# Iridoid Glycosides from *Lagotis alutacea*

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*Z. Naturforsch.* **2014**, *69b*, 835 – 838 / DOI: 10.5560/ZNB.2014-4093
Received April 25, 2014

One new iridoid glycoside, lagotisoside F (**1**), was isolated from *Lagotis alutacea*, along with three known analogs, lagotisoside D (**2**), 6-*O*-$\alpha$-L-(4″-*O*-(*E*)-cinnamoyl) rhamnopyranosyl-catapol (**3**) and globularin (**4**). Their structures were elucidated by extensive spectroscopic analysis and by comparison with data reported in the literature. Compounds **1**–**4** were evaluated for cytotoxic activity against HL-60, MCF-7, A549, SW480, and SMMC-7721 cells and exhibited no appreciable activity with $IC_{50}$ values above 40 $\mu$M.

*Key words: Logatis alutacea*, Iridoid Glycosides, Lagotisoside F

## Introduction

The genus *Lagotis* (Scrophulariaceae) is represented with 17 species in China, mostly growing in the southwestern part of the country on mountains of 3000 meters above sea level or higher [1]. Several species such as *L. alutacea*, *L. glauca*, *L. yunnanesis*, and *L. brachystachya* have long been used in Tibetan folk medicine for the treatment of fever, high blood pressure, and acute and chronic hepatitis [2, 3]. In the literature, the chemical compositions of two species of *Lagotis* have been studied. Flavonoids were isolated from *L. brachystachya* [4], while phenylpropanoid glycosides and iridoid glycosides were found in *L. stolonifera* [5]. In our efforts to seek structurally interesting compounds from medicinal plants growing on the Yunnan-Tibet Plateau, we investigated the chemical constituents of *L. yunnanesis*, and have found a series of iridoid glycosides [6 – 8]. As part of continued investigations on the genus *Lagotis*, *L. alutacea* has been examined. To the best of our knowledge, no phytochemical study on *L. alutacea* has been reported as yet. As a result, one new iridoid glycoside, lagotisoside F (**1**), was isolated from this plant, together with three known analogs, lagotisoside D (**2**) [8], 6-*O*-$\alpha$-L-(4″-*O*-(*E*)-cinnamoyl) rhamnopyranosylcatapol (**3**) [9] and globularin (**4**) [10] (Fig. 1). Their structures were elucidated by extensive spectroscopic analysis and by comparison with data reported in the literature. Herein, we report on the isolation and structural elucidation of the new iridoid glycoside **1** from this plant.

## Results and Discussion

Lagotisoside F (**1**) was isolated as a colorless amorphous powder, $[\alpha]_D^{24} = -168.50$ ($c = 0.345$, MeOH). Its molecular formula was determined as $C_{32}H_{42}O_{17}$ by HR-FAB-MS (found 698.2425, calcd. 698.2422). The IR spectrum showed characteristic absorptions of OH (3375 $cm^{-1}$, br), of an $\alpha$, $\beta$-unsaturated ester (1710 and 1635 $cm^{-1}$) and an aromatic-ring (1598 and 1508 $cm^{-1}$). The UV absorptions at 222 (3.59), 303 (3.18) and 312 (3.74) nm also confirmed the presence of these unsaturated functional groups.

The $^1$H NMR spectrum (Table 1) of **1** indicated the presence of a catalpol unit (H-1, H-3 to H-10, H-1′ to H-6′) combined with a rhamnose unit (H-1″ to H-6″) and a 3,4-disubstituted-(*E*)-cinnamoyl moiety (H-2‴, H-5‴, H-6‴, H-7‴, and H-8‴). The

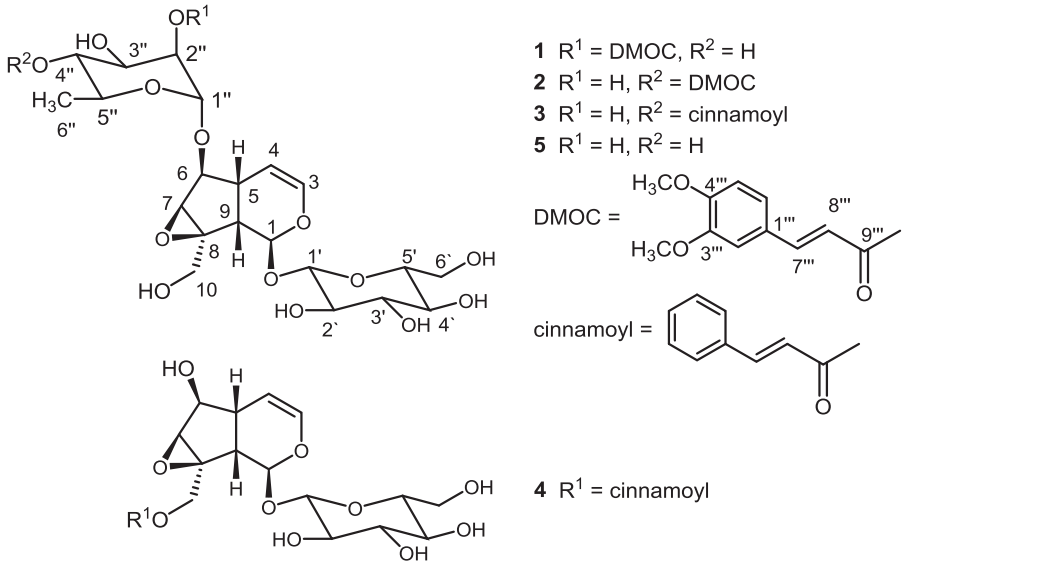


Fig. 1. The structures of compounds **1**–**5**.

two olefinic protons of the cinnamoyl moiety at $\delta = 7.66$ (d, $J = 15.6$ Hz, H-7‴) and $\delta = 6.47$ ppm (d, $J = 15.6$ Hz, H-8‴) with a coupling constant of 15.6 Hz indicated *trans*-configuration of the double bond. An anomeric proton at $\delta = 5.08$ (d, $J = 2.0$ Hz, H-1″) and a methyl group at $\delta = 1.33$ ppm (d, $J = 6.0$ Hz, H-6″) in the $^1$H NMR spectrum, as well as $^{13}$C NMR signals at $\delta = 97.8$ (C-1″) and 18.2 ppm (C-6″) suggested the presence of a rhamnose moiety. Based on the coupling constant of the anomeric proton ($J = 2.0$ Hz), an $\alpha$-rhamnose was confirmed. In the case of a $\beta$-rhamnose, the coupling constant normally is approximately 4.2 Hz [11]. The position of the 3,4-disubstituted-(*E*)-cinnamoyl moiety was determined by comparison of the $^1$H and $^{13}$C NMR spectra with those of unsubstituted 6-*O*-$\alpha$-L-rhamnopyranosylcatalpol (**5**) [12] (Fig. 1, Table 1). The C-2″ signal of **1** was shifted downfield by 1.9 ppm; the H-2″ signal was also shifted downfield by 1.96 ppm, whereas the signals of H-3″, H-4″ and H-5″ were shifted downfield by 0.11, 0.01 and 0.02 ppm, respectively. These features were compatible with the attachment of the acyl group to the C-2 of rhamnose. This assignment was confirmed by the HMBC spectrum.

In the HMBC spectrum (Fig. 2), the correlations of $\delta_H = 5.20$ (H-2″) to $\delta_C = 168.6$ (C-9‴) confirmed that

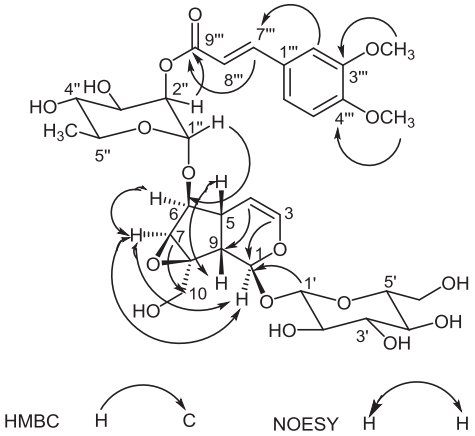


Fig. 2. The key HMBC and NOESY correlations of lagotiside D (**1**).

the 3,4-disubstituted-(*E*)-cinnamoyloxy moiety was substituted at the C-2″ position of rhamnose. The correlations of $\delta_H = 4.81$ (H-1′) to $\delta_C = 95.27$ (C-1) suggested that $\beta$-D-glucose was substituted at C-1, while $\delta_H = 5.08$ (H-1″) to $\delta_C = 84.3$ (C-6) indicated that $\alpha$-L-rhamnose was substituted at C-6. The correlations between $\delta_H = 3.86$ ($OCH_3$) to $\delta_C = 150.69$ (C-3‴) and of $\delta_H = 3.86$ ($OCH_3$) to $\delta_C = 152.87$ (C-4‴) suggested an (*E*)-configuration of the 3,4-dimethoxy-

(*E*)-cinnamoyl moiety. NOESY experiments were also conducted, and the key correlations are shown in Fig. 2. The correlations between H-1 and H-6, H-1 and H-7, H-6 and H-7, as well as H-5 and H-9 suggested that the relative configurations of C-1, C-6, C-7, C-5, and C-9 in compound **1** are identical to that of catalpol [10].

Therefore, the structure of compound **1** was elucidated as 6-*O*-$\alpha$-L-(2″-*O*-*E*-3‴,4‴-dimethoxycinnamoyl)rhamnopyranosylcatalpol, named lagotisoside F.

Compounds **1**–**4** were evaluated *in vitro* against a panel of human tumor cell lines, including leukemia (HL-60), breast carcinoma (MCF-7), lung carcinoma (A549), colon carcinoma (SW480), and myeloid liver carcinoma (SMMC-7721). The compounds lacked activities against all tumor cell lines investigated at the concentration of 40 $\mu$M.

## Conclusion

In summary, one new iridoid glycoside, lagotisoside F (**1**), and another four known analogs were isolated from *L. alutacea*. Compounds **1**–**4** were evaluated for cytotoxic activity against HL-60, MCF-7, A549, SW480, and SMMC-7721 cells and exhibited no appreciable activity against these tested cell lines with $IC_{50}$ values above 40 $\mu$M.

## Experimental Section

### *General*

Melting points (uncorrected): XT-4 melting point apparatus; $[\alpha]_D$ values: Jasco 20C digital polarimeter; UV spectra: UV-210A spectrometer; $\lambda_{max}$ in nm; IR spectra: Bio-Rad FTS-135 spectrometer; 1D and 2D NMR spectra: DRX-400

Table 1. $^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectral data for **1** and **5** ($CD_3OD$, TMS, $\delta$ in ppm, $J$ in Hz).

| No. | **1** | | **5** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 5.09 (d, 1H, $J$ = 8.9) | 95.3 (d) | 5.07 (d, 1H, $J$ = 8.9) | 95.2 (d) |
| 3 | 6.39 (dd, 1H, $J$ = 6.0, 1.8) | 142.4 (d) | 6.35 (dd, 1H, $J$ = 6.0, 1.8) | 142.2 (d) |
| 4 | 5.07 (dd, 1H, $J$ = 6.0, 5.5) | 103.6 (d) | 5.05 (dd, 1H, $J$ = 6.0, 5.0) | 103.6 (d) |
| 5 | 2.44 (m, 1H) | 37.3 (d) | 2.40 (m, 1H) | 37.4 (d) |
| 6 | 4.02 (dd, 1H, $J$ = 8.0, 2.0) | 84.3 (d) | 3.99 (dd, 1H, $J$ = 8.0, 2.0) | 83.7 (d) |
| 7 | 3.64 (d, 1H, $J$ = 2.0) | 59.6 (d) | 3.62 (d, 1H, $J$ = 2.0) | 59.4 (d) |
| 8 | | 66.7 (s) | | 67.0 (s) |
| 9 | 2.58 (dd, 1H, $J$ = 9.2, 8.0) | 43.3 (d) | 2.54 (dd, 1H, $J$ = 9.2, 8.0) | 43.4 (d) |
| 10 | 3.83 (d, 1H, $J$ = 13.2) | 61.5 (t) | 3.81 (d, 1H, $J$ = 13.0) | 61.5 (t) |
| | 4.17 (d, 1H, $J$ = 13.2) | | 4.13 (d, 1H, $J$ = 13.0) | |
| 1′ | 4.81 (d, 1H, $J$ = 8.0) | 99.8 (d) | 4.77 (d, 1H, $J$ = 8.0) | 99.8 (d) |
| 2′ | 3.30 (dd, 1H, $J$ = 9.0, 8.0) | 74.9 (d) | 3.25 (dd, 1H, $J$ = 9.0, 8.0) | 74.9 (d) |
| 3′ | 3.45 (dd, 1H, $J$ = 9.0, 8.0) | 77.7 (d) | 3.40 (dd, 1H, $J$ = 9.0, 8.0) | 77.8 (d) |
| 4′ | 3.28 (dd, 1H, $J$ = 10.0, 8.0) | 71.7 (d) | 3.24 (dd, 1H, $J$ = 10.0, 8.0) | 71.8 (d) |
| 5′ | 3.32 (m, 1H) | 78.6 (d) | 3.30 (m, 1H) | 78.7 (d) |
| 6′ | 3.61 (dd, 1H, $J$ = 12.0, 6.0) | 63.0 (t) | 3.61 (dd, 1H, $J$ = 12.0, 6.0) | 63.0 (t) |
| | 3.97 (dd, 1H, $J$ = 12.0, 2.0) | | 3.91 (dd, 1H, $J$ = 12.0, 2.0) | |
| 1″ | 5.08 (d, $J$ = 2.0) | 97.8 (d) | 4.92 (d, $J$ = 2.0) | 100.4 (d) |
| 2″ | 5.20 (dd, 1H, $J$ = 3.0, 2.0) | 74.2 (d) | 3.84 (dd, 1H, $J$ = 3.0, 2.0) | 72.3 (d) |
| 3″ | 3.78 (dd, 1H, $J$ = 9.0, 3.0) | 70.6 (d) | 3.67 (dd, 1H, $J$ = 9.0, 3.0) | 72.4 (d) |
| 4″ | 3.37 (t, 1H, $J$ = 10.0) | 71.5 (d) | 3.38 (t, 1H, $J$ = 10.0) | 73.9 (d) |
| 5″ | 3.63 (m, 1H) | 70.3 (d) | 3.63 (m, 1H) | 71.9 (d) |
| 6″ | 1.33 (d, 3H, $J$ = 6.3) | 18.2 (d) | 1.30 (d, 3H, $J$ = 6.3) | 17.9 (q) |
| 1‴ | | 128.7 (s) | | |
| 2‴ | 7.20 (m, 1H) | 111.4 (d) | | |
| 3‴ | | 150.7 (s) | | |
| 4‴ | | 152.9 (s) | | |
| 5‴ | 6.95 (d, 1H, $J$ = 8.4) | 112.6 (d) | | |
| 6‴ | 7.15 (d, 1H, $J$ = 8.4) | 124.4 (d) | | |
| 7‴ | 7.71 (d, 1H, $J$ = 15.6) | 147.2 (d) | | |
| 8‴ | 6.47 (d, 1H, $J$ = 15.6) | 116.2 (d) | | |
| 9‴ | | 168.6 (s) | | |
| MeO-3‴ | 3.85 (s, 3H) | 56.6 (q) | | |
| MeO-4‴ | 3.86 (s, 3H) | 56.6 (q) | | |

instrument; $SiMe_4$ as internal reference, $\delta$ in ppm, $J$ in Hz; EI-MS: VG Autospec-3000 mass spectrometer.

*Plant material*

*L. alutacea* was collected in Shangri-la Country, Yunnan Province, China, in November 2009. The plant was identified by Associate Prof. Qing-Song Yang, School of Chemical and Biological Technology, Yunnan Minzu University. A voucher specimen (09-1101) was deposited in the Key Laboratory of Ethnic Medicine Resource Chemistry, Yunnan Minzu University, Yunnan, China.

*Extraction and isolation*

The dried whole plants (5 kg) were extracted four times with 95 % EtOH (4 × 20 L) at room temperature for 7 days, and the combined extracts were concentrated *in vacuo*. The residue was suspended in $H_2O$, and then partitioned with petroleum ether (4 × 2 L), EtOAc (4 × 2 L) and *n*-BuOH (4 × 2 L), successively. The *n*-BuOH extract (436 g) was subjected to chromatography over silica gel, eluting with EtOAc-MeOH (80 : 1 → 0 : 1), to afford eight fractions (A–H). Fraction F (160 g) was purified by silica gel chromatography eluted with $CHCl_3$-MeOH (80 : 0 → 0 : 1) to give six fractions (1 – 6). Fr. 3 was repeatedly chromatographed over a silica gel column eluting with $CHCl_3$-MeOH (50 : 0 → 0 : 1), to yield compounds **1** (12 mg), **2** (92 mg),**3** (16 mg), and **4** (150 mg).

*Lagotisoside F* (**1**). Colorless amorphous powder. M. p.: 155 – 157 °C. $[\alpha]_D^{24} = -167.30$ ($c$ = 0.126, MeOH). – UV (MeOH): $\lambda_{max}(\lg \varepsilon_{max})$ = 222 (3.59), 303 (3.18) and 312 (3.74). – IR (KBr): $\nu$ = 3375, 1710, 1655, 1635, 1598, 1508, 1365, 1220, 1150, 1040, 830. – MS (EI, 70 eV): $m/z$ = 698 $[M]^+$, 683, 638 $[M–2 \times OCH_3]^+$, 325, 207 $[C_6H_3(OMe)_2CH{=}CH\text{-}COOH–1]^+$, 147, 80. – HRMS ((+)-FAB): $m/z$ = 698.2425 (calcd. 698.2422 for $C_{32}H_{42}O_{17}$, $[M]^+$). – $^1H$ and $^{13}C$ NMR ($CD_3OD$): see Table 1.

*Acknowledgement*

This work was supported by the Natural Science Foundation of China (21162042) and Yunnan Province (2010CD090), the Program for Innovative Research Teams (in Science and Technology) of the University of Yunnan Province (IRTSTYN), and the Green Chemistry and Functional Materials Research for Yunnan Innovation Team (2011HC008).

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