

Communication

## A New Phenolic Amide from the Roots of *Paris verticillata*

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**Abstract:** A new phenolic amide **8**, together with the nine known phenolic compounds **1-7**, **9** and **10** were isolated from the MeOH extract of the roots of *Paris verticillata*. The structure of the new compound **8** was determined to be 1-*N*-feruloylaminobutyl-4-*p*-hydroxybenzamide by spectroscopic methods. The isolated compounds were tested for cytotoxicity against four human tumor cell lines using the SRB assay.

**Keywords:** *Paris verticillata*, Liliaceae, cytotoxicity, 1-*N*-feruloyl-aminobutyl-4-*p*-hydroxybenzamide

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### Introduction

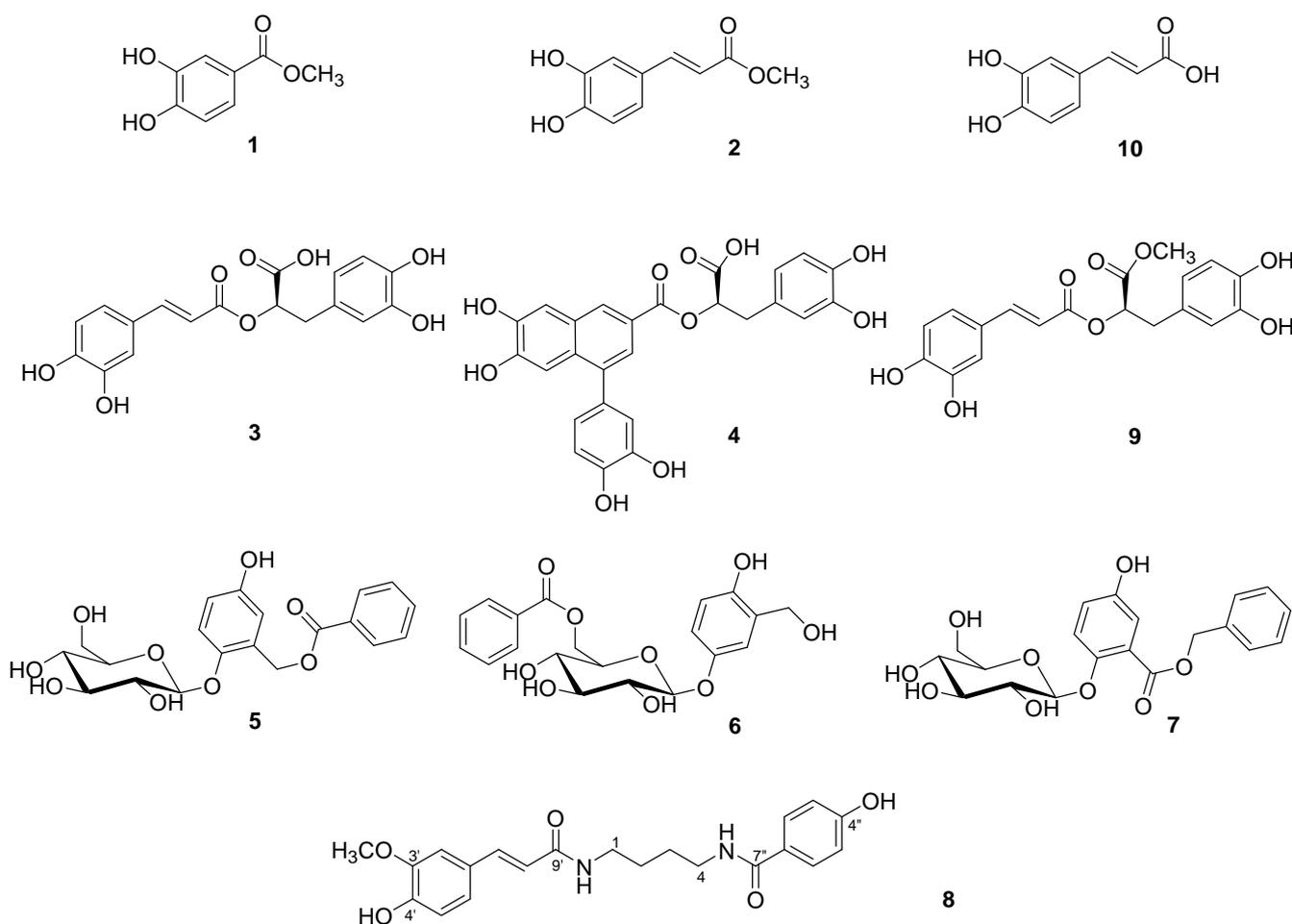
The perennial herb *Paris verticillata* BIEB. (Liliaceae) is found in Korean valleys and has been used in Korean folk medicine as a treatment for asthma and chronic bronchitis [1]. Steroids and flavonol glycosides have been isolated from *P. verticillata* [2]. In continuation of our search for biologically active compounds from Korean medicinal plant sources, we have investigated the constituents of the roots of *P. verticillata*. Repeated column chromatographic separation of the MeOH extract of this plant led to the isolation of a new phenolic amide **8**, as well as nine known phenolic

constituents (**1** - **7**, **9** and **10**, Figure 1). The known compounds are reported from this plant source for the first time. This paper deals with the isolation of the phenolic constituents, their cytotoxicity and the structure determination of the new phenolic amide.

## Results and Discussion

The known compounds (Figure 1) were identified as methyl 3,4-dihydroxybenzoate (**1**) [3], methyl caffeate (**2**) [4], rosmarinic acid (**3**) [5], globoidnan A (**4**) [6], salireposide (**5**) [7], xylosmacin (**6**) [8], trichocarpin (**7**) [9], methyl rosmarinate (**9**) [5] and caffeic acid (**10**) [10] by comparing their spectroscopic data with those reported in the literature.

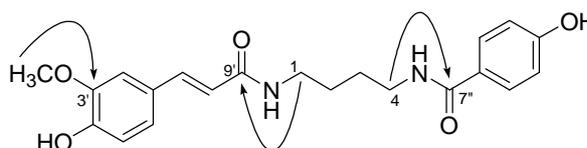
**Figure 1.** The structures of the isolated compounds **1-10** from *P. verticillata*.



Compound **8** was obtained as a yellowish gum. Its molecular formula  $C_{21}H_{25}O_5N_2$  was determined by HR FAB-MS ( $m/z$ ) 385.1782  $[M+H]^+$ , (calcd. 385.1758). The IR spectrum showed a hydroxyl group at  $3334\text{ cm}^{-1}$  and an amide group at  $1575\text{ cm}^{-1}$ . The  $^1\text{H-NMR}$  spectrum showed two benzene rings [ $\delta$  7.70 (2H, d,  $J = 8.5\text{ Hz}$ , H-2'', 6'') and 7.13 (1H, d,  $J = 2.0\text{ Hz}$ , H-2'), 7.03 (1H, dd,  $J = 8.0, 2.0\text{ Hz}$ , H-6'), 6.82 (2H, d,  $J = 8.5\text{ Hz}$ , H-3'', 5'') and 6.80 (1H, d,  $J = 8.0\text{ Hz}$ , H-5')] and one double bond with *trans* coupling [ $\delta$  7.44 (1H, d,  $J = 15.5\text{ Hz}$ , H-7') and 6.43 (1H, d,  $J = 15.5\text{ Hz}$ , H-8')]. The  $^{13}\text{C-NMR}$  spectrum exhibited 21 carbon signals, including two amide signals at  $\delta$  169.0 (C-7''), and 168.1

(C-9'), fourteen olefinic carbon signals at  $\delta$  160.8 (C-4''), 148.7 (C-4'), 148.1 (C-3'), 140.8 (C-7'), 129.0 (2C-2'', 6''), 127.1 (C-1'), 125.4 (C-1''), 122.0 (C-6'), 117.6 (C-8'), 115.3 (C-5'), 114.9 (2C-3'', 5'') and 110.5 (C-2'), four aliphatic carbon signals at  $\delta$  39.3 (C-4), 39.1 (C-1), 26.9 (C-3) and 26.8 (C-2), and one methoxy signal at  $\delta$  55.2 (OMe). The correlation of  $\delta$  3.35 (2H, t,  $J = 7.0$  Hz, H-1) with 168.1 (C-9'),  $\delta$  3.40 (2H, t,  $J = 7.0$  Hz, H-4) with 169.0 (C-7''), and  $\delta$  3.89 (OMe) with 148.1 (C-3') in the HMBC spectrum of **8** confirmed the connectivities of *p*-hydroxybenzamide and feruloyl amide group with the *n*-butyl chain (Figure 2). Thus, the structure of new compound **8** was determined to be 1-*N*-feruloyl-aminobutyl-4-*p*-hydroxybenzamide.

**Figure 2.** Key HMBC correlations of **8** ( $^1\text{H} \rightarrow ^{13}\text{C}$ )



### Biological activity

The isolated compounds were tested for cytotoxicity against four human tumor cell lines using the sulforhodamin B (SRB) assay. Compounds **1**, **2**, **4** and **9** showed mild cytotoxicity against only SK-OV-3 (ovary malignant ascites), with  $\text{ED}_{50}$  values of 10.14, 9.13, 12.27 and 7.22  $\mu\text{M}$ , respectively. The other compounds exhibited little activity ( $\text{ED}_{50} > 30 \mu\text{M}$ ) against the tested cancer cell lines.

## Experimental

### General

NMR spectra were recorded on a Varian Unity Inova 500Nb spectrometer. MS spectra were recorded on a JEOL JMS-700 mass spectrometer. Preparative HPLC used a Knauer K1001 instrument equipped with a refractive index detector, a UV detector and an Econosil silica 5  $\mu\text{m}$  column (length: 250 mm, I.D 22 mm, Alltech) and an Econosil C<sub>18</sub> 5  $\mu\text{m}$  column (length: 250 mm, I.D 4.6 mm, Alltech). For open column chromatography, silica gel (Merck, 70-230), ODS (Cosmosil 140 C<sub>18</sub>) and Sephadex LH-20 (Pharmacia) were used. Low pressure liquid chromatography was carried out over Merck LiChroprep Lobar-A Si 60 or Merck LiChroprep Lobar-A RP-C18 columns with an FMI QSY pump (Isco).

### Plant material

The roots of *P. verticillata* (1.5 kg) were collected at Mt. O-Dae, Gang Won province, S. Korea in October 2002. A voucher specimen (SKKU-2002-04) was deposited at the College of Pharmacy, Sungkyunkwan University, Korea.

### Extraction and Isolation

The dried and chopped root of *P. verticillata* (1.5 kg) was extracted three times with MeOH at room temperature. The MeOH extract (180 g) was suspended in distilled water (1.6 L) and successively partitioned with *n*-hexane, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc and *n*-butanol, followed by evaporation, to yield 5.2, 3.5, 4.4 and 20 g fractions, respectively. The EtOAc fraction (4.4 g) was chromatographed over a silica gel column using a gradient solvent system (CHCl<sub>3</sub>-MeOH = 5:1 → 0:1) to give five subfractions (E1- E5). The E1 fraction (0.2 g) was chromatographed over silica gel (CHCl<sub>3</sub>-MeOH = 10:1), Sephadex LH-20 filtration (MeOH) and purified using HPLC (RP C-18 resin, 30% MeOH) to yield **1** (7 mg) and **2** (15 mg). The E2 fraction (1.4 g) was chromatographed over RP C-18 resin (50% MeOH), silica gel (CHCl<sub>3</sub>-MeOH = 7:1) and purified using HPLC (RP C-18 resin, 45% MeOH) to yield **3** (15 mg), **4** (4 mg), **5** (5 mg), **6** (6 mg) and **7** (5 mg). The E3 fraction (1.2 g) was chromatographed over silica gel (CHCl<sub>3</sub>-MeOH = 10:1), LPLC (RP C-18 resin, 40% MeOH) and purified using HPLC (silica gel, hexane-CHCl<sub>3</sub>-MeOH = 3:5:1 and 2:5:1) to yield **8** (4 mg), **9** (25 mg) and **10** (34 mg).

*Spectroscopic data for 1-N-feruloyl-aminobutyl-4-ρ-hydroxybenzamide (8)*: Yellowish gum; UV (MeOH) λ<sub>max</sub> nm (log ε): 317 (3.446) and 247 (3.631); IR ν<sub>max</sub>: 3334, 2936, 1575 and 1128 cm<sup>-1</sup>; HR FAB-MS (*m/z*) 385.1782 [M+H]<sup>+</sup>, (C<sub>21</sub>H<sub>25</sub>O<sub>5</sub>N<sub>2</sub> calcd. 385.1758); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 500 MHz): δ 7.70 (2H, d, *J* = 8.5 Hz, H-2'', 6''), 7.44 (1H, d, *J* = 15.5 Hz, H-7'), 7.13 (1H, d, *J* = 2.0 Hz, H-2'), 7.03 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.82 (2H, d, *J* = 8.5 Hz, H-3'', 5''), 6.80 (1H, d, *J* = 8.0 Hz, H-5'), 6.43 (1H, d, *J* = 15.5 Hz, H-8'), 3.89 (3H, s, OMe), 3.40 (2H, t, *J* = 7.0 Hz, H-4), 3.35 (2H, t, *J* = 7.0 Hz, H-1), 1.68 (4H, m, H-2, 3); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): δ 169.0 (C-7''), 168.1 (C-9'), 160.8 (C-4''), 148.7 (C-4'), 148.1 (C-3'), 140.8 (C-7'), 129.0 (2C-2'', 6''), 127.1 (C-1'), 125.4 (C-1''), 122.0 (C-6'), 117.6 (C-8'), 115.3 (C-5'), 114.9 (2C-3'', 5''), 110.5 (C-2'), 55.2 (OMe), 39.3 (C-4), 39.1 (C-1), 26.9 (C-3), 26.8 (C-2).

### Test for in vitro cytotoxicity.

A sulforhodamin B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines [11]. The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma) and HCT (colon adenocarcinoma).

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*Sample Availability:* Milligram quantities of compounds **2**, **3**, **9** and **10** are available from the authors.

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