5′). 3.58 (1H, d, J = 2.0, H-3), 0.92 (3H, s, H-23), 0.74 (3H, s, CH_3-24), 0.76 (3H, s, CH_3-25), 0.90 (3H, s, CH_3-26), 1.16 (3H, s, CH_3-27), 0.92 (3H, s, CH_3-29), 0.98 (3H, s, CH_3-30). ESI-MS *m/z* 619 $[M + H]^+$.



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