Biochemical Systematics and Ecology 61 (2015) 424–428

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Chemical constituents from *Lagopsis supina* (Steph.) Ik.-Gal. ex Knorr

Jing Zhang [a, b], Zheng Huang [a, b], Hui-Xia Huo [a, b], Yue-Ting Li [a, b], Dao-Ran Pang [a, b], Jiao Zheng [a], Qian Zhang [a], Yun-Fang Zhao [a], Peng-Fei Tu [a], Jun Li [a, *]

[a] *Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, People's Republic of China*
[b] *School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, People's Republic of China*

ARTICLE INFO

Article history:
Received 14 May 2015
Received in revised form 10 July 2015
Accepted 11 July 2015
Available online 20 July 2015

Keywords:
Lagopsis supina
Labiatae
Phenylethanoid glycoside
Phenylmethanoid glycoside
Megastigmane glycoside
Monoterpenoid glycoside

ABSTRACT

Phytochemical investigation of the whole plants of *Lagopsis supina* (Steph.) Ik.-Gal. ex Knorr. led to the isolation of 18 compounds (**1**–**18**), including ten phenylethanoid glycosides (**1**–**10**), one phenylmethanoid glycoside (**11**), four megastigmane glycosides (**12**–**15**), and three monoterpenoid glycosides (**16**–**18**). Lagopsides A (**1**) and B (**2**) were identified as new phenylethanoid glycosides. This is the first report of compounds **7**, **11**, **12**, **15**, and **16** from the Labiatae family, while compounds **4**–**6**, **8**–**10**, **13**–**14**, and **17**–**18** were isolated from the genus *Lagopsis* for the first time. The chemotaxonomic significance of these isolated compounds was summarized.

1. Subject and source

The genus *Lagopsis* belongs to the Labiatae family, and three *Lagopsis* species are native to China. *Lagopsis supina* is a perennial herbaceous plant and widely distributed in northeast Asia (Editorial Committee of CAS "Flora of China", 1977). The whole plant, also known as "Xiazhicao" in Chinese, has been used in traditional Chinese medicine for the treatment of gynecologic disorders such as menorrhagia, irregular menstruation, and painful menstruation, as well as edema in acute and chronic nephritis (Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 1999). The whole plants of *L. supina* were collected from Beijing Botanical Garden, in April 2012, and the plant material was authenticated by one of the authors (P.-F. Tu). A voucher specimen (No. JLI-LS-201204) is deposited at Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine (Beijing, China).

2. Previous work

Previous chemical investigations on the whole plants of *Lagopsis supina* have revealed the presence of phenylethanoid glycosides (Yang et al., 2001), flavonoid glycosides (Li et al., 2002), and labdane diterpenoids (Li et al., 2014) in *L. supina*.

* Corresponding author.
E-mail address: drlj666@163.com (J. Li).

http://dx.doi.org/10.1016/j.bse.2015.07.010
0305-1978/

3. Present study

The air-dried and powdered whole plants of *L. supina* (3.0 kg) were extracted with acetone (20 L × 3) and MeOH (12 L × 2) by maceration at room temperature for 2 days. After filtration, combination, and solvent evaporation, the residue (313.8 g) was dissolved in 90% aqueous MeOH and successively partitioned with *n*-hexane and CH_2Cl_2 to afford *n*-hexane (71.0 g), CH_2Cl_2 (30.4 g), and water (197.5 g) extracts, respectively. The water extract (197.5 g) was subjected to D101 macroporous resin column chromatography (CC), eluting with H_2O, 20%, 40%, 60%, and 80% aqueous EtOH, to give five fractions (A–E). Fr. B (13.3 g) was subjected to Sephadex LH-20 CC, eluting with MeOH–H_2O (1:1, v/v), to yield eight subfractions (B1–B8). B3 (1.6 g) was applied to Sephadex LH-20 CC followed by separation over silica gel CC to afford 16 subfractions (B3a–B3p). B3f (32.8 mg) was purified by semipreparative RP-HPLC eluting with isocratic 15% aqueous MeOH to yield **12** (8.2 mg, $t_R = 22.4$ min) and **13** (9.4 mg, $t_R = 26.4$ min). B3i (37.5 mg) was purified by semipreparative RP-HPLC eluting with isocratic 15% aqueous MeOH to give **14** (10.3 mg, $t_R = 31.5$ min). B3j (45.0 mg) was purified by semipreparative RP-HPLC using 9% aqueous ACN as mobile phase to obtain **16** (12.1 mg, $t_R = 19.7$ min). B3k (43.8 mg) was purified by semipreparative RP-HPLC eluting with isocratic 9% aqueous ACN to yield **15** (7.2 mg, $t_R = 25.3$ min). B4 (7.1 g) was chromatographed over silica gel CC eluting with a stepwise gradient of EtOAc-EtOH-H_2O (40:2:1 → 5:2:1, v/v) to afford eight subfractions (B4a – B4h). B4c (0.5 g) was subjected to Sephadex LH-20 CC eluting with MeOH to give 11 subfractions (B4c1–B4c11). B4c4 (0.2 g) was applied to RP-C_{18} CC followed by purification on semipreparative RP-HPLC (isocratic 11% aqueous ACN) to afford **17** (15.5 mg, $t_R = 61.3$ min) and **18** (14.8 mg, $t_R = 67.4$ min). Fr. C (15.0 g) was chromatographed over silica gel CC, eluting with a stepwise gradient of EtOAc-EtOH-H_2O (40:2:1 → 5:2:1, v/v) to yield 15 subfractions (C1–C15). C3 (3.7 g) was fractionated into 8 portions (C3a–C3h) by Sephadex LH-20 CC eluted with MeOH. C3d (0.2 g) was chromatographed over silica gel CC followed by semipreparative RP-HPLC using an isocratic elution of 21% aqueous ACN to obtain **5** (8.2 mg, $t_R = 12.0$ min). C9 (2.3 g) was applied to Sephadex LH-20 CC eluting with MeOH to give 13 subfractions (C9a – C9m). C9c (0.4 g) was chromatographed on silica gel CC followed by semipreparative RP-HPLC eluting with a gradient of 25%–66% aqueous MeOH within 24 min to yield **9** (20.3 mg, $t_R = 19.8$ min) and **2** (4.1 mg, $t_R = 22.5$ min). C10 (0.6 g) was subjected to Sephadex LH-20 CC eluting with MeOH to afford **4** (48.6 mg) and six subfractions (C10a–C10f). C10c (67.0 mg) was purified by semipreparative RP-HPLC eluting with a gradient of 10%–38% aqueous ACN within 24 min to give **8** (14.7 mg, $t_R = 17.6$ min). C10d (53.7 mg) was purified by semipreparative RP-HPLC eluting with a gradient of 10%–45% aqueous ACN within 24 min to afford **10** (11.5 mg, $t_R = 15.0$ min). Fr. D (3.1 g) was subjected to Sephadex LH-20 CC eluting with MeOH to yield 14 subfractions (D1–D14). D6 and D7 (0.5 g) were combined and chromatographed over silica gel CC followed by semipreparative RP-HPLC (isocratic 26% aqueous ACN) to afford **3** (9.5 mg, $t_R = 9.7$ min), **11** (8.5 mg, $t_R = 22.7$ min), **7** (20.6 mg, $t_R = 24.5$ min), **6** (18.2 mg, $t_R = 35.3$ min), and **1** (4.0 mg, $t_R = 52.6$ min), respectively.

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{21}$ − 27 (*c* 0.1, MeOH). Its molecular formula was determined as $C_{33}H_{40}O_{16}$ by negative HRESIMS, which gave a quasi-molecular ion peak at *m/z* 691.2239 $[M-H]^-$ (calcd for $C_{33}H_{39}O_{16}$ 691.2244). The IR spectrum of **1** showed absorption bands at 3422, 1705, 1600, and 1509 cm^{-1} ascribable to hydroxyls, ester carbonyls, and aromatic rings. The ^1H NMR spectrum of **1** exhibited the characteristic signals belonging to 3,4-dihydroxyphenylethyl, *trans*-caffeoyl moieties, and 2-butenoyl group: two sets of *ortho*- and *meta*-coupled ABX-type aromatic protons [δ_H 7.06 (1H, d, $J = 1.5$ Hz, H-2‴), 6.96 (1H, dd, $J = 8.0$, 1.5 Hz, H-6‴), 6.78 (1H, d, $J = 8.0$ Hz, H-5‴); δ_H 6.69 (1H, d, $J = 1.5$ Hz, H-2), 6.68 (1H, d, $J = 8.0$ Hz, H-5), 6.56 (1H, dd, $J = 8.0$, 1.5 Hz, H-6)], four *trans*-olefinic protons [δ_H 7.58 (1H, d, $J = 16.0$ Hz, H-7‴), 6.98 (1H, dd, $J = 15.5$, 6.0 Hz, H-3‴), 6.27 (1H, d, $J = 16.0$ Hz, H-8‴), 5.84 (1H, dd, $J = 15.5$, 1.5 Hz, H-2‴)], and two methylene protons [δ_H 3.95, 3.73 (each 1H, m, H-8), 2.79 (2H, m, H-7)]. The ^1H and ^{13}C NMR spectroscopic data (Table 1) of **1** were similar to those of acteoside (**3**) (Li et al., 2005). The main difference was the presence of an additional 2-butenoyl group [δ_C 167.8, 147.2, 123.2, 18.2; δ_H 6.98 (1H, dd, $J = 15.5$, 6.0 Hz), 5.84 (1H, dd, $J = 15.5$, 1.5 Hz), 1.80 (3H, dd, $J = 7.0$, 1.5 Hz)] in **1**. The deshielded H_2-6′ resonance (δ_H 4.20) of glucose moiety indicated that the 2-butenoyl group was located at C-6′. This deduction was confirmed by the HMBC correlation between H_2-6′ and the carbonyl carbon (δ_C 167.8, C-1‴). Connectivities between aglycone and sugars in **1** were further confirmed by an HMBC experiment, which showed long-range correlations from δ_H 4.40 (H-1′) to δ_C 72.7 (C-8), from δ_H 5.20 (H-1″) to δ_C 81.5 (C-3′), and from δ_H 5.00 (H-4′) to δ_C 168.2 (C-9‴) (Fig. 1). Thus, the structure of compound **1** was established as 2-(3,4-dihydroxyphenyl)-ethanol-*O*-α-L-rhamnopyranosyl-(1 → 3)-4-*O*-*trans*-caffeoyl-6-*O*-(2-butenoyl)-β-D-glucopyranoside and named lagopside A.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{21}$ − 60 (*c* 0.1, MeOH). Its molecular formula was determined as $C_{34}H_{44}O_{17}$ by negative HRESIMS, which gave a deprotonated ion at *m/z* 723.2513 $[M-H]^-$ (calcd for $C_{34}H_{43}O_{17}$ 723.2506). The IR spectrum of **2** showed absorption bands at 3422, 1701, 1636, and 1541 cm^{-1} ascribable to hydroxyls, ester carbonyls, and aromatic rings. The ^1H NMR spectrum of **2** exhibited the characteristic signals belonging to phenylethyl and *trans*-caffeoyl moieties: ABX-type aromatic protons [δ_H 7.06 (1H, d, $J = 2.0$ Hz, H-2‴), 6.96 (1H, dd, $J = 8.5$, 2.0 Hz, H-6‴), 6.78 (1H, d, $J = 8.5$ Hz, H-5‴)], two *trans*-olefenic protons [δ_H 7.60 (1H, d, $J = 16.0$ Hz, H-7‴), 6.28 (1H, d, $J = 16.0$ Hz, H-8‴)], mono-substituted phenyl protons [δ_H 7.27 (4H, m, H-2, 3, 5, 6), 7.19 (1H, m, H-4)], and two methylene protons [δ_H 4.12, 3.81 (each 1H, m, H-8), 2.96 (2H, t, $J = 7.0$ Hz, H-7)]. In the ^1H NMR spectrum, three anomeric proton signals were observed at δ_H 4.40 (1H, d, $J = 7.5$ Hz, H-1′), 5.49 (1H, br s, H-1″), and 4.32 (1H, d, $J = 7.0$ Hz, H-1‴), respectively, indicating the presence of three sugar moieties in compound **2**. The ^1H and ^{13}C NMR data (Table 1) of **2** were similar to those of jionoside C (**6**) (Li et al., 2005). The main difference was the presence of an α-L-arabinopyranosyl moiety in **2**. The significantly deshielded C-2″ resonance (δ_C 83.0; $\Delta\delta_C$ +10.8) suggested the arabinose moiety was linked to C-2″ of rhamnose moiety, which was supported by the HMBC correlation between H-1‴ (δ_H 4.32) and C-2″ (δ_C 83.0). Connectivities between aglycone and sugars in **2** were further