New Feruloyl Tyramine Glycosides from *Stephania hispidula* Yamamoto

Tadahiro Yahagi,[a] Yumiko Yamashita,[a] Akihiro Daikonnya,[a] Jin-bin Wu,[b] and Susumu Kitanaka*,[a]

[a] *College of Pharmacy, Nihon University; 7–7–1 Narashinodai, Funabashi, Chiba 274–8555, Japan: and* [b] *Institute of Pharmaceutical Chemistry, China Medicinal University; 404 Taichung, Taiwan.*

Received October 29, 2009; accepted December 9, 2009

Three new feruloyl tyramine glycosides, *N-cis*-feruloyl tyramine-4‴-*O*-β-D-glucopyranoside (1), *N-trans*-feruloyl tyramine-4‴-*O*-β-D-glucopyranoside (2), and *N-trans*-feruloyl tyramine-4′-*O*-β-D-glucopyranoside (3), along with six known compounds, *N-trans*-feruloyl-3‴-methoxydopamine-4′-*O*-β-D-glucopyranoside (4), haitinosporine (5), tubocurine (6), fuzitine (7), (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside (8), and (−)-lyoniresinol-2α-*O*-β-D-glucopyranoside (9), were isolated from the stem of *Stephania hispidula* Yamamoto. The structures were elucidated by spectroscopic and chemical analysis.

Key words *Stephania hispidula*; Menispermaceae; feruloyl tyramine glycoside

Stephania hispidula Yamamoto, belongs to the family Menispermaceae and is native to Taiwan. The stem of this plant is used in traditional medicine as an anodyne, for detoxification, and to treat inflammation and rheumatoid arthritis.[1,2] There have been no reports on the constituents of *S. hispidula*. This paper describes the isolation and structural determination of three new feruloyl tyramine glycosides **1**—**3**, namely, *N-cis*-feruloyl tyramine-4‴-*O*-β-D-glucopyranoside (**1**), *N-trans*-feruloyl tyramine-4‴-*O*-β-D-glucopyranoside (**2**), and *N-trans*-feruloyl tyramine-4′-*O*-β-D-glucopyranoside (**3**), together with one known feruloyl dopamine glycoside, *N-trans*-feruloyl-3‴-methoxydopamine-4′-*O*-β-D-glucopyranoside (**4**), three known isoquinoline alkaloids, haitinosporine (**5**), tubocurine (**6**), and fuzitine (**7**), and two known lignan glycosides, (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside (**8**) and (−)-lyoniresinol-2α-*O*-β-D-glucopyranoside (**9**), from the stem of *S. hispidula*.

Results and Discussion

The stems of *S. hispidula* were extracted with 80% aqueous methanol. The methanolic extract was suspended in H_2O and partitioned with *n*-hexane. As shown Chart 1, the aqueous layer was subjected to Diaion HP-20, normal-phase, and reverse-phase column chromatographies, and finally HPLC to afford three new feruloyl tyramine glycosides (**1**—**3**) and six known compounds (**4**—**9**).

N-cis-Feruloyl tyramine-4‴-*O*-β-D-glucopyranoside (**1**) was isolated as a yellow amorphous powder, and its molecular formula was determined as $C_{24}H_{29}NO_9$ by high-resolution fast atom bombardment mass spectroscopy (HR-FAB-MS). Its IR spectrum exhibited characteristic absorption bands for a hydroxyl group (3318 cm^{-1}), conjugated carbonyl group (1650 cm^{-1}), and conjugated double bond (1510 cm^{-1}). Acid hydrolysis of **1** afforded D-glucose as determined by comparing the HPLC retention time of the hydrolysis product with that of an authentic sample. The ^{1}H-NMR spectrum (Table 1) indicated the presence of one 1,4-disubstituted aromatic ring at δ_H 7.10 (2H, d, J=8.6 Hz, H-2‴, 6‴) and δ_H 7.00 (2H, d, J=8.6 Hz, H-3‴, 5‴); one 1,3,4-trisubstituted aromatic ring at δ_H 7.34 (1H, d, J=1.7 Hz, H-2′), δ_H 6.91 (1H, dd, J=8.2, 1.7 Hz, H-6′), and δ_H 6.73 (1H, d, J=8.2 Hz, H-5′); one *cis*-olefin at δ_H 6.60 (1H, d, J=12.7 Hz, H-3) and δ_H 5.80 (1H, d, J=12.7 Hz, H-2); and one methoxyl proton at δ_H 3.80 (3H). From the coupling constant of the anomeric proton of **1** at δ_H 4.94 (1H, d, J=7.6 Hz, Glc-1), C-1 of the D-glucopyranose was determined to be in the β-configuration. Analysis of the ^{13}C-NMR (Table 1) and distortionless enhancement by polarization transfer (DEPT) spectra revealed the presence of one carbonyl group, one methoxyl group, three methylenes, fourteen methines, and five quaternary carbons. All protonated carbons were assigned by analysis of the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC). Furthermore, the ^{1}H–^{1}H correlation spectroscopy (COSY) (Fig. 1) spectrum displayed connectivity between nonequivalent methylene protons at δ_H 3.40 (H-1″) and δ_H 2.73 (H-2″), and between olefinic protons at H-2 and H-3, respectively. Heteronuclear multiple bond connection (HMBC) analysis of **1** (Fig. 1) showed long-range correlations between H-2‴ and carbons at δ_C 157.7 (C-4‴) and 82.7 (C-2″); H-2″ and δ_C 130.7 (C-2‴); H-1″ and a carbonyl at δ_C 170.4 (C-1); H-3 and C-1, δ_C 124.7 (C-6′), and δ_C 113.9 (C-2′); H-2′ and δ_C 148.5 (C-3′) and δ_C 138.3 (C-3); and the methoxyl protons and C-3′. Nuclear Overhauser enhancement correlated spec-

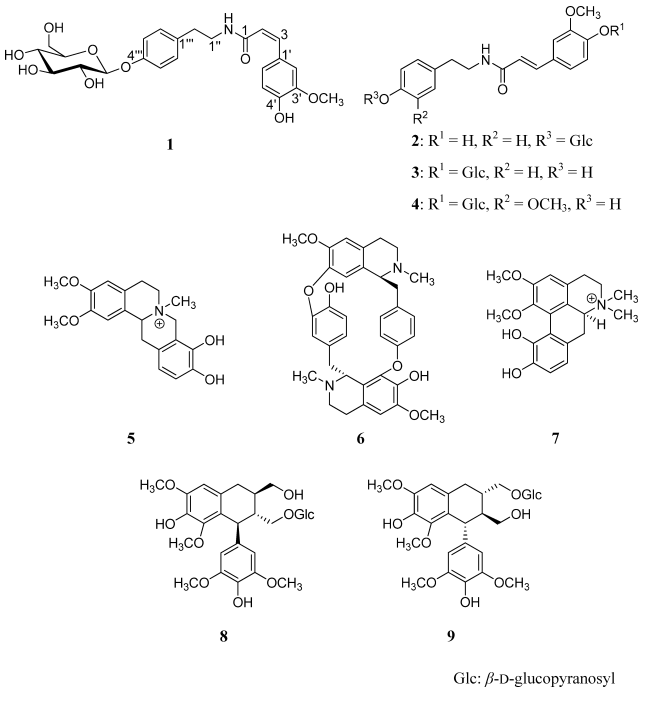

Chart 1

* To whom correspondence should be addressed. e-mail: kitanaka.susumu@nihon-u.ac.jp

Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) Data for Compounds **1**—**3** (MeOH-d_4)

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		170.4		169.2		165.1
2	5.80 d (12.7)	121.4	6.38 d (15.4)	118.7	6.48 d (15.7)	120.4
3	6.60 d (12.7)	138.3	7.43 d (15.4)	142.1	7.32 d (15.7)	138.4
1′		128.5		128.2		128.8
2′	7.34 d (1.7)	113.9	7.11 d (1.7)	111.5	7.17 br d (1.7)	110.7
3′		148.5		149.3		149.0
4′		148.5		149.9		147.6
5′	6.73 d (8.2)	115.8	6.79 d (8.4)	116.5	7.09 d (8.6)	115.1
6′	6.91 dd (8.2, 1.7)	124.7	7.01 dd (8.4, 1.7)	123.2	7.07 br dd (8.6, 1.7)	121.0
1″	3.40 t (7.2)	42.1	3.48 t (7.2)	42.3	3.33 t (7.2)	40.5
2″	2.73 t (7.2)	35.5	2.80 t (7.2)	35.8	2.65 t (7.2)	34.4
1‴		134.3		134.4		129.5
2‴, 6‴	7.10 d (8.6)	130.7	7.16 d (8.6)	130.7	7.01 d (8.5)	129.5
3‴, 5‴	7.00 d (8.6)	117.8	7.04 d (8.6)	117.8	6.68 d (8.5)	115.1
4‴		157.7		157.8		155.6
Glc-1	4.94 d (7.6)	102.4	4.94 d (7.6)	102.4	4.96 d (7.2)	99.7
2	3.39 (overlap)	77.9	3.39 (overlap)	78.0	3.17 (overlap)	76.8
3	3.45 (overlap)	78.1	3.45 (overlap)	78.1	3.45 (overlap)	77.0
4	3.38 (overlap)	74.9	3.38 (overlap)	74.9	3.26 (overlap)	73.1
5	3.40 (overlap)	71.4	3.40 (overlap)	71.4	3.26 (overlap)	69.6
6	3.88 dd (12.0, 1.9)	62.5	3.88 dd (12.0, 1.9)	62.5	3.65 dd (11.4, 2.3)	60.6
	3.69 dd (12.0, 5.5)		3.69 dd (12.0, 5.5)		3.42 dd (11.4, 6.2)	
OCH_3	3.80 s	56.4	3.87 s	56.4	3.79 s	56.5

Chemical shifts are given in ppm; coupling constants J (in parentheses) are given in Hz.

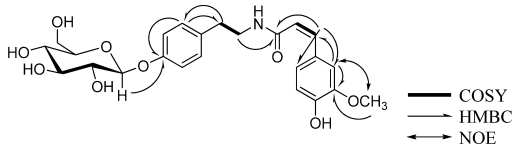

Fig. 1. Characteristic Correlations Observed in COSY, HMBC, and NOESY Spectra of Compound **1**

troscopy (NOESY) analysis (Fig. 1) further confirmed that the location of the methoxyl was at C-3′ based on the correlation between the methoxyl protons and H-2′. On the basis of the above analysis, combined with the ^{13}C-NMR spectrum ($\delta_{C\text{-}1''}$ 170.4, $\delta_{C\text{-}1}$ 42.1) and the molecular formula of **1**, it was deduced that C-1″ and C-1 were linked by a nitrogen atom. The current analysis and comparison with literature data suggested that **1** was a feruloyl tyramine derivative.[3—5] Moreover, the HMBC correlation between the anomeric proton at Glc-1 and C-4‴ indicated that the β-D-glucose was located at C-4‴. Thus, the structure of **1** was established to be *N-cis*-feruloyl tyramine-4‴-*O*-β-D-glucopyranoside.

N-trans-Feruloyl tyramine-4‴-*O*-β-D-glucopyranoside (**2**) was isolated as a yellow amorphous powder, and its molecular formula was determined as $C_{24}H_{29}NO_9$ by HR-FAB-MS. The IR and NMR spectra of **2** closely resembled that of **1**, except that the coupling constant (J=15.4 Hz) of the olefin proton signal was larger. This indicated that the configuration of the olefin in the aglycone of **2** is *trans*. Furthermore, **2** was a geometrical isomer of **1**. Thus, the structure of **2** was elucidated as *N-trans*-feruloyl tyramine-4‴-*O*-β-D-glucopyranoside.

N-trans-Feruloyl tyramine-4′-*O*-β-D-glucopyranoside (**3**) was isolated as a yellow amorphous powder, and its molecular formula was determined as $C_{24}H_{29}NO_9$ by HR-FAB-MS. The MS, IR and NMR spectra were similar to those of **1** and **2**. However, the HMBC correlations between the anomeric proton at δ_H 4.96 (Glc-1) and δ_C 147.6 (C-4′) indicated that the β-D-glucosyl unit was located at C-4′. Thus, the structure of **3** was determined to be *N-trans*-feruloyltyramine-4′-*O*-β-D-glucopyranoside. Compounds **1**—**3** are the first reported feruloyl tyramine glycosides to contain D-glucose.

Compounds **4**—**9** were identified as *N-trans*-feruloyl-3‴-methoxydopamine-4′-*O*-β-D-glucopyranoside (**4**),[6] haitinosporine (**5**),[7] tubocurine (**6**),[8] fuzitine (**7**),[9,10] (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside (**8**),[11,12] and (−)-lyoniresinol-2α-*O*-β-D-glucopyranoside (**9**)[13] by comparison of the physical and spectral data with those described in the literature. These were isolated from the stem of *S. hispidula* for first time.

Experimental

General Experimental Procedure Optical rotation (OR) was measured in MeOH on a P-1020 polarimeter (JASCO Co., Ltd., Tokyo, Japan). The UV spectra were obtained in MeOH on a V-550 spectrophotometer (JASCO Co., Ltd., Tokyo, Japan), and the IR spectra were recorded on a IR A-2 spectrophotometer (JASCO Co., Ltd., Tokyo, Japan). The NMR spectra were recorded on an ECA-600 spectrometer (JEOL Ltd., Tokyo, Japan), with tetramethylsilane (TMS) as an internal standard. MS data were obtained on a GCmate spectrometer (JEOL Ltd., Tokyo, Japan). For column chromatography, silica gel 60N (Kanto Chemical Co., Inc., Tokyo, Japan), YMC GEL ODS-A (YMC Co., Ltd., Kyoto, Japan), and Diaion HP-20 column (Mitsubishi Chemical Co., Ltd., Tokyo, Japan) were used. TLC was performed on TLC plates (thickness: 0.25 mm, Merck Co., Ltd., Tokyo, Japan), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in EtOH and Dragendorff reagent. HPLC was performed on a JASCO PU-1580 apparatus (JASCO Co., Ltd., Tokyo, Japan) equipped with a JASCO UV-1575 detector (JASCO Co., Ltd., Tokyo, Japan) and Shodex OR-2 OR detector (Showa Denko K. K., Tokyo, Japan). Cosmosil 5C_{18}-MS-II (Φ10×250 mm, Nacalai Tesque Co., Kyoto, Japan) was used for preparative purpose. CAPCELL PAK NH_2 (Φ4.6×250 mm, Shiseido Co., Ltd., Tokyo, Japan) was used for confirmation of the glycosyl moiety.

Plant Materials The stems of *S. hispidula* YAMAMOTO were purchased

in "Shan you Qing Cao hang" of Taiwan, and identified by Prof. Zhang Xian Zhe, China Medicinal University, in September 2008. Voucher specimens (NK08010) have been deposited at College of Pharmacy, Nihon University.

Extraction and Isolation The stems of *S. hispidula* Yamamoto (2.0 kg) were extracted three times with 80% aqueous MeOH. Evaporation of the solvent under reduced pressure from the combined extract afforded the methanolic extract (161 g). The extract was suspended in H_2O (1 : 1 v/v) and partitioned with *n*-hexane (3×1 : 1 v/v). Removal of the solvents afforded the *n*-hexane extract (39.6 g) and the aqueous extract (121.3 g). The aqueous extract was chromatographed on Diaion HP-20 with H_2O (Fr. A), 30% MeOH (Fr. B), 50% MeOH (Fr. C), 70% MeOH (Fr. D), and 100% MeOH (Fr. E) as eluent, successively. The Fr. B (49.4 g) was subjected to silica gel column chromatography, [$CHCl_3$–MeOH (100 : 0→0 : 100 v/v)]. The fractions (300 ml each) were combined according to the results of TLC monitoring into 7 fractions, [Fr. B-1 (83.1 mg), B-2 (129 mg), B-3 (79.8 mg), B-4 (995 mg), B-5 (6.16 g), B-6 (3.96 g), B-7 (9.71 mg)]. Compounds **5** (856 mg) and **7** (361 mg) were crystallized from Fr. B-5 and B-7, respectively. Fr. B-5 was subjected to octadecylsilanized silica gel (ODS) column chromatography [MeOH–H_2O (10 : 90→100 : 0 v/v)] to yield 11 fractions [Fr. B-5-A (196 mg), B-5-B (571 mg), B-5-C (1.95 g), B-5-D (164 mg), B-5-E (333 mg), B-5-F (168 mg), B-5-G (272 mg), B-5-H (591 mg), B-5-I (100 mg), B-5-J (72.6 mg), B-5-K (95.1 mg)]. Fr. B-5-E was purified by HPLC with CH_3CN–H_2O (14 : 86 v/v) to afford **8** (23.2 mg) and **9** (10.6 mg). Fr. B-5-H was purified by HPLC with CH_3CN–H_2O (15 : 85 v/v) to afford **1** (10.6 mg), **2** (11 mg), **3** (8.7 mg), and **4** (3.3 mg).

N-cis-Feruloyl Tyramine-4‴-*O*-*β*-D-glucopyranoside (**1**): Yellow amorphous powder. IR (KBr) ν_{max} cm^{-1}: 3318, 2925, 1650, 1610, 1511, 1230, 1076. FAB-MS (positive mode) *m*/*z*: 476 $[M+H]^+$. HR-FAB-MS (positive mode) *m*/*z*: 476.1921 $[M+H]^+$ (Calcd for $C_{24}H_{30}NO_9$, 476.1920). UV λ_{max} (MeOH) nm (log ε): 223 (3.33), 282 (3.05), 315 (3.04). ^{1}H- and ^{13}C-NMR spectral data presented in Table 1.

N-trans-Feruloyl Tyramine-4‴-*O*-*β*-D-glucopyranoside (**2**): Yellow amorphous powder. IR (KBr) ν_{max} cm^{-1}: 3317, 2926, 1650, 1610, 1510, 1230, 1076. FAB-MS (positive mode) *m*/*z*: 476 $[M+H]^+$. HR-FAB-MS (positive mode) *m*/*z*: 476.1921 $[M+H]^+$ (Calcd for $C_{24}H_{30}NO_9$, 476.1920). UV λ_{max} (MeOH) nm (log ε): 223 (3.34), 283 (3.08), 314 (3.06). ^{1}H- and ^{13}C-NMR spectral data presented in Table 1.

N-trans-Feruloyl Tyramine-4′-*O*-*β*-D-glucopyranoside (**3**): Yellow amorphous powder. IR (KBr) ν_{max} cm^{-1}: 3434, 2931, 1654, 1617, 1513, 1259, 1083. FAB-MS (positive mode) *m*/*z*: 476 $[M+H]^+$. HR-FAB-MS (positive mode) *m*/*z*: 476.1922 $[M+H]^+$ (Calcd for $C_{24}H_{30}NO_9$, 476.1920). UV λ_{max} (MeOH) nm (log ε): 219 (3.44), 287 (3.38), 315 (3.33). ^{1}H- and ^{13}C-NMR spectral data presented in Table 1.

Acid Hydrolysis of 1 A solution of **1** (2 mg) in 10% HCl was heated at 100 °C for 1 h. After cooling, the reaction mixture was neutralized with 10% NaOH and partitioned between $CHCl_3$ and H_2O. The H_2O layer was concentrated under reduced pressure. The H_2O extract was analyzed by HPLC under the following condition: solvent, CH_3CN–H_2O (75 : 25 v/v); flow rate, 0.8 ml/min; detection, OR. The identification of D-glucose present in the water layer was carried out by comparing the retention time and polarity of the hydrolysis product with those of an authentic sample: t_R (min) 17.6 (D-glucose, positive polarity).

Acknowledgment This work was supported by the "Academic Frontier" Project for Private Universities: matching fund subsidy from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), 2007—2010.

References

1) Luo H. S., "Menispermaceae. Delectis Florae Republicae Popularis Sinicae, Agendae Academiae Sinicae Edita. Flora Republicae Popularis Sinicae," Tomus 30, Science Press, Beijing, 1996, pp. 1—81.
2) Nien Y. C., Kuang H. C., "The Illustrated Medicinal Plants of Taiwan," 5, SMC Publishing Inc., Taipei, 1998, p. 77.
3) Fukuda N., Yonemitsu M., Kimura T., *Chem. Pharm. Bull.*, **31**, 156—161 (1983).
4) Ji M., Shannon H. J., Sidney M. H., *Bioorg. Med. Chem.*, **12**, 3885—3889 (2004).
5) Hyang R. K., Hye Y. M., Yeon H. J., Sang K. L., Nam S. L., Eun K. S., *Arch. Pharm. Res.*, **28**, 1224—1227 (2005).
6) Xiang Y., Li Y. B., Zhang J., Li P., Yao Y. Z., *Acta Pharm. Sin.*, **42**, 618—620 (2007).
7) Guo Y. Y., Lin L. B., Shen J., *Acta Pharm. Sin.*, **33**, 350—354 (1998).
8) Bick R. C., *J. Chem. Soc.*, **1960**, 2402—2407 (1960).
9) Zhang W. D., Han G. Y., Liang H. Q., *Acta Pharm. Sin.*, **27**, 670—673 (1992).
10) Yu M. L., Jun S. Y., Qing H. L., *Chem. Pharm. Bull.*, **52**, 454—455 (2004).
11) Piyanuch T., Rutt S., Rapepol B., Robert V., *Fitoterapia*, **75**, 623—628 (2004).
12) Khald M. M., Mostafa A. F., Matsunami K., Mohamed S. K., Otsuka H., *ARKIVOC*, **8**, 63—70 (2007).
13) Ohashi K., Watanabe H., Okumura Y., Tahan U., Kitagawa I., *Chem. Pharm. Bull.*, **42**, 1924—1926 (1994).