Two New Iridoid Glycosides from *Lagotis yunnanensis*

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Two new iridoid glycosides, named lagotisoside D (**1**) and E (**2**), were isolated from *Logatis yunnanensis*. The structures of the two compounds were established as 6-*O*-α-L-(4″-*O*-*E*-3‴,4‴-dimethoxycinnamoyl)rhamnopyranosylcatalpol (**1**) and 6-*O*-α-L-(4″-*O*-*Z*-3‴,4‴-dimethoxycinnamoyl)rhamnopyranosylcatalpol (**2**) on the basis of spectral analysis.

Key words: Logatis yunnanensis, Iridoid Glycosides, Lagotisoside D, Lagotisoside E

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 m height above sea level or higher [1]. Several species such as *L. yunnanesis*, *L. glauca*, *L. integra*, and *L. brachystachya* have long been used in Tibetan folkloric 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]. As part of our studies of medicinal plants growing on the Yunnan-Tibet Plateau, *L. yunnanesis* has been examined. In previous papers [6 – 9], we reported several glycosides from *L. yunnanensis*. A continuation of our studies on the same plant led to the isolation of iridoid glycosides. Here we report on the isolation and structural elucidation of two new iridoid glycosides **1** and **2** (Fig. 1) from this plant.

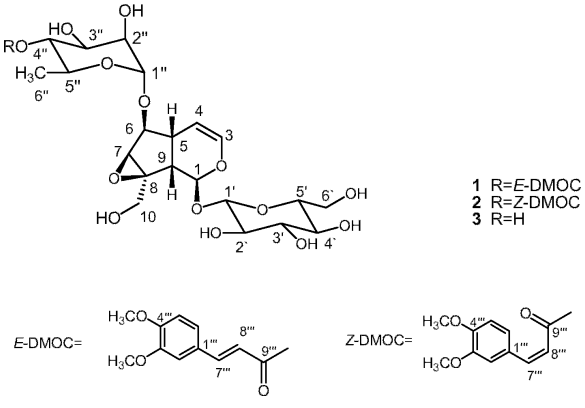

Fig. 1. The structures of compounds **1** – **3**.

Results and Discussion

Lagotisoside D (**1**) was isolated as a white 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 698.2449, calcd. 698.2422). The IR spectrum showed characteristic absorptions for OH (3375 cm^{-1}, br), α,β-unsaturated ester groups (1710 and 1635 cm^{-1}), and aromatic rings (1598 and 1508 cm^{-1}). The UV absorptions at 222 (4.20), 303 (4.42) and 312 (4.56) nm also confirmed the existence of these unsaturated functional groups.

The ^{1}H NMR spectrum (Table 1) of **1** indicated 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 two olefinic protons of the cinnamoyl moiety at δ = 6.49 (d, J = 15.9 Hz, H-8‴) and δ = 7.71 (d, J = 15.9 Hz, H-7‴) with a coupling constant of 15.9 Hz indicated a *trans* config-

Table 1. ^{1}H NMR data (500 MHz, $CDCl_3$) for **1** – **3**.

Position	**1**	**2**	**3**
1(CH)	5.08 (d, *J* = 8.9 Hz)	5.07 (d, *J* = 8.9 Hz)	5.07 (d, *J* = 8.9 Hz)
3(CH)	6.38 (dd, *J* = 6.0, 1.8 Hz)	6.38 (dd, *J* = 6.0, 1.8 Hz)	6.35 (dd, *J* = 6.0, 1.8 Hz)
4(CH)	5.06 (dd, *J* = 6.0, 5.0 Hz)	5.04 (dd, *J* = 6.0, 5.0 Hz)	5.05 (dd, *J* = 6.0, 5.0 Hz)
5(CH)	2.43 (m)	2.43 (m)	2.40 (m)
6(CH)	4.03 (dd, *J* = 8.0, 2.0 Hz)	4.01 (dd, *J* = 8.0, 2.0 Hz)	3.99 (dd, *J* = 8.0, 2.0 Hz)
7(CH)	3.62 (d, *J* = 2.0 Hz)	3.62 (d, *J* = 2.0 Hz)	3.62 (d, *J* = 2.0 Hz)
9(CH)	2.56 (dd, *J* = 9.2, 8.0 Hz)	2.55 (dd, *J* = 9.2, 8.0 Hz)	2.54 (dd, *J* = 9.2, 8.0 Hz)
10 (CH_2)	3.81 (d, *J* = 13.2 Hz)	3.81 (d, *J* = 13.2 Hz)	3.81 (d, *J* = 13.0 Hz)
	4.15 (d, *J* = 13.2 Hz)	4.15 (d, *J* = 13.2 Hz)	4.13 (d, *J* = 13.0 Hz)
1′ (CH)	4.78 (d, *J* = 7.8 Hz)	4.79 (d, *J* = 7.8 Hz)	4.77 (d, *J* = 8.0 Hz)
2′ (CH)	3.26 (dd, *J* = 9.1, 7.8 Hz)	3.26 (dd, *J* = 9.1, 7.8 Hz)	3.25 (dd, *J* = 9.0, 8.0 Hz)
3′ (CH)	3.41 (dd, *J* = 9.1, 8.0 Hz)	3.41 (dd, *J* = 9.1, 8.0 Hz)	3.40 (dd, *J* = 9.0, 8.0 Hz)
4′ (CH)	3.25 (dd, *J* = 10.0, 8.0 Hz)	3.25 (dd, *J* = 10.0, 8.0 Hz)	3.24 (dd, *J* = 10.0, 8.0 Hz)
5′ (CH)	3.31 (m)	3.31 (m)	3.30 (m)
6′ (CH_2)	3.61 (dd, *J* = 12.0, 6.0 Hz)	3.60 (dd, *J* = 12.0, 6.0 Hz)	3.61 (dd, *J* = 12.0, 6.0 Hz)
	3.90 (dd, *J* = 12.0, 2.0 Hz)	3.93 (dd, *J* = 12.0, 2.0 Hz)	3.91 (dd, *J* = 12.0, 2.0 Hz)
1″ (CH)	4.98 (d, *J* = 2.0 Hz)	4.96 (d, *J* = 2.0 Hz)	4.92 (d, *J* = 2.0 Hz)
2″ (CH)	3.85 (dd, *J* = 3.0, 2.0 Hz)	3.85 (dd, *J* = 3.0, 2.0 Hz)	3.84 (dd, *J* = 3.0, 2.0 Hz)
3″ (CH)	3.70 (dd, *J* = 9.0, 3.0 Hz)	3.70 (dd, *J* = 9.0, 3.0 Hz)	3.67 (dd, *J* = 9.0, 3.0 Hz)
4″ (CH)	5.12 (t, *J* = 10.0 Hz)	5.12 (t, *J* = 10.0 Hz)	3.38 (t, *J* = 10.0 Hz)
5″ (CH)	3.66 (m)	3.66 (m)	3.63 (m)
6″ (CH_3)	1.31 (d, *J* = 6.3 Hz)	1.32 (d, *J* = 6.3 Hz)	1.30 (d, *J* = 6.3 Hz)
2‴ (CH)	7.22 (d, *J* = 1.9 Hz)	7.79 (d, *J* = 1.9 Hz)	
5‴ (CH)	7.18 (dd, *J* = 8.4, 1.9 Hz)	6.96 (dd, *J* = 8.4, 1.9 Hz)	
6‴ (CH)	6.48 (d, *J* = 8.4 Hz)	7.18 (d, *J* = 8.4 Hz)	
7‴ (CH)	7.71 (d, *J* = 15.9 Hz)	6.94 (d, *J* = 12.9 Hz)	
8‴ (CH)	6.48 (d, *J* = 15.9 Hz)	5.96 (d, *J* = 12.9 Hz)	
3‴ (MeO)	3.85 (s)	3.83 (s)	
4‴ (MeO)	3.86 (s)	3.84 (s)	

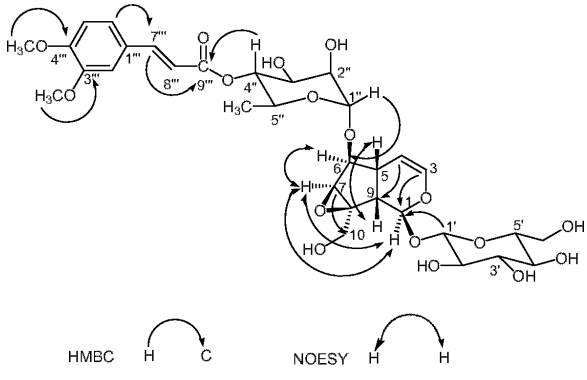

Fig. 2. The key correlations in the HMBC and NOESY spectra of lagotisoside D (**1**).

uration of the double bond. An anomeric proton at δ = 4.94 (d, *J* = 2.0 Hz, H-1″) and a methyl group at δ = 1.31 (d, *J* = 6.3 Hz, H-6″) in the ^{1}H-NMR spectrum, as well as ^{13}C NMR signals at δ = 100.7 (C-1″) and 18.5 (C-6″) suggested the presence of a rhamnose moiety. Based on the coupling constant of the anomeric proton (*J* = 2.0 Hz), an α-rhamnose was confirmed. In the case of a β-rhamnose, the coupling constant normally appears at approximately 4.2 Hz [10]. 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*-α-L-rhamnopyranosylcatalpol (**3**) [11] (Tables 1 and 2). The H-4″ signal of **3** was shifted downfield by 1.74 ppm; the C-4″ signal was also shifted downfield by 1.9 ppm, whereas the signals of C-3″ and C-5″ were shifted upfield by 1.6 and 1.2 ppm, respectively. These features were only compatible with the attachment of the acyl group to the C-4 of rhamnose. This assignment was confirmed by an HMBC spectrum.

In the HMBC spectrum (Fig. 2), the correlations of δ_H = 5.12 (H-4″) to δ_C = 169.08 (C-9‴) confirmed that the 3, 4-disubstituted-(*E*)-cinnamoyl moiety was substituted at the C-4″ position of rhamnose. The correlations of δ_H = 4.78 (H-1′) to δ_C = 95.63 (C-1) suggested that β-D-glucose was substituted at C-1 position, while that of δ_H = 4.98 (H-1″) to δ_C = 63.36 (C-6) indicated that α-L-rhamnose was substituted at C-6 position. The correlations between δ_H = 3.85 (OCH_3) to δ_C = 142.66 (C-3‴) and δ_H = 3.86 (OCH_3) to δ_C = 153.23 (C-4‴) suggested that the cinnamoyl is 3, 4-dimethoxy-(*E*)-cinnamoyl. NOESY experiments were also conducted,

and the key correlations are indicated in Fig. 2. The correlations between H-1 and H-6, H-1 and H-7, H-6 and H-7 as well as between H-5 and H-9 suggested that the relative configuration of C-1, C-6, C-7, C-5 and C-9 in compound **1** are similar to that of catalpol [12].

Therefore, the structure of compound **1** was elucidated as 6-*O*-α-L-(4″-*O*-*E*-3‴,4‴-dimethoxycinnamoyl)rhamnopyranosylcatalpol.

Lagotisoside E (**2**) was isolated as a white amorphous powder, $[\alpha]_D^{24} = -172.32$ (c = 0.204, MeOH). Its molecular formula was found to be the same determined by HR-FAB-MS as that of **1** (found 698.2445, calcd. 698.2422 for $C_{32}H_{42}O_{17}$). The other spectroscopic data were also similar to those of **1**, except for the coupling constants of two olefinic protons, δ_H = 5.96 (d, J = 12.9 Hz, H-8‴) and 6.91 (d, J = 12.9 Hz, H-7‴), from which a *cis*-form of the double bond in the acyl moiety was assumed. Therefore, the structure of **2** was elucidated as 6-*O*-α-L-(4″-*O*-*Z*-3‴,4‴-dimethoxycinnamoyl)rhamnopyranosylcatalpol.

Experimental Section

General

Melting points (uncorrected): XT-4 melting point apparatus; $[\alpha]_D$ values: JASCO 20C digital polarimeter; UV spectra: UV-210A spectrometer; λ_{max} in nm; IR spectra: Bio-Red FTS-135 spectrometer; 1D and 2D NMR spectra: DRX-500 instrument; $SiMe_4$ as internal reference, δ in ppm, J in Hz; EI-MS: VG Autospec-3000 mass spectrometer.

Plant material

L. yunnanesis was collected in Deqin Country, Yunnan Province, P.R. China, in September 2001. The plant was identified by Prof. Zhi-Hao Hu, School of Life Science, Yunnan University. A voucher specimen (No. 01-Y001) was deposited in Key Laboratory of Medicinal Chemistry for Natural Resources, Ministry of Education, Yunnan, P.R. China.

Extraction and isolation

The dried whole plants (8 kg) were extracted four times with 95 % EtOH (4 × 20 L) at r. t. for 9 d, and the combined extracts were concentrated *in vacuo*. The residue was suspended in H_2O, and then partitioned with $CHCl_3$ (4 × 1.5 L) and *n*-BuOH (6 × 1.5 L), successively. The *n*-BuOH extract (897 g) was subjected to chromatography over silica gel, eluting with $CHCl_3$/MeOH/H_2O (8 : 1 : 0.1, 6 : 1 : 0.1, 4 : 1 : 0.15, 2 : 1 : 0.2, 1 : 1 : 0.4 and 0 : 1 : 0), to afford seventeen fractions (A – Q).

Fraction G (30 g) was purified by silica gel chromatography eluted with $CHCl_3$/MeOH (60 : 0 → 0 : 1) to give five fractions (1 – 5). Fr. 4 was repeatedly chromatographed over a silica gel column eluting with $CHCl_3$/MeOH (40 : 0 → 0 : 1), then purified by RP-18 column chromatography eluting with MeOH/H_2O (8 : 2 → 1 : 1), and finally purified by HPLC (MeOH/H_2O 35 : 65, 8 m min^{-1}, UV detector, 320 nm) to yield **2** ($t_R$between 340 ∼ 355 mL, 5 mg) and **1** (t_R between 370 ∼ 400 mL, 10 mg).

Table 2. ^{13}C NMR data (125 MHz, $CDCl_3$) for **1**–**3**.

Position	1	2	3
C(1)	95.6 (d)	95.6 (d)	95.2 (d)
C(3)	142.7 (d)	142.7 (d)	142.2 (d)
C(4)	104.0 (d)	104.0 (d)	103.6 (d)
C(5)	37.7 (d)	37.7 (d)	37.4 (d)
C(6)	84.2 (d)	84.2 (d)	83.7 (d)
C(7)	59.8 (d)	59.8 (d)	59.4 (d)
C(8)	67.0 (s)	67.0 (s)	67.0 (s)
C(9)	43.7 (d)	43.7 (d)	43.4 (d)
C(10)	64.9 (t)	64.9 (t)	61.5 (t)
C(1′)	100.1 (d)	100.1 (d)	99.8 (d)
C(2′)	75.2 (d)	75.2 (d)	74.9 (d)
C(3′)	78.1 (d)	78.1 (d)	77.8 (d)
C(4′)	71.8 (d)	71.8 (d)	71.8 (d)
C(5′)	79.0 (d)	79.0 (d)	78.7 (d)
C(6′)	63.4 (t)	63.4 (t)	63.0 (t)
C(1″)	100.7 (d)	100.7 (d)	100.4 (d)
C(2″)	72.2 (d)	72.2 (d)	72.3 (d)
C(3″)	70.8 (d)	70.8 (d)	72.4 (d)
C(4″)	75.8 (d)	75.8 (d)	73.9 (d)
C(5″)	70.7 (d)	70.7 (d)	71.9 (d)
C(6″)	18.5 (q)	18.5 (q)	17.9 (q)
C(1‴)	129.3 (s)	128.7 (s)	
C(2‴)	111.7 (d)	111.6 (d)	
C(3‴)	151.1 (s)	149.7 (s)	
C(4‴)	153.2 (s)	151.7 (s)	
C(5‴)	124.4 (d)	124.0 (d)	
C(6‴)	117.1 (d)	117.7 (d)	
C(7‴)	147.1 (d)	147.0 (d)	
C(8‴)	113.1 (d)	115.2 (d)	
C(9‴)	169.1 (s)	168.5 (s)	
MeO (3‴)	57.0 (q)	56.5 (q)	
MeO (4‴)	56.9 (q)	56.5 (q)	

*Lagotisoside D (**1**).* White amorphous powder. $[\alpha]_D^{24} = -168.50$ (c = 0.345, MeOH). – UV (MeOH): λ_{max} ($\lg \varepsilon_{max}$) = 222 (4.20), 303 (4.42), 312 (4.56). – IR (KBr): ν = 3375, 2974, 1710, 1655, 1635, 1598, 1508, 1365, 1220, 1150, 1040, 830. – MS (EI, 70 eV): m/z (%) = 698 $[M]^+$ (2.0), 683 (2.5), 638 $[M–2 \times OCH_3]^+$ (10.5), 325 (4.8), 207 $[C_6H_3(OMe)_2CH{=}CH–COOH–1]^+$ (8.8), 147 (100), 80 (3.4). – HR-FABMS: m/z = 698.2449 (calcd. 698.2422 for $C_{32}H_{42}O_{17}$, $[M]^+$). – ^{1}H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2.

*Lagotisoside E (**2**).* White amorphous powder. $[\alpha]_D^{24} = -172.32$ (c = 0.204, MeOH). – UV (MeOH): λ_{max} ($\lg \varepsilon_{max}$) = 227 (4.22), 300 (4.39), 312 (4.58). – IR (KBr): ν = 3375, 2974, 1713, 1654, 1633, 1600, 1559, 1510, 1367, 1220, 1152, 1040, 832. – MS (EI, 70 eV): m/z (%) = 698 (2.0)

$[M]^+$, 683 (2.5), 638 (10.5) $[M-2\times OCH_3]^+$, 325 (4.8), 207 (8.8) $[C_6H_3(OMe)_2CH{=}CH{-}COOH{-}1]^+$, 147 (100), 80 (3.4). – HR-FABMS: m/z = 698.2445 (calcd. 698.2422 for $C_{32}H_{42}O_{17}$ $[M]^+$). – ^{1}H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2.

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[1] Institute of Botany, Chinese Academy of Sciences, *Flora Reipublicae Popularis Sinicae*, Vol. 67, Part 2, Science Press, Beijing, **1979**, pp. 325 – 338.
[2] Gansu Health Bureau, *Handbook of Traditional Chinese Medicines in Gansu*, Gansu People Press, Lanzhou, **1974**, pp. 121 – 129.
[3] Yunnan Medicinal Material Company, *Index Chinese Medicines Resources Yunnanensis*, Science Press, Beijing, **1993**, pp. 486 – 490.
[4] Y. Z. Chen, H. D. Zhang, S. M. Zhang, Q. H. Yang, *Chem. J. Chin. Univ*. **1989**, *10*, 260 – 261.
[5] I. Calis, D. Tasdemir, A. D.Wright, O. Sticher, *Helv. Chim. Acta* **1991**, *74*, 1273 – 1277.
[6] X. D. Yang, J. F. Zhao, S. X. Mei, H. B. Zhang, L. Li, *Fitoterapia* **2003**, *74*, 506 – 507.
[7] X. D. Yang, Z. Y. Li, S. X. Mei, J. F. Zhao, H. B. Zhang, L. Li, *J. Asian Nat. Prod. Res*. **2003**, *5*, 223 – 226.
[8] X. D. Yang, S. X. Mei, J. F. Zhao, G. P. Li, H. B. Zhang, L. Li, *Chin. Chem. Lett*. **2003**, *14*, 936 – 938.
[9] X. D. Yang, J. F. Zhao, S. X. Mei, J. X. Pu, H. B. Zhang, L. Li, *Chin. Chem. Lett*. **2004**, *15*, 49 – 51.
[10] J. M. Yue, S. N. Chen, S. P. Yang, C. Q. Fan, Z. W. Lin, H. D. Sun, *Acta Botanica Sinica* **2001**, *43*, 1199 – 1201.
[11] T. Miyase, C. Akahori, H. Kohsaka, A. Ueno, *Chem. Pharm. Bull*. **1991**, *39*, 2944 – 2951.
[12] R. K. Chaudhuri, O. Sticher, *Helv. Chim. Acta* **1981**, *64*, 3 – 15.

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