http://dx.doi.org/10.5012/bkcs.2012.33.4.1253

# Structure-Guided Identification of Novel Phenolic and Phenolic Amide Allosides from the Rhizomes of *Cimicifuga heracleifolia*

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*Received December 20, 2011, Accepted January 12, 2012*

Two phenolic allopyranosides and two phenolic amide allopyranosides, along with eight known phenolic compounds, including cimicifugic acids, shomaside B, fukiic acid, isoferulic acid, and piscidic acid, were isolated from the *n*-butanolic extract of rhizomes of *Cimicifuga heracleifolia.* On-line spectroscopic data for UV, NMR, and MS from a combination of LC-NMR and LC-MS techniques directly and rapidly provided sufficient structural information to identify and confirm all the structures of major phenolic compounds in the extract, in addition to their HPLC profiles. This combined analytic information was then used as a dereplication tool for structure-guided screening in order to isolate unknown phenolic compounds in the extract. Successive fractionation and purification using semi-preparative HPLC acquired four unknown allopyranosides, and their structures were identified as *cis*-ferulic acid 4-*O*-*β*-D-allopyranoside, *trans*-ferulic acid 4-*O*-*β*-D-allopyranoside, *trans*-feruloyltyramine 4-*O*-*β*-D-allopyranoside, and *trans*-feruloyl-(3-*O*-methyl)dopamine 4-*O*-*β*-D-allopyranoside, based on a subsequent spectroscopic interpretation.

**Key Words :** *Cimicifuga heracleifolia*, *cis*-Ferulic acid 4-*O*-*β*-D-allopyranoside, *trans*-Ferulic acid 4-*O*-*β*-D-allopyranoside, *trans*-Feruloyltyramine 4-*O*-*β*-D-allopyranoside, *trans*-Feruloyl-(3-*O*-methyl)dopamine 4-*O*-*β*-D-allopyranoside

## Introduction

Plants of the genus *Cimicifuga*, a member of the family Ranunculaceae, are perennial plants distributed widely in Asia, Europe, and North America; and the dried rhizomes of most *Cimicifuga* species are commonly used as a traditional medicine and a herbal dietary supplement.[1,2] Numerous studies pertaining to this herbal drug have been performed on their phytochemical and bioactive constituents, and have included biological and pharmacological characterizations and clinical trials.[2] Also, Cimicifugae Rhizoma originating from *Cimicifuga heracleifolia* Komarov and *Cimicifuga dahurica* Maxim., have been traditionally utilized as antipyretic, analgesic, and anti-inflammatory drugs in China, Korea, and Japan.[2-4] However, although several biological and chemical characterizations have been previously performed on the *C. heracleifolia* extract,[5-7] the phytochemical aspects of the plant extract of this species have not yet been extensively investigated.

In the study of natural products chemistry, conventional bioassay-guided fractionation procedures often lead to the isolation of phytochemically or pharmacologically already-known substances. Hence, various LC-coupled spectroscopic methods have been considerably applied to preliminary identification step at an early stage in order to distinguish target substances in the crude extracts.[8] HPLC-hyphenated spectroscopy with LC-NMR and LC-MS was applied in an attempt to acquire the detailed structural information of each LC peak from the crude extract of *Cimicifuga heracleifolia* rhizomes. The directly coupled HPLC, *i.e*, $^1$H NMR spectroscopy and mass spectrometry, is known as a straightforward and efficient analytical technique that is increasingly being used to determine the structures of natural products, without the necessity of time-consuming and laborious isolations.[9-11] This combined method allows plant extracts to be screened not just for biological activity but at the same time for structural classes, which plays a significant role in the isolation of natural products having an unknown structure.[12] To date, a number of applications of LC-NMR combined with LC-MS to the identification or characterization of drug metabolism products in biofluids, natural products in crude plant extracts, and isomeric mixtures produced by chemical reactions have been described and summarized.[13-15]

As part of our ongoing research pertaining to the identification of bioactive and novel natural products, the on-line structural elucidation of phenolic constituents *via* LC-NMR and LC-MS was carried out in an *n*-butanolic extract of *C. heracleifolia* extract in order to avoid isolating known substances during structure-guided natural product screening. A further isolation and purification step followed this derepli-

cation procedure, which enabled the tracing of unknown phenolic constituents. Here, we describe in detail the application of LC-NMR and LC-ESIMS to the screening of novel phenolic and phenolic amide glycosides (**3**-**6**) from the rhizomes of *C. heracleifolia*, in addition to their isolation and structural elucidation and the rapid characterization of eight previously known major phenolic compounds (**1**, **2**, **7**-**12**).

## Experimental Section

**General Experimental Procedures.** Optical rotation data were measured using a JASCO DIP 1000 digital polarimeter. $^1$H, $^{13}$C, and 2D-NMR experiments were recorded using either a Varian Unity INOVA 500 or a Varian VNMRS 600 MHz NMR spectrometer. Here, LC-$^1$H NMR was performed on a Varian VNMRS 600 MHz NMR spectrometer ($^1$H: 600.006 MHz) hyphenated to a Varian ProStar HPLC system using a 150 μL triple-resonance microflow cryogenic probe. LC-MS was measured on a Varian 320-MS TQ mass spectrometer coupled to a Varian ProStar HPLC system in negative and positive ion mode electrospray ionization (ESI). High resolution mass (ESIMS) and LC-MS/MS spectra were measured on a Waters Synapt HDMS TOF mass spectrometer operating in negative electrospray ion mode. IR spectra were recorded using a Shimadzu IR Prestige-21 spectrometer, and UV spectra were obtained using an Optizen 3220UV spectrometer. Note that an analytical HPLC was performed on an Agilent HP1100 series, comprised of a degasser, a binary mixing pump, a column oven, and a DAD detector, using a Waters SunFire$^{TM}$ (4.6 mm × 150 mm, 5 μm) column. A semi-preparative HPLC was carried out on a Waters multisolvent delivery system that was connected to a DECASSIT$^{TM}$, 6342 degasser, using a Waters SunFire$^{TM}$ Prep C18 (10 mm × 150 mm and 19 mm × 150 mm, 5 μm) columns in conjunction with MeCN and $H_2O$ containing 0.1% HCOOH.

**Plant Material.** Rhizomes of *Cimicifuga heracleifolia* were purchased from a local herbal store, Hansol Pharm. Co., Ltd. (Gwangju, Korea) in December, 2006, which were imported from China. These plant materials were identified by Prof. Dong Young Rhyu, Department of Medicinal Plant Resources, Mokpo National University. A voucher specimen (No. GTI Cimicifuga Rhizoma-2006-Bat. 001) was subsequently deposited in the GIST Technology Institute at the Gwangju Institute of Science and Technology.

**Extraction Procedure.** The dried rhizomes (3.0 kg) were exhaustively extracted (three times) using 80% MeOH at room temperature. The resultant MeOH extract was evaporated under 40 °C at reduced pressure, and this extract (330.4 g) was then suspended in $H_2O$ and partitioned with *n*-hexane, EtOAc, *n*-BuOH, and $H_2O$, successively. The organic extracts, including the *n*-BuOH extract (37.0 g), were stored at −20 °C.

**LC-MS Method.** The HPLC method was initiated with 95% $D_2O$ containing 0.1% HCOOH/5% MeCN, followed by a gradient to 35% $D_2O$ containing 0.1% HCOOH/65% MeCN over 35 min (total run time 45 min), and its flow rate of 1.0 mL/min was split into 95% for UV detection (280 nm) and 5% for MS detection.

**LC-NMR Method.** 1D $^1$H NMR spectra for the *n*-BuOH extract were obtained both in stopped-flow and continuous-flow modes. For the stopped-flow mode $^1$H NMR, the HPLC method was initiated with 95% $D_2O$ containing 0.1% HCOOH/5% MeCN, followed by a gradient to 35% $D_2O$ containing 0.1% HCOOH/65% MeCN over 35 min (total run time 45 min), with a flow rate of 1.0 mL/min and UV detection at 280 nm. The mother liquor sample (50 μL) was injected onto a Waters SunFire$^{TM}$ C18 (5 μm, 4.6 mm × 150 mm) reversed-phase column. The standard WET1D sequence was used for the pre-saturation of the $^1$H frequency in HOD, MeCN, and HCOOH. Data were acquired at a 9 kHz sweep width using 33 K time domain points at an acquisition time of 1.82 s. Variable numbers of scans (128-512) were used based on the relative concentration of each compound within the probe flow cell. $^1$H NMR spectra were then referenced to the MeCN resonance (1.96 ppm). The continuous-flow LC-NMR experiment was performed under the same gradient conditions, except the flow rate was changed to 0.2 mL/min and the total run time was 180 min. $^1$H NMR spectra were collected by 32 scans each, continuously obtained during chromatographic elution. The retention times ($t_R$) of each LC peak were as follows: **1** (32.4 min), **2** (46.8 min), **7** (119.1 min), **8** (120.1 min), **9** (164.8 min), **10** (165.8 min), **11** (176.6 min), and **12** (177.4 min).

**Isolation of Allosides.** An aliquot (10.21 g) of the *n*-BuOH extract was separated using the semi-preparative HPLC on the peak-based fractionation, based on the following conditions: initiation with 88% $H_2O$ containing 0.1% HCOOH/12% MeCN, followed by a gradient to 55% $H_2O$ containing 0.1% HCOOH/45% MeCN for 40 min, at a flow rate of 4.0 mL/min and UV detection at 280 nm. Compounds **3** (4.0 mg) and **4** (3.3 mg) were purified by HPLC with a gradient solvent system; 95% $H_2O$ containing 0.1% HCOOH/5% MeCN to 85% $H_2O$ containing 0.1% HCOOH/15% MeCN for 70 min [$t_R$ of each compound: **3** (23.67 min) and **4** (32.28 min)]. Compounds **5** (1.3 mg) and **6** (4.0 mg) were further chromatographed at a gradient eluent; 90% $H_2O$ containing 0.1% HCOOH/10% MeCN to 75% $H_2O$ containing 0.1% HCOOH/25% MeCN for 70 min [$t_R$: **5** (32.84 min) and **6** (34.26 min)].

***cis*-Ferulic acid 4-*O*-*β*-D-allopyranoside (3):** Brownish amorphous powder, $[\alpha]_D$ +72.6° (*c* 0.2, MeOH); UV $\lambda_{max}$ ($H_2O$) (log ε) 269 (6.04), 303 (5.86) nm; IR (KBr) $\nu_{max}$: 3451, 1634, 667 cm$^{-1}$; $^1$H and $^{13}$C NMR spectra ($D_2O$, 600 MHz): See Table 1; HRESIMS *m/z* 355.1094 $[M-H]^-$ (calcd for $C_{16}H_{19}O_9$, 355.1029).

***trans*-Ferulic acid 4-*O*-*β*-D-allopyranoside (4):** Brownish amorphous powder, $[\alpha]_D$ +175.2° (*c* 0.1, MeOH); UV $\lambda_{max}$ ($H_2O$) (log ε) 267 (6.01), 302 (5.76) nm; IR (KBr) $\nu_{max}$: 3445, 1634, 709.8 cm$^{-1}$; $^1$H and $^{13}$C NMR spectra ($D_2O$, 600 MHz): See Table 1; HRESIMS *m/z* 355.1028 $[M-H]^-$ (calcd for $C_{16}H_{19}O_9$, 355.1029).

***trans*-Feruloyltyramine 4-*O*-*β*-D-allopyranoside (5):**

Brownish amorphous powder, $[\alpha]_D$ −268.2° (*c* 0.1, MeOH); UV $\lambda_{max}$ (MeOH) (log ε) 230 (6.29), 283 (6.07), 312 (5.95) nm; IR (KBr) $\nu_{max}$: 3364, 2945, 2833, 1261, 1032 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra ($CD_3OD$, 600 MHz): See Table 2; HRESIMS *m/z* 474.1763 $[M-H]^-$ (calcd for $C_{24}H_{28}NO_9$, 474.1764).

***trans*-Feruloyl-(3-*O*-methyl)dopamine 4-*O*-β-D-allopyranoside (6):** Brownish amorphous powder, $[\alpha]_D$ −15.0° (*c* 0.2, MeOH); UV $\lambda_{max}$ (MeOH) (log ε) 228 (6.19), 283 (5.92), 313 (5.78) nm; IR (KBr) $\nu_{max}$: 3389, 2945, 2833, 1261, 1032, 916, 665 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra ($CD_3OD$, 600 MHz): See Table 2; HRESIMS *m/z* 504.1866 $[M-H]^-$ (calcd for $C_{25}H_{30}NO_{10}$, 504.1870).

## Results and Discussion

Dried rhizomes of *C. heracleifolia* were extracted with 80% MeOH and fractionated by successive partitions with *n*-hexane, EtOAc, and *n*-BuOH to obtain three crude extracts. Based on preliminary analytical scale HPLC-DAD experimental results, the *n*-BuOH-soluble extract was finally selected for further LC-NMR and LC-MS studies. An initial fast LC-NMR experiment was performed in continuous-flow mode. 1D LC-$^1H$ NMR slices corresponding to all the peaks were extracted, and the 2D on-flow LC-$^1H$ NMR chromatogram was then analyzed, despite its relatively low signal-to-noise ratio. In addition, to obtain more structural information, stopped-flow LC-NMR detection was carried out to acquire satisfactory $^1H$ NMR spectra of the main compounds **1**, **2**, **7**-**12** (Figure 1). A subsequent LC-ESIMS

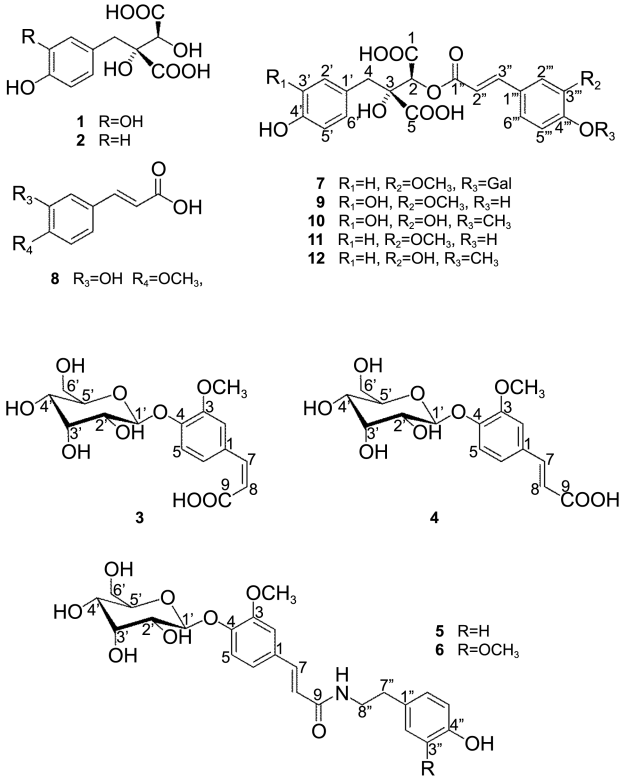


**Figure 1.** Chemical Structures of Compounds **1**-**12**.

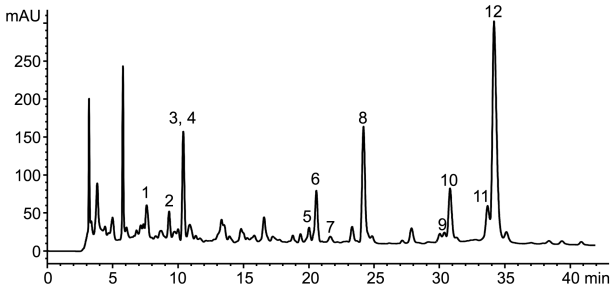


**Figure 2.** HPLC profile of the *n*-BuOH fraction obtained from Cimicifugae Rhizoma. HPLC conditions were as follows; Column: SunFire C18 (4.6 mm × 150 mm, 5 μm). Eluent: 88% $H_2O$ containing 0.1% HCOOH/12% MeCN followed by a gradient to 55% $H_2O$ containing 0.1% HCOOH/45% MeCN over 40 min. Flow rate: 1.0 mL/min. UV: 280 nm. Retention times ($t_R$) of each LC peak: **1** (7.60 min), **2** (9.32 min), **3** and **4** (10.41 min), **5** (20.03 min), **6** (20.58 min), **7** (21.66 min), **8** (24.19 min), **9** (30.38 min), **10** (30.81 min), **11** (33.69 min), and **12** (34.18 min).

analysis of this crude mixture revealed a series of closely related compounds, which could then be identified by their quasi-molecular ion peaks $[M-H]^-$. The LC-$^1H$ NMR spectra of fukiic acid (**1**),[16] piscidic acid (**2**),[16] shomaside B (7),[17] isoferulic acid (**8**),[18] cimicifugic acid A (**9**),[16] cimicifugic acid B (**10**),[16] cimicifugic acid E (**11**),[16] and cimicifugic acid F (**12**),[16] allowed for direct identification *via* a comparison with previously reported conventional NMR data. Since the spectra exclusively exhibited aromatic signals, there was no overlap with the solvent signals of $H_2O$ and MeCN. The remaining LC peaks, except for **1**-**12**, were separately collected in the modified HPLC condition (Figure 2) based on both the LC-NMR and LC-MS data, and then analyzed using an off-line NMR after purification.

Phenolic glycosides **3** and **4** were isolated as a pale brownish amorphous powder. They exhibited highly similar UV absorption bands at 269 and 303 nm (**3**) and at 267 and 302 nm (**4**) with IR absorption bands corresponding to hydroxyl (3451 $cm^{-1}$ in **3** and 3445 $cm^{-1}$ in **4**) and carbonyl (1634 $cm^{-1}$). Their molecular formulas were then found to correspond to $C_{16}H_{20}O_9$ based on the HRESIMS spectra of both compounds, which were obtained in negative ion mode (*m/z* 355.1094 $[M-H]^-$ of **3**, and *m/z* 355.1028 $[M-H]^-$ of **4**. calcd for $C_{16}H_{19}O_9$, 355.1029) (Figure 2).

In the $^1H$ and $^{13}C$ NMR spectra (Table 1), compound **3** showed proton signals corresponding to a 1,3,4-trisubstitued benzene ring at $\delta_H$ 7.21 (1H, *d*, *J* = 1.8 Hz, H-2), 7.06 (1H, *d*, *J* = 8.7 Hz, H-5), and 6.99 (1H, *dd*, *J* = 8.7, 1.8 Hz, H-6), with a *cis*-double bond at $\delta_H$ 6.78 (1H, *d*, *J* = 12.6 Hz, H-7), 5.90 (1H, *d*, *J* = 12.6 Hz, H-8), and a methoxyl proton at $\delta_H$ 3.76 (3H, *s*, 3-OMe), as well as a carboxyl carbon at $\delta_C$ 172.4 (C-9). These data indicated that **3** had either an isoferuloyl or feruloyl moiety. In addition, seven oxygen-bearing proton signals assignable to a glycosidic moiety were observed at $\delta_H$ 5.30 (1H, *d*, *J* = 8.4 Hz), 3.69 (1H, *dd*, *J* = 8.4, 3.0 Hz), 4.18 (1H, *t*, *J* = 3.0 Hz), 3.64 (1H, *dd*, *J* = 10.2, 3.0 Hz), 3.83 (1H, *ddd*, *J* = 10.2, 5.4, 2.4 Hz), 3.80 (1H, *dd*, *J* = 12.6, 2.4 Hz), and 3.63 (1H, *dd*, *J* = 12.6, 5.7 Hz), with six relevant carbon signals at $\delta_C$ 98.0 (CH), 69.8

**Table 1.** NMR data for phenolic allosides **3** and **4**[a]

| Position | **3** | | | **4** | | |
|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | HMBC (H→C) | $\delta_H$ | $\delta_C$ | HMBC (H→C) |
| 1 | - | 130.1 | - | - | 128.9 | - |
| 2 | 7.21 (1H, *d*, 1.8) | 112.9 | 3, 4, 6, 7 | 6.99 (1H, *m*)[b] | 111.1 | 3, 4, 6, 7 |
| 3 | - | 148.0 | - | - | 148.4 | - |
| 4 | - | 145.9 | - | - | 147.4 | - |
| 5 | 7.06 (1H, *d*, 8.7) | 115.0 | 1, 3, 4 | 6.99 (1H, *m*)[b] | 115.1 | 1, 3, 4 |
| 6 | 6.99 (1H, *dd*, 8.7, 1.8) | 122.8 | 2, 4, 7 | 6.99 (1H, *m*)[b] | 122.7 | 2, 4, 7 |
| 7 | 6.78 (1H, *d*, 12.6) | 139.1 | 1, 2, 6, 9 | 7.35 (1H, *d*, 16.2) | 145.6 | 1, 2, 6, 9 |
| 8 | 5.90 (1H, *d*, 12.6) | 120.2 | 1, 7, 9 | 6.15 (1H, *d*, 16.2) | 115.9 | 1, 7, 9 |
| 9 | - | 172.4 | - | - | 171.0 | - |
| 1' | 5.30 (1H, *d*, 8.4) | 98.0 | 4 | 5.27 (1H, *d*, 7.8) | 97.9 | 4 |
| 2' | 3.69 (1H, *dd*, 8.4, 3.0) | 69.8 | 1' | 3.68 (1H, *dd*, 7.8, 3.0) | 69.8 | 1' |
| 3' | 4.18 (1H, *t*, 3.0) | 71.0 | 1', 2', 4', 5' | 4.17 (1H, *t*, 3.0) | 70.9 | 1', 2', 4', 5' |
| 4' | 3.64 (1H, *dd*, 10.2, 3.0) | 66.3 | 5', 6' | 3.63 (1H, *dd*, 10.2, 3.0) | 66.4 | 5', 6' |
| 5' | 3.83 (1H, *ddd*, 10.2, 5.4, 2.4) | 73.7 | 4' | 3.83 (1H, *ddd*, 10.2, 5.4, 2.4) | 73.7 | 4' |
| 6' | 3.80 (1H, *dd*, 12.6, 2.4) | 60.7 | 4', 5' | 3.79 (1H, *dd*, 12.1, 2.4) | 60.7 | 4', 5' |
| | 3.63 (1H, *dd*, 12.6, 5.7) | | 4', 5' | 3.62 (1H, *dd*, 12.1, 6.0) | | 4', 5' |
| Me | 3.76 (3H, *s*) | 55.7 | 3 | 3.70 (3H, *s*) | 55.6 | 3 |

[a]NMR data were observed at 600 ($^1$H) and 150 ($^{13}$C) MHz in $D_2O$ (δ in ppm, *J* in Hz). Assignments were verified by 2D NMR spectra with HSQC and HMBC. [b]Aromatic protons could not be analyzed due to severe signal overlap.

(CH), 71.0 (CH), 66.3 (CH), 73.7 (CH), and 60.7 ($CH_2$). This glycosidic moiety was identified as β-D-allopyranoside, due to the large vicinal coupling constants of H-1 (*J* = 8.4 Hz) and splitting patterns of H-2 through H-5. Specifically, the triplet of H-3 with a small *J* value (3.0 Hz) indicated that this sugar proton was equatorially positioned. Indeed, the proton NMR data of β-D-allopyranosyl group is in good agreement with those of cyanogenic allopyranosides in the same NMR solvent (MeOH-$d_4$).[19] The 2D NMR spectra including HSQC and HMBC displayed a methoxyl group attached to C-3, which indicated that the aglycone moiety is ferulic acid. Further HMBC correlations of H-1 to C-4 confirmed that an allopyranosyl group is linked to C-4 of ferulic acid. From these results, the structure of **3** was elucidated to be *cis*-ferulic acid 4-*O*-β-D-allopyranoside.

The $^1$H and $^{13}$C NMR spectra of **4** were closely similar to those of *cis*-ferulic acid 4-*O*-β-D-allopyranose (**3**), though a *trans*-olefinic moiety was observed at $\delta_H$ 7.35 (1H, *d*, *J* = 16.2 Hz, H-7), 6.15 (1H, *d*, *J* = 16.2 Hz, H-8) and $\delta_C$ 145.6 (C-7, CH), 115.9 (C-8, CH). As a result, the positions of methoxyl and allopyranose were identified to be C-3 and C-4, respectively, based on the HMBC correlations of H-1 at δ 5.27 (1H, *d*, *J* = 7.8 Hz) to C-4 at $\delta_C$ 147.4 and a methoxyl at $\delta_H$ 3.70 (3H, *s*) to C-3 at $\delta_C$ 148.4, which are identical with those of **3**. Thus, the structure of **4** was elucidated to be *trans*-ferulic acid 4-*O*-β-D-allopyranoside.

Compound **5** was obtained as a pale brownish amorphous powder, and its molecular formula was established as $C_{24}H_{29}NO_9$ by using the HRESIMS spectra obtained in negative ion mode (*m/z* 474.1763 $[M-H]^-$, calcd for $C_{24}H_{28}NO_9$, 474.1764) (Figure 3), based on the 24 carbon signals in the $^{13}$C NMR spectrum. The spectrum showed UV maximal absorptions at 230, 283 and 312 nm, and intensive IR absorption peaks at 3364, 2945, 2833, and 1032 $cm^{-1}$.

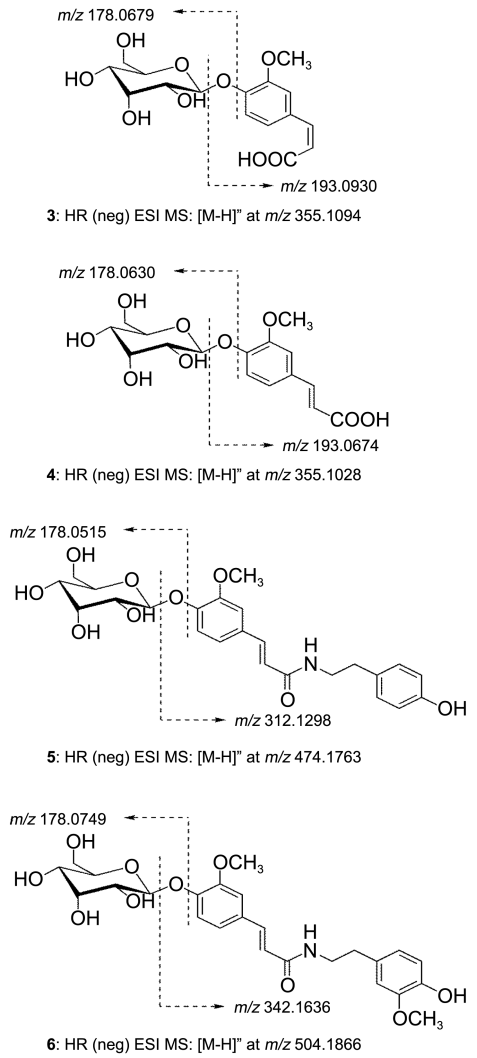


**Figure 3.** MS/MS fragmentation of **3-6**.

**Table 2.** NMR data for phenolic allosides **5** and **6**[a]

| Position | **5** | | | **6** | | |
|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | HMBC (H→C) | $\delta_H$ | $\delta_C$ | HMBC (H→C) |
| 1 | - | 131.0 | - | - | 131.0 | - |
| 2 | 7.18 (1H, *d*, 1.8) | 112.4 | 1, 4, 6, 7 | 7.17 (1H, *d*, 1.8) | 112.4 | 1, 3, 4, 6, 7 |
| 3 | - | 151.0 | - | - | 151.1 | - |
| 4 | - | 149.9 | - | - | 149.9 | - |
| 5 | 7.17 (1H, *d*, 7.8) | 117.4 | 1, 3, 4 | 7.17 (1H, *d*, 9.0) | 117.4 | 1, 3, 6 |
| 6 | 7.13 (1H, *dd*, 7.8, 1.8) | 122.9 | 2, 4, 7 | 7.12 (1H, *dd*, 9.0. 1.8) | 122.8 | 2, 4, 7 |
| 7 | 7.46 (1H, *d*, 15.9) | 141.5 | 2, 7, 8, 9 | 7.46 (1H, *d*, 15.9) | 141.5 | 1, 2, 6, 8, 9 |
| 8 | 6.48 (1H, *d*, 15.9) | 120.5 | 9 | 6.48 (1H, *d*, 15.9) | 120.5 | 1, 9 |
| 9 | - | 169.0 | - | - | 169.0 | - |
| 1' | 5.31 (1H, *d*, 7.8) | 100.4 | 4 | 5.31 (1H, *d*, 7.8) | 100.4 | 4 |
| 2' | 3.65 (1H, *dd*, 7.8, 3.0) | 72.1 | 1' | 3.65 (1H, *dd*, 7.8, 3.0) | 72.1 | 1', 3' |
| 3' | 4.16 (1H, *t*, 3.0) | 73.1 | 1' | 4.16 (1H, *t*, 3.0) | 73.1 | 1', 2' |
| 4' | 3.61 (1H, *dd*, 9.6. 3.0) | 68.7 | | 3.61 (1H, *dd*, 9.6. 3.0) | 68.7 | 5' |
| 5' | 3.85 (1H, *m*) | 76.0 | 4' | 3.85 (1H, *m*) | 76.0 | 4' |
| 6' | 3.86 (1H, *dd*, 12.3, 2.4) | 62.9 | 4' | 3.86 (1H, *m*) | 62.9 | 4' |
| | 3.68 (1H, *dd*, 12.3, 5.7) | | 5' | 3.68 (1H, *dd*, 12.3, 5.7) | | 4', 5' |
| 3-OMe | 3.89 (3H, *s*) | 56.8 | 3 | 3.88 (3H, *s*) | 56.8 | 3 |
| 3"-OMe | - | - | - | 3.83 (3H, *s*) | 56.5 | 3" |
| 1" | - | 131.4 | - | - | 132.1 | - |
| 2" | 7.06 (1H, *d*, 8.4) | 130.9 | 1", 4", 7" | 6.82 (1H, *d*, 1.8) | 113.5 | 3", 4", 6", 7" |
| 3" | 6.72 (1H, *d*, 8.4) | 116.4 | 4" | - | 149.1 | - |
| 4" | - | 157.1 | - | - | 146.2 | - |
| 5" | 6.72 (1H, *d*, 8.4) | 116.4 | 4" | 6.72 (1H, *d*, 8.1) | 116.3 | 1", 3", 4" |
| 6" | 7.06 (1H, *d*, 8.4) | 130.9 | 1", 4", 7" | 6.67 (1H, *dd*, 8.1, 1.8) | 122.4 | 2", 4", 7" |
| 7" | 2.76 (2H, *t*, 7.5) | 35.9 | 1", 2", 6", 8" | 2.77 (2H, *t*, 7.2) | 36.3 | 1", 2", 6", 8" |
| 8" | 3.47 (2H, *t*, 7.5) | 42.7 | 9", 1", 7" | 3.49 (2H, *t*, 7.2) | 42.6 | 9", 1", 7" |

[a]NMR data were observed at 600 ($^1$H) and 150 ($^{13}$C) MHz in MeOH-$d_4$ ($\delta$ in ppm, $J$ in Hz). Assignments were verified by 2D NMR spectra with HSQC and HMBC

The $^1$H NMR and $^{13}$C NMR spectra (Table 2) displayed 4-hydroxyphenethyl moiety signals at $\delta_H$ 7.06 (2H, *d*, $J$ = 8.4 Hz), 6.72 (2H, *d*, $J$ = 8.4 Hz), and 2.76 (2H, *t*, $J$ = 7.5 Hz), and 3.47 (2H, *t*, $J$ = 7.5 Hz), with corresponding carbon signals at $\delta_C$ 130.9, 116.4, 35.9, and 42.7. In addition, proton and carbon NMR signals quite similar to those of *trans*-ferulic acid 4-*O*-$\beta$-D-allopyranoside (**4**) were also shown, except for the upfield-shifted resonance ($\delta_C$ 169.0) of the carbonyl group. These observations suggest that this compound is a phenolic amide that contains a functional group (-CONH-) consisting of a feruloyl allopyranose and 4-hydroxyphenethylamine moiety. Note that the presence of an amide bond was unambiguously confirmed by the NMR spectra of **5** in DMSO-$d_6$. The proton signal corresponding to NH was observed at $\delta_H$ 8.07 (1H, *t*, $J$ = 5.7 Hz) in the $^1$H NMR spectrum using DMSO-$d_6$, which is not correlated with any carbon in the HSQC spectrum. In addition, this NH proton signal showed long-range correlations with C-8 methylene carbon at $\delta_C$ 40.6 and C-9 carbonyl at $\delta_C$ 165.1 in the HMBC NMR spectrum with DMSO-$d_6$. The HMBC correlations unambiguously confirmed the attached position of allopyranose and a methoxyl group; and the configuration of allopyranose was identified to be b based on the $J$ value (7.8 Hz) of the H-1 anomeric proton. From these results, the structure of **5** was identified as being that of *trans*-feruloyltyramine 4-*O*-$\beta$-D-allopyranoside.

Compound **6** was also isolated as pale brownish amorphous powder, and its molecular formula was established as $C_{25}H_{31}NO_{10}$ based on the HRESIMS spectra obtained in negative mode (*m/z* 504.1866 $[M-H]^-$, calcd for $C_{25}H_{30}NO_{10}$, 504.1870) (Figure 3) with 25 carbon signals. The proton signal of NH was also shown at $\delta$ 8.05 (1H, *t*, $J$ = 5.4 Hz) in the $^1$H NMR spectrum with DMSO-$d_6$. Its UV and IR data exhibited closely similar values to those of **5** at 228, 283, and 313 nm, and at 3389, 2945, 2833, and 1032 cm$^{-1}$, respectively. The NMR spectral data (Table 2) also showed allopyranosyl and *trans*-feruloyl moieties identical to those of **5**. However, the ABX aromatic proton signals -two methylene and one methoxyl signals corresponding to *O*-methyldopamine, instead of a 4-hydroxyphenethylamine group- were observed at $\delta_H$ 6.82 (1H, *d*, $J$ = 1.8 Hz), 6.72 (1H, *d*, $J$ = 8.1 Hz), and 6.67 (1H, *dd*, $J$ = 8.1, 1.8 Hz), and at 2.77 (2H, *t*, $J$ = 7.2 Hz), 3.49 (2H, *t*, $J$ = 7.2 Hz) and 3.83 (3H, *s*). 2D NMR experiments with HSQC and HMBC were then performed to elucidate the unambiguous structure of **6**. The allopyranose configuration was established to be $\beta$-form, based on the large vicinal coupling constants (7.8 Hz) of the anomeric proton, with the linked position being identified as

C-4 in a *trans*-feruloyl moiety. In the HMBC spectrum, long-range coupling was shown between methoxyl at $\delta_H$ 3.83 to C-3 of a dopamine moiety, and correlations of H-8 ($\delta_H$ 3.49; 2H, *t*, *J* = 7.2 Hz) to C-9 ($\delta_C$ 169.0) were also exhibited. Thus, the structure of **6** was identified to be *trans*-feruloyl-(3-*O*-methyl)dopamine 4-*O*-$\beta$-D-allopyranoside.

In this study, a fast and effective structural screening was successfully accomplished by combining LC-NMR and LC-ESIMS to identify known phenolic compounds using a single injection of *C. heracleifolia* extract with no subsequent isolation procedure. The acquired LC profiles and structural information for the known constituents were then applied to efficiently dereplicate unknown trace phenolic derivatives in an identical extract. The subsequent peak-based fractionation and purification for the selected LC peaks using semi-preparative HPLC then led to the isolation of novel phenolic and phenolic amide glycosides.

**Acknowledgments.** We gratefully thank the Gwangju branch of the Korea Basic Science Institute (KBSI) for running NMR and HRESIMS and LC-MS/MS experiments. This work was supported by a Korea Research Foundation Grant funded by the Korean government (KRF-2008-220-E00042).

## References

1. Jiang, J. W.; Qi, C. D. *Dictionary of Medicinal Plants*; Tianjin Science and Technology Press: Tianjin, China, 2005; p 175.
2. Li, J. X.; Yu, Z. Y. *Curr. Med. Chem.* **2006**, *13*, 2927.
3. Shibata, M.; Sakurai, N.; Onoda, M. *Yakugaku Zasshi* **1977**, *97*, 911.
4. Shibata, M.; Yamatake, Y.; Amagaya, Y.; Fukushima, M. *Yakugaku Zasshi* **1975**, *95*, 539.
5. Hirabayashi, T.; Ochiai, H.; Sakai, S.; Nakajima, K.; Terasawa, K. *Planta Med.* **1995**, *61*, 221.
6. Lee, K. H.; Lee, W. J.; Yang, S. J.; Huh, J. W.; Choi, J.; Hong, H. N.; Hwang, O.; Cho, S. W. *Mol. Cells* **2004**, *17*, 509.
7. Li, J. X.; Kadota, S.; Hattori, M.; Yoshimachi, S.; Shiro, M.; Oogami, N.; Mizuno, H.; Namba, T. *Chem. Pharm. Bull.* **1993**, *41*, 832.
8. Vogler, B.; Klaiber, I.; Roos, G.; Walter, C. U.; Hiller, W.; Sandor, P.; Kraus, W. *J. Nat. Prod.* **1998**, *61*, 175.
9. Höltzel, A.; Schlotterbeck, G.; Albert, G.; Bayer, E. *Chromatographia* **1996**, *42*, 499.
10. Schneider, B.; Zhao, Y.; Blitzke, T.; Schmitt, B.; Nookandeh, A.; Sun, X.; Stöckigt, J. *Phytochem. Anal.* **1998**, *9*, 237.
11. Spring, O.; Buschmann, H.; Vogler, B; Schilling, E. E.; Spraul, M.; Hoffmann, M. *Phytochemistry* **1995**, *39*, 609.
12. Gu, Z. M.; Zhaou, D.; Wu, J.; Shi, G.; Zeng, L.; McLaughlin, J. L. *J. Nat. Prod.* **1997**, *60*, 242.
13. Hostettmann, K.; Potterat, O.; Wolfender, J. L. *Pharm. Ind.* **1997**, *59*, 339.
14. Wolfender, J. L.; Ndjoko, K.; Hostettmann, K. *Phytochem. Anal.* **2001**, *12*, 2.
15. Wolfender, J. L.; Ndjoko, K.; Hostettmann, K. *Curr. Org. Chem.* **1998**, *2*, 575.
16. Takahira, M.; Kusano, A.; Shibano, M.; Kusano, G.; Sacurai, N.; Nagai, M.; Miyase, T. *Chem. Pharm. Bull.* **1998**, *46*, 362.
17. Iwanaga, A.; Kusano, G.; Warashina, T.; Miyase, T. *J. Nat. Prod.* **2010**, *73*, 573.
18. Kruse, S. O.; Lohning, A.; Pauli, G. F.; Winterhoff, H.; Nahrstedt, A. *Planta Med.* **1999**, *65*, 763.
19. Seigler, D. D.; Pauli, G. F.; Nahrstedt, A.; Leen, R. *Phytochemistry* **2002**, *60*, 873.