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A new feruloyl tyramine glycoside from the roots of *Achyranthes bidentata*

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[ABSTRACT] AIM: To study the chemical constituents of the roots of *Achyranthes bidentata* Bl.. **METHODS:** The chemical constituents were isolated and purified by macroporous adsorptive resin D101, silica gel, and ODS column chromatographies and preparetive HPLC. Their structures were elucidated on the basis of 1D and 2D NMR analyses. **RESULTS:** Two feruloyl tyramine glycosides and seven triterpenoid saponins were obtained and identified as *N-trans*-feruloyl-3-methoxytyramine-4′-*O*-*β*-D-glucopyranoside (**1**), *N-trans*-feruloyl-3-methoxytyra mine-4-*O*-*β*-D-glucopyranoside (**2**), PJS-1 (**3**), chikusetsusaponin IVa (**4**), oleanolic acid 3-*O*-[*β*-D-glucuronopy ranoside-6-*O*-methyl ester]-28-*O*-*β*-D-glucopyranoside (**5**), oleanolic acid 3-*O*-[*β*-D-glucuronopyran-oside-6-*O*-ethyl ester]-28-*O*-*β*-D-glucopyranoside (**6**), oleanolic acid 3-*O*-[*β*-D-glucuronopyranoside-6-*O*-butyl ester]-28-*O*-*β*-D-glucopyranoside (**7**), ginsenoside R_0 (**8**) and hederagenin-28-*O*-*β*-D-glucopyranosyl ester (**9**). **CONCLUSION:** Compound **1** is a new feruloyl tyramine glycoside, while compounds **2** and **9** are reported from *A. bidentata* for the first time.

[KEY WORDS] *Achyranthes bidentata* Bl.; Feruloyl tyramine glycoside; Triterpenoid saponin

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1 Introduction

Achyranthes bidentata Bl., a member of *Amaranthaceae*, is an erect perennial herb distributed and grown in hilly districts of India, China, Japan and Java. The dried roots of *A. bidentata*, named Niuxi in Chinese, is an important medicinal herb documented in Chinese Pharmacopeia[1]. The main efficacies of Niuxi include dissipating blood stasis, nourishing the liver and kidney, strengthening the bones and muscles. It is usually prescribed by practitioners of Traditional Chinese Medicines (TCMs) for the treatment of osteodynia of lumbar and knees, spasm and flaccidity of limbs[2]. The modern pharmacological studies showed that the *A. bidentata* possessed immunostimulant[3-4], uteri-excitant and antifertility[5-6], antitumor[7], analgestic, antibacterial, anti-inflammatory[8], anti-osteoporosis[9] activities. Previous phytochemical investigations on *A. bidentata* resulted in the isolation of polysaccharide[3-4], triterpenoid saponins[10-11] and phytoecdysones[12-14]. However, for a long period of time, feruloyl tyramine compounds were barely reported. As a part of our search for anti-osteoporosic activity natural products from the roots of *A. bidentata*, a new feruloyl tyramine glycoside was isolated. Herein, we report the isolation and structural elucidation of *N-trans*-feruloyl-3-methoxytyramine-4′-*O*-*β*-D-glucopyranoside.

2 Experimental

2.1 *General experimental procedures*

Melting points were measured on Kofler micromelting point apparatus (uncorrected). Optical rotations were measured with a PE-241 digital polarimeter. IR spectra were recorded on an IR-47 spectrometer. NMR spectra were recorded on Bruker DPX 400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR), respectively. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard, and coupling constants are given in Hz. The HRESIMS analyses were conducted on IonSpec Ultima 7.0T FTICR. Preparative HPLC (Waters, Delta 600-2487) was performed on Pegasil ODS II (5 μm, 10 mm × 250 mm, Senshu Pak, Japan). Macroporous absorption resin (D101

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Crosslinked Polystyrene, Nankai, Tianjin, China) was employed for column chromatography. Silica gel (75−150 μm) for column chromatography and silica gel H for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China. ODS-A (120 A, 50 μm) was obtained from YMC Co..

2.2 *Plant material*

The roots of *A. bidentata* were purchased from Medicinal Materials Planting Base of Anhui University of Traditional Chinese Medicine in 2007, and authenticated by Prof. WANG Zhen-Yue of Heilongjiang University of Chinese Medicine. A voucher specimen (No. 20071062) is deposited at the Herbarium of Heilongjiang University of Chinese Medicine, Harbin, China.

2.3 *Extraction and isolation*

The air dried roots of A. bidentata (12 kg) were ground to the particle size through standard mesh sieve No.10 and extracted with 95% EtOH (3 × 10 L) for 2 h. The EtOH extracts (5.5 kg) was concentrated under reduced pressure and fractioned by D101 macroporous resin column (8 cm × 60 cm) eluted with H_2O, 50% and 95% EtOH-H_2O to give three fractions (H_2O fraction, 50% EtOH-H_2O fraction and 95% EtOH-H_2O fraction). The 50% EtOH-H_2O fraction (108 g) was subjected to repeated column chromatography on silica gel with a gradient of CH_2Cl_2/MeOH (30 : 1 to 3 : 1) solvents as eluents to afford eight fractions: Fr. 1−8. Fr. 3 (18.3 g) continues silica gel chromatography eluted with CH_2Cl_2/MeOH (20 : 1 to 10 : 1) to afford a number of sub-fractions A1−A4. Compounds **1** (15.2 mg) and **2** (17.4 mg) were obtained by preparative HPLC chromatography of the sub-fraction A2 (1.8 g) eluted with MeOH/H_2O (1 : 4). F6 (32.5 g) was subjected to column chromatography on silica gel with CH_2Cl_2/MeOH (8 : 1 to 3 : 1) to afford a number of sub-fractions B1-B7. B3 (4.1 g)was separated on ODS-A column with MeOH/H_2O (1 : 5 to 1 : 0) for elution, to produce eight sub-fractions (C1-C8). The sub-fraction C6 (0.9 g) was purified by Prep. HPLC with MeOH/H_2O (4:1) to afford **3** (25.5 mg), and **9** (14.2 mg), respectively. Similarly, compounds **4** (26.1mg) and **8** (18.6 mg) were obtained by ODS column chromatography of the sub-fraction C7 (1.5 g) eluted with MeOH/H_2O (17:20). Fr.7 (4.5 g) continues silica gel column chromatography eluted with CH_2Cl_2/MeOH (5 : 1 to 3 : 1) to afford a number of sub-fractions D1-D4.The sub-fraction D1 (1.5 g) was also passed over ODS column chromatography with MeOH/H_2O (4 : 1) and finally purified by preparative HPLC with MeOH/H_2O (4 : 1) to afford **5** (15.8 mg), **6** (15.3 mg) and **7** (16.0 mg), respectively.

3 Results and Discussion

Compound 1 Yellow amorphous powder, mp 190−191 °C, $[\alpha]_D^{20}$ +2.4 (*c* 0.25, MeOH), positive to Molish reaction. Its molecular formula $C_{25}H_{31}NO_{10}$ was determined by HRESI-MS([M + H]$^+$ 506.194 6, Calcd. for 506.194 8). The acid hydrolysis of **1** liberated D-glucose, which was identified by HPLC using an optical rotation detector[15]. The IR spectrum displayed absorption bands for an amido NH at 3 200 cm^{-1}, a hydroxyl group at 3 320 cm^{-1}, a carbonyl group at 1 650 cm^{-1}, a double bond at 1 510 cm^{-1} and two benzene rings at 1 580 cm^{-1} and 1 520 cm^{-1}. UV maxima (log ε) occurred at 198 nm (4.20), 288 nm (4.30) and 313 nm (4.05), consistent with a cinnamamide chromophore.

The ^{1}H NMR spectrum (400 MHz, in C_5D_5N) indicated the presence of one *trans*-olefin at δ 8.10 (1H, d, *J* = 15.6 Hz, H-7) and 6.87 (1H, d, *J* = 15.6 Hz, H-8); two sets of ABX aromatic proton signals at δ 7.16 (1H, d, *J* = 1.6 Hz, H-2), 7.13 (1H, d, *J* = 8.0 Hz, H-5) and 7.18 (1H, dd , *J* =1.6, 8.0 Hz, H-6) and δ 6.95 (1H, d, *J* = 2.0 Hz, H-2′), 7.47(1H, d, *J* = 8.0 Hz, H-5′) and 6.81 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′); one NH proton signal at δ 8.66 (1H, t, *J* = 5.6 Hz, N-H); two methoxy proton signals at δ 3.65 (6H, s, 3, 3′-OCH_3). From the coupling constant of the anomeric proton of **1** at δ 5.62(1H, d, *J* = 7.2Hz, Glc-1″), C-1″ of the D-glucopyranose was determined to be in the *β*-configuration. Signals at δ 2.98 (2H, t, *J* = 6.8 Hz, H-7′) and 3.86 (2H, dt, *J* = 5.6, 6.8 Hz, H-8′) indicated the presence of a -CH_2-CH_2- structure fragment according to ^{1}H NMR and ^{1}H-^{1}H COSY spectrum. The ^{13}C NMR spectrum also revealed the presence of two aromatic rings, one *trans*-double bond, one carbonyl group, two methoxy groups, three methylene groups and one glucopyranosyl group in compound **1**.

In the HMBC spectrum, some key long-range correlations were observed between the H-7 signal and the C-1, C-2, C-8 and C-9; between the H-8 signal and the C-1, C-9 and C-8′; between the H-7′ signal and the C-1′, C-2′ and C-6′; between the H-8′ signal and C-9; and between two methoxy proton signals and C-3 and C-3′, respectively. The connection position of the glucopyranosyl group was established unambiguously by a HMBC experiment in which long-range correlations between H-1″ of the glucopyranosyl group and C-4′. At the same time, two methoxyls at δ 3.65 were placed at C-3 and C-3′, respectively, on the basis of their NOE interactions with H-2 (δ 7.16) and H-2′ (δ 6.95). The NOE interaction of the anomeric proton with H-5′ (δ 7.47) also comfirmed the placement of glucosyl units on C-4′.

For all the remaining assignments of ^{1}H NMR and ^{13}C NMR see Table 1, and for all the remains of HMBC correlation see Fig.1. On the basis of the above data, compound **1** was elucidated as *N-trans*-feruloyl-3-methoxytyramine-4'-*O*-*β*-D-glucopyranoside.

Compound 2 Yellow amorphous powder, mp 191−192 °C, $[\alpha]_D^{20}$ +2.0 (*c* 0.18, MeOH), produced positive reactions to Molish reagent, had the molecular formula $C_{25}H_{31}NO_{10}$ determined by HRESI-MS ([M + H]$^+$ 506.194 9, Calcd. for 506.194 8). IR (KBr): 3 200, 3 318, 1 650, 1 511, 1 580, 1 514 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 198 (4.15), 286 (4.30), 312 (4.10).

Analysis of the ^{1}H and ^{13}C NMR data provided evidence that **2** possessed a *trans*-olefin, two sets of ABX, two methoxy and a monosaccharide moiety. Detailed comparison of chemical shifts of compound **2** with **1** indicated that the spec-