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Phytochemical and chemotaxonomic study on *Lagotis brevituba* (Scrophulariaceae)

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ABSTRACT

A phytochemical investigation of *Lagotis brevituba* led to the isolation of 16 compounds, including five phenylpropanoids (**1**–**5**), eight flavonoids (**6**–**13**), one iridoid (**14**), one phenolic compound (**15**) and one triterpene (**16**). The structures of these compounds were identified by spectroscopic methods and a comparison of their data with those reported in the literature. This is the first report of compounds **1**, **2**, **7**–**13** and **15** from the genus *Lagotis*. The chemotaxonomic significance of these compounds has also been summarized.

1. Subject and source

The genus *Lagotis* consists of about 30 species, which are widely distributed throughout the northern hemisphere (Editorial Committee of Flora of China (1979)). *Lagotis brevituba* is a perennial species that grows in mountainous regions at altitudes in the range of 3800–4800 m. This plant is endemic to the Qinghai, Gansu and Xizang Provinces of China and has been used for centuries in traditional Tibetan medicine for the treatment of hypertension and nephritis. The *L. brevituba* plants used in this study were collected from the Qilian mountains (E 100° 19′ 6.8″, N 38° 7′ 23.8″, 3900 m), Qinghai Province, China, in September 2014, and subsequently identified by Professor Mei Lijuan (Northwest Institute of Plateau Biology (NWIPB), Chinese Academy of Sciences, Xining, China). A voucher specimen (2014090101) was deposited in the herbarium of NWIPB, Xining, China.

Abbreviations: NWIPB, Northwest Institute of Plateau Biology.

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2. Previous work

Previous chemical investigations on plants belonging to the genus *Lagotis* resulted in the isolation of a range of structurally diverse compounds, including phenylpropanoids (Zheng and Shi, 2004; Yang et al., 2007a,b; Xi et al., 2009), flavonoids (Yang et al., 2004, 2007a,b), iridoids (Yang et al., 2005a,b,c), and triterpenes (Feng et al., 2000).

3. Present study

The *L. brevituba* plants were air-dried and powdered in a mill. The resulting powdered material (1 kg) was extracted three times with 95% EtOH at 70 °C and the combined EtOH extracts were evaporated under reduced pressure at 50 °C to give 80 g of residue. The residue was dissolved in water and then partitioned successively with petroleum ether, EtOAc and n-butanol. The n-butanol-soluble extract (31 g) was purified by column chromatography over AB-8 macroporous resin eluting with a linear gradient of water-EtOH (100:0 to 0:100, v/v) to yield four fractions (Fr. 1–4). Fr. 2 (12 g) was separated by column chromatography on Sephadex LH 20 eluting with CH_2Cl_2–MeOH (1:1, v/v) to give seven sub-fractions (Fr. 2-1 to 2-7). Fr. 2-1 (600 mg) was purified by column chromatography on silica gel eluted with a gradient of increasing concentrations of MeOH in CH_2Cl_2 to give two fractions (Fr. 2-1-1 and Fr. 2-1-2). Fr. 2-1-2 (400 mg) was further purified by column chromatography on silica gel eluting with CH_2Cl_2–MeOH (8:2, v/v) to give compounds **1** (130 mg) and **2** (81 mg). Fr. 2-4 (6 g) was purified by column chromatography on silica gel eluting with a gradient elution of CH_2Cl_2–MeOH (9:1 to 5:5, v/v), followed by semi-preparative HPLC (column, HC RP-C18, 10 μm, 250 × 20 mm; mobile phase, water-CH_3CN, 73:27 (v/v); flow rate, 15 mL/min; detection, 254 nm) to provide compounds **3** (2140 mg), **4** (173 mg) and **5** (58 mg). Fr. 2-6 (100 mg) was separated by column chromatography on silica gel eluting with CH_2Cl_2–MeOH (7:3, v/v) to afford compounds **6** (34 mg) and **7** (21 mg). Fr. 3 (3 g) was subjected to column chromatography on silica gel eluting with CH_2Cl_2 containing an increasing amount of MeOH to yield five sub-fractions (Fr. 3-1 to Fr. 3-5). Compound **8** (28 mg) was precipitated from Fr. 3-2 using MeOH. Fr. 3-3 (151 mg) was purified by semi-preparative HPLC (column, HC RP-C18, 10 μm, 250 × 20 mm; mobile phase, water-CH_3CN, 80:20 (v/v); flow rate, 15 mL/min; detection, 210 nm) to obtain compounds **9** (11 mg), **10** (83 mg) and **11** (6 mg). Fr. 3-4 (153 mg) was repeatedly purified by column chromatography on silica gel to afford compounds **12** (28 mg) and **13** (39 mg). Fr. 4 (3.2 g) was chromatographed on silica gel eluting with CH_2Cl_2–MeOH (9:1, v/v) to give three sub-fractions (Fr. 4-1 to Fr. 4-3). Fr. 4-2 (92 mg) was chromatographed on silica gel eluting with CH_2Cl_2–MeOH (9.5:0.5, v/v) to give compounds **14** (21 mg) and **15** (8 mg). Fr. 4-3 (60 mg) was purified by semi-preparative HPLC (column, HCE RP-C18, 7 μm, 250 × 20 mm; mobile phase, water-CH_3CN, 85:15 (v/v); flow rate, 15 mL/min; detection, 210 nm) to yield compound **16** (51 mg). The structures of the isolated compounds were elucidated based on a comparison of their spectroscopic data (ESI-MS, NMR) with those reported in the literature (Fig. 1). These compounds were therefore determined as CampneosideII (**1**) (Saimaru and Orihara, 2010), Suspensaside (**2**) (Ming et al., 1999), Verbascoside (**3**) (Kanchanapoom et al., 2001), Echinacoside (**4**) (Liu and Jia, 1991), Martynoside (**5**) (Pendota et al., 2015), Apigenin-7-O-β-D-glucuronide (**6**) (Eshbakova et al., 2014), Apigenin-7-O-β-D-Glucuronide methyl ester (**7**) (Lee et al., 2002), Quercetin (**8**) (Maranhão et al., 2013), Diosmetin-7-O-β-D-glucuronide methyl ester (**9**) (Du and Cui, 2007), Luteolin-7-O-glucuronide-6″-methyl ester (**10**) (Makino et al., 1998), 6″-O-(4-hydroxybenzoyl) hyperoside (**11**) (Chulia et al., 1995), 5,4′-dihydroxy-3′-methoxy flavone-7-O-β-D-galacturonide (**12**) (Feng et al., 2008), 5,4′-Dihydroxy-3′-methoxy flavone-7-O-6″-n-butyryl-β-D-glucopyranoside (**13**) (Chu et al., 2007), Pedicutricoside A (**14**) (Kobayashi et al., 1985), Apocynin (**15**) (Jung et al., 2002), ArveninsI (**16**) (Kawahara et al., 2004).

4. Chemotaxonomic significance

The current phytochemical study reports the isolation and structural elucidation of 16 compounds from the n-butanol-soluble extract of *L. brevituba*, including five phenylpropanoids (**1**–**5**), eight flavonoids (**6**–**13**), one iridoid (**14**), one phenolic compound (**15**) and one triterpene (**16**). Notably, this is the first report of compounds **1**, **2**, **7**–**13** and **15** from the genus *Lagotis*. Phenylpropanoids and flavonoids were isolated as the major components of *L. brevituba*. These compounds also represent the main components of several other species of *Lagotis* grown on the Qinghai–Tibet Plateau, including *Lagotis brachystachya*, *Lagotis ramalana* and *Lagotis alutacea* (Chen et al., 1989; Yang et al., 2007a,b; Liu et al., 2015). The high level of consistency observed for the chemical constituents of *Lagotis* from the Qinghai-Tibet Plateau could be attributed to similarities in their growth environment such as light and temperature.

Compounds **3**, **4** and **6** were previously extracted from *L. ramalana* (Yang et al., 2007a,b). Compound **3**, which was isolated as the major component of *L. ramalana* (Yang et al., 2007a,b), was also isolated as the main component of *L. brevituba* in the current study in a much higher yield of 2140 mg. Therefore, the isolation of compounds **3**, **4** and **6** from *L. brevituba* revealed the existence of a close chemotaxonomic relationship between these two species of *Lagotis*, especially in terms of their major component compound **3**. This compound could therefore be of great chemotaxonomic significance and serve as a valuable chemotaxonomic marker for *L. brevituba* and *L. ramalana*. Although compounds **7**, **9**, **10** and **12** were initially isolated from plants belonging to this genus, the skeleton types of apigenin and luteolin have been reported in *L. ramalana* (Yang et al., 2007a,b), which further confirms the chemotaxonomic relationship between *L. brevituba* and *L. ramalana*. Interestingly, compound **15** has a similar structure to vanillin, which was isolated from *L. brachystachy* and *L. alutacea* (Yang et al., 2005a,b,c; Liu et al., 2015). The isolation of compound **15** therefore suggests that these three species of *Lagotis* share a common biosynthetic pathway. Compound **1** was obtained from *Paulownia coreana* (Kim et al., 2008); compound **5** was obtained from