Chemistry of Natural Compounds, Vol. 43, No. 5, 2007

ELLAGIC ACID GLYCOSIDES FROM THE STEM BARK OF *Aphananthe aspera*

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Five ellagic acid glycosides were isolated from Aphananthe aspera *and their structures were identified as 3-*O*-methylellagic acid-4′-*O*-α-L-rhamnopyranoside (**1**), 3-*O*-methylellagic acid-4′-*O*-β-D-xylopyranoside (**2**), 3,3′-di-*O*-methylellagic acid-4′-*O*-β-D-xylopyranoside (**3**), 3,3′, 4-tri-*O*-methylellagic acid-4′-*O*-β-D-glucopyranoside (**4**), and 3,3′-di-*O*-methylellagic acid-4′-*O*-α-L-rhamnopyranoside (**5**) on the basis of spectroscopic analysis. Compound **1** is new, and all the compounds were isolated for the first time from the title plant.*

Key words: *Aphananthe aspera*, ellagic acid glycosides; structure elucidation

The stem bark of *Aphananthe aspera* (Thunb.) Planch. (Ulmaceae) has been used as a traditional medicine in the treatment of inflammation and ache by local inhabitants [1]. However, this plant has not been chemically studied. In searching for bioactive substances, we have analyzed the title plant collected from Guangxi, in the southern part of China. Five ellagic acid glycosides, 3-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**1**), 3-*O*-methylellagic acid-4′-*O*-*β*-D-xylopyranoside (**2**), 3,3′-di-*O*-methylellagic acid-4′-*O*-*β*-D-xylopyranoside (**3**), 3,3′, 4-tri-*O*-methylellagic acid-4′-*O*-*β*-D-glucopyranoside (**4**), and 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**5**) were isolated from the 95% EtOH extracts of this plant.

Compound **1** was obtained as white needle crystals. Its negative ion HR-ESIMS suggested a molecule of $C_{21}H_{18}O_{12}$. Its ultraviolet (UV) spectrum (λ_{max} 254.2, 353.9 nm) was similar to that of ellagic acid [2], suggesting that **1** has an ellagic acid skeleton. Its infrared (IR) spectrum showed a hydroxy band at 3422 cm^{-1}, a carbonyl band at 1716 cm^{-1}, and absorptions for the aromatic ring at 1607 and 1500 cm^{-1}. The ^{1}H NMR spectrum of **1** revealed two protons as singlets at δ 7.48 and 7.61, assignable to H-5 and H-5′, respectively. The ^{1}H NMR spectrum of **1** also showed an aromatic methoxy at δ 4.01 (3H, s). The sugar was identified as *α*-L-rhamnose by co-paper chromatographied with authentic sugars using *n*-BuOH: AcOH : H_2O (4 : 1 : 5) as solvent. The position of the glycosidic linkage to the aglycone was confirmed on the basis of HMBC (Fig.1) and NOESY experiments. The HMBC spectrum of **1** showed that the anomeric proton of rhamnose (δ 5.45, H-1″) correlated with C-4′ (δ 152.6) of ellagic acid, which in turn, correlated with H-5′ (δ 7.61). Furthermore, NOESY experiment revealed clearly that the anomeric proton of rhamnose (δ 5.45, H-1″) correlated with H-5′ (δ 7.61) of the ellagic acid. This interaction is only possible when the sugar residue is linked at C-4′. The position of the methoxyl linkage to the ellagic acid was deduced from the HMBC experiment. The chemical shift of the methyl carbon (δ 61.1) of **1** was similar to that of the 3-*O*-methyl derivative (δ 60~61) but different from that of the 4-*O*-methyl derivative (δ 56~57), suggesting that the methoxyl group is located at C-3 or C-3′ [2]. The presence of the methoxyl group at C-3 was confirmed by HMBC experiment, in which the H-5 signal (δ 7.48) showed a cross peak with C-3 (δ 136.2), and the C-3 signal, in turn, showed a cross peak with the 3-methoxyl signal (δ 4.01). These observations indicated unequivocally that **1** is 3-*O*-methyl-ellagic acid-4′-*O*-*α*-L-rhamnopyranoside.

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 0009-3130/07/4305-0558

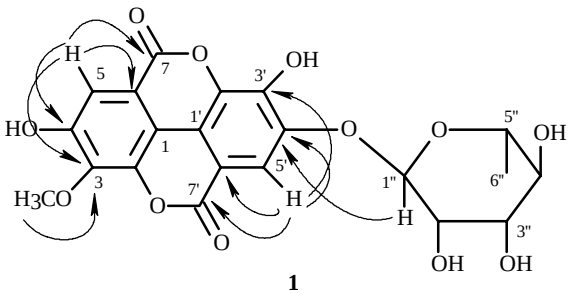

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Fig. 1. Selected key HMBC correlations (H→C) of compound **1**.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. UV spectra were recorded on a Varian Cary 300 Bio spectrophotometer; IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer; NMR spectra were measured on a Bruker DRX-400 (400MHz for ^{1}H and 100 MHz for ^{13}C spectra) spectrometer. Chemical shifts were expressed in δ values with reference to TMS as internal standard, and coupling constants (J) were given in Hz. ESI-MS were carried out on a Finnigan MAT 95 instrument.

Plant Material. *Aphananthe aspera* was collected from Liu Zhou, Guang Xi Province, the People's Republic of China and was identified by Prof. Hua Peng of the Kunming Institute of Botany, Chinese Academy of Science. A voucher specimen has been deposited in the Herbarium of the Shanghai Institute of Materia Medica.

Extraction and Isolation. The air-dried powdered stem bark of *A. aspera* (1.0 kg) was extracted with 95% EtOH (8 L × 3, 2 days each) at room temperature. After removal of solvent in *vacuo*, an extract of 100 g was obtained, which was partitioned between various organic solvents and water to afford EtOAc-soluble (12.3 g) and *n*-BuOH-soluble (30.2 g) fractions.

The *n*-BuOH-soluble fraction was chromatographied on a silica gel column using eluents of increasing polarity, from $CHCl_3$ to CH_3OH. The fraction eluted with $CHCl_3$-CH_3OH (9 : 1) was further purified by ODS-18 column chromatography using MeOH-H_2O (8:2) as eluent to yield compounds **3** and **4**. The fraction eluted with $CHCl_3$-CH_3OH (8:2) was further purified by Sephadex LH-20 column chromatography using MeOH as eluent to yield compounds **2** and **5**. The fraction eluted with $CHCl_3$-CH_3OH (7:3) was further purified by ODS-18 column chromatography using MeOH-H_2O (3:2) as eluent to afford compound **1**.

3-*O*-Methylellagic acid-4′-*O*-α-L-rhamnopyranoside (1), white needle crysals, mp 249~251°C; ESI-MS (negative) *m/z*: 461 $[M-1]^-$; HR-ESIMS *m/z*: 461.0769 $[M-1]^-$ (calcd. for $C_{21}H_{17}O_{12}$, 461.0798); ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, δ, ppm, J/Hz): 4.01 (3H, s, 3-CH_3), 5.45 (1H, s, H-1″), 7.61 (1H, s, H-5′), 7.48 (1H, s, H-5); ^{13}C NMR (DMSO-d_6, 100 MHz): 107.2 (C-1), 140.1 (C-2), 136.2 (C-3), 146.5 (C-4), 111.4 (C-5), 111.7 (C-6), 158.7 (C-7), 114.3 (C-1′), 141.2 (C-2′), 141.9 (C-3′), 152.6 (C-4′), 111.6 (C-5′), 113.0 (C-6′), 158.8 (C-7′), 100.1 (C-1″), 70.1 (C-2″), 70.0 (C-3″), 71.7 (C-4″), 69.8 (C-5″), 17.9 (C-6″), 61.1 (3-CH_3).

3-*O*-Methylellagic acid-4′-*O*-β-D-xylopyranoside (2), white needle crysals, mp >300°C; ESI-MS (negative) *m/z*: 447 $[M-1]^-$; ^{1}H NMR and ^{13}C NMR spectrum data corresponded well to those reported [3].

3,3′-Di-*O*-methylellagic acid-4′-*O*-β-D-xylopyranoside (3), white needle crysals, mp 227~229°C; ESI-MS (negative) *m/z*: 461 $[M-1]^-$; ^{1}H NMR and ^{13}C NMR spectrum data were in accordance with those reported [3].

3,3′,4-Tri-*O*-methylellagic acid-4′- *O*-β-D-glucopyranoside (4), prism crystals, mp 266~268°C; ESI-MS (negative) *m/z*: 505 $[M-1]^-$. ^{1}H NMR and ^{13}C NMR spectrum data corresponded well to those reported [2].

3,3′-Di-*O*-methylellagic acid-4′-*O*-α-L-rhamnopyranoside (5), prism crystals, mp 186°C (dec); ESI-MS (negative) *m/z*: 475 $[M-1]^-$. ^{1}H NMR and ^{13}C NMR spectrum data were in accordance with those reported [4].

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