DOI 10.5012/bkcs.2011.32.8.3175

New Phenylpropanoid Glycosides from *Eurya emarginata* (Thunb.) Makino†

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Received March 8, 2011, Accepted May 26, 2011

Key Words : *Eurya emarginata*, Eutigoside D, Eutigoside E, Isolation, Structure determination

Eurya emarginata (Thunb.) Makino belonging to Theaceae is an evergreen branched shrub with coriaceous and emarginated leaves. These trees are distributed along the coastal regions in the southern parts of Korea including Jeju Island. The genus *Eurya* contains more than 100 species worldwide, but only two species have been described in Korea.[1] The leaves of *E. emarginata* and *E. japonica* have been used in Korean traditional medicine to treat arthritis.[2] Previous biological studies of the extract of *E. emarginata* reported the anti-cancer,[3] anti-inflammation[4] and radiation protection[5] activities, and identified phenolic glycosides as the active constituents. As part of an ongoing study aimed at identifying bioactive compounds from plants in Jeju Island,[6] this study conducted phytochemical investigations on this plant.

From the ethanol extract of the leaves and twigs of *E. emarginata*, new phenylpropanoid glycosides, eutigosides D (**1**) and E (**2**), were isolated along with five known compounds, eutigoside B (**3**), eutigoside C (**4**), cinnamic acid (**5**), rengyolone (**6**) and cleroindicin B (**7**) (Fig. 1). We herein report the structure elucidation and antioxidative activities of isolates **1**-**7**.

Compound **1** was obtained as viscous liquid with molecular ion peak at *m*/*z* 446.1577 in HR-FAB-MS, indicating a molecular formula of $C_{23}H_{26}O_9$ (11 unsaturations). The ^{13}C NMR spectrum revealed only 20 carbon peaks suggesting the presence of a symmetric moiety, which was readily identified as a phenyl group based on the characteristic aromatic ^1H and ^{13}C NMR signals appearing in narrow δ ranges (Tables 1 and 2). The *trans*-olefinic structure was also clearly characterized by the large coupling constant (J = 16.0 Hz) of the sp^2 peaks at δ 7.76 (H-7") and 6.52 (H-8") ppm. This olefin was determined to be connected to both the phenyl and a carbonyl (C-9") groups by an inspection of the heteronuclear multiple bond correlation (HMBC) spectrum, which established a cinnamoyl substructure. The other aromatic protons were characterized to have the ABX spin system in the ^1H NMR spectrum (Table 1). For example, the signal for H-5 showed a coupling constant of ^3J = 8.5 Hz, which confirmed the *ortho* coupling to H-6. The signal for H-6 showed *ortho* and *meta* coupling to H-2 with ^4J = 2.8 Hz. The remaining substructure –OCH_2CH_2- was confirmed by its ^1H, ^{13}C and COSY NMR data. The HMBC cross signal of H-8/C-1 indicated the linkage of this ethylene unit to C-1 of the aromatic ring (Fig. 2). The presence of a sugar moiety in compound **1** is also clearly recognized by the ^1H and ^{13}C NMR spectra. As protons of the sugar exhibited relatively large coupling constants due to their axial H/H interactions, this hexose was assumed to be glucose. This was confirmed by the hydrolysis of the compound **1** with aq. HCl followed by the comparative TLC analysis of the aqueous phase to the authentic D-glucose. As the anomeric

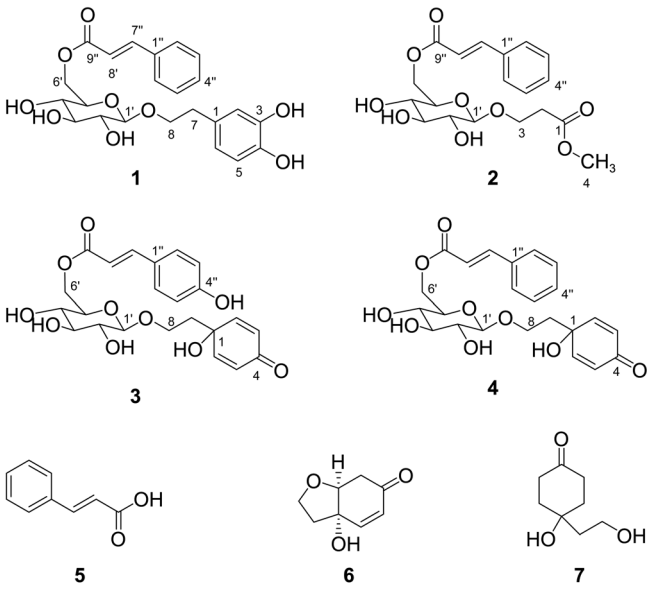

Figure 1. Structures of compounds **1**-**7** isolated from *E. emarginata*.

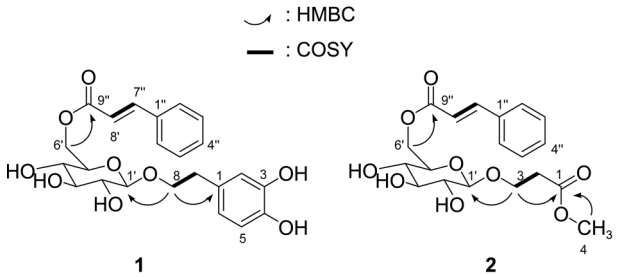

Figure 2. Key HMBC and COSY correlations for compounds **1** and **2**.

†This paper is dedicated to Professor Eun Lee on the occasion of his honourable retirement.

Table 1. ^{1}H NMR spectral data of compounds **1**, **2** and **4**[a]

Position	**1**[b]	**2**[c]	**4**[b]
1			
2	6.61 (d, 2.8)	2.62 (m)	6.69 (d, 9.0)
3		4.04 (m)	6.05 (d, 9.0)
		3.85 (m)	
4		3.63 (s)	
5	6.58 (d, 8.5)	–	6.05 (d, 9.0)
6	6.48 (dd, 8.5, 2.8)	–	6.69 (d, 9.0)
7	2.88 (t, 7.4)	–	2.04 (t, 5.0)
8	3.98 (m)	–	3.96 (m)
	3.77 (m)		3.66 (m)
1'	4.35 (d, 7.8)	4.32 (d, 7.8)	4.33 (d, 7.8)
2'	3.22 (dd, 9.0, 7.8)	3.36 (m)	3.37 (m)
3'	3.38 (m)	3.50 (m)	3.38 (m)
4'	3.36 (m)	3.44 (m)	3.22 (dd, 8.0, 8.0)
5'	3.55 (dd, 9.0, 6.0)	3.57 (m)	3.52 (m)
6'	4.50 (dd, 11.8, 2.0)	4.46 (dd, 11.8, 2.0)	4.53 (dd, 11.8, 2.2)
	4.32 (dd, 11.8, 2.0)	4.32 (dd, 11.8, 2.0)	4.34 (dd, 11.8, 2.2)
1''			
2''	7.51 (m)	7.48 (m)	7.76 (m)
3''	7.37 (m)	7.34 (m)	7.40 (m)
4''	7.38 (m)	7.34 (m)	7.41 (m)
5''	7.37 (m)	7.34 (m)	7.40 (m)
6''	7.51 (m)	7.48 (m)	7.76 (m)
7''	7.67 (d, 16.0)	7.67 (d, 16.0)	7.72 (d, 16.2)
8''	6.52 (d, 16.0)	6.45 (d, 16.0)	6.55 (d, 16.2)
9''			

[a]δ values are in ppm, and mulitiplicity and coupling constants (Hz) are noted in parentheses.
[b]CD_3OD was used as a solvent.
[c]$CDCl_3$ was used as a solvent.

peak at δ 4.35 (H-1') showed a large coupling constant (J = 7.8 Hz), the glucose was suggested to be in a β-configuration. The final connections of the substructures were established by the HMBC spectrum. The cross peak of H-6'/C-9 showed that the cinnamoyl group was bonded to C-6' of the glucose. The HMBC correlation of H-8 to C-1' indicated the position of the oxyethylene group to anomeric carbon (C-1') (Fig. 2). Therefore, compound **1** was identified as a new compound, 6'-*O*-cinnamoyl-1'-*O*-[2-(3,4-dihydroxyphenyl)ethyl]-β-glucopyronoside. This compound was named eutigoside D because its structure shares the cinnamoyl glucose unit with eutigoside C (**4**)[3] isolated by this laboratory. Tables 1 and 2 summarize the ^{1}H and ^{13}C NMR spectral values of eutigoside C (**4**).

Compound **2** was also obtained as a viscous liquid with a pseudo molecular ion $[M + Na]^+$ peak at m/z 419.1319 in HR-FAB-MS, indicating a molecular formula of $C_{19}H_{24}O_9$ (8 unsaturations). This compound exhibited 17 carbon peaks in the ^{13}C NMR spectrum suggesting two carbons in symmetric environments similar to compound **1**. From a comparative inspection of the ^{1}H and ^{13}C NMR spectra, compound **2** was readily determined to have a cinnamoyl glucose unit, as observed in compounds **1** and **4** (Tables 1 and 2). Identification of the remaining C_4 substructure, $-CH_2CH_2CO_2CH_3$, was also accomplished by the ^{1}H, ^{13}C and COSY spectral data. This subunit was connected to anomeric oxygen of the glucose, as determined by the H-1''/C-3 HMBC correlation (Fig. 2). The glucose unit was also identified by the hydrolysis of compound **2**, and a subsequent TLC comparison of the hydrosylate to the authentic D-glucose. Therefore, compound **2** was identified as methyl 3-(6'-*O*-cinnamoyl)-β-glucopyranosylpropanoate, a new compound named as eutigoside E.

Eutigosides B (**3**) and C (**4**), which were originally isolated from the leaves of *E. tigang*,[7] were identified by spectroscopic methods and a comparison of their data with the literature values. The other known compounds, cinnamic acid (**5**), rengyolone (**6**)[8] and cleroindicin (**7**)[9] were also identified using similar methods. Compounds **5**-**7** were isolated for the first time from *E. emarginata*.

To indentify the anti-oxidative properties, the DPPH radical scavenging[10] and xanthine oxidase inhibitory[11] activities were examined for isolates **1**-**7**. For the DPPH radical scavenging test, the compound **1** showed moderate activity with SC_{50} of 40.4 μg/mL. A water soluble vitamin E derivative, Trolox, was used as the positive control (SC_{50} 7.9 μg/mL). The other compounds exhibited very low activities with a SC_{50} > 200 μg/mL. In studies of xanthine oxidase inhibition, compounds **2** and **4** showed relatively higher activities with IC_{50} values of 289.9 and 205.6 μg/mL,

Table 2. ^{13}C NMR spectral data of compounds **1**, **2** and **4**[a]

Position	**1**[b]	**2**[c]	**4**[b]
1	127.0 (s)	172.2 (s)	69.2 (s)
2	118.3 (d)	34.6 (t)	154.3 (d)
3	151.1 (s)	65.3 (t)	127.8 (d)
4	149.4 (s)	51.9 (q)	187.8 (s)
5	114.8 (d)	–	127.8 (d)
6	116.9 (d)	–	154.3 (d)
7	32.1 (t)	–	41.0 (t)
8	71.1 (t)	–	65.9 (t)
1'	104.6 (d)	103.1 (d)	104.4 (d)
2'	75.3 (d)	74.1 (d)	75.4 (d)
3'	77.9 (d)	76.0 (d)	77.9 (d)
4'	71.7 (d)	70.0 (d)	71.7 (d)
5'	75.0 (d)	73.3 (d)	75.0 (d)
6'	64.9 (t)	63.5 (t)	64.8 (t)
1''	135.6 (s)	134.2 (s)	135.7 (s)
2''	130.0 (d)	128.9 (d)	129.9 (d)
3''	129.3 (d)	128.2 (d)	129.2 (d)
4''	131.5 (d)	130.4 (d)	130.8 (d)
5''	129.3 (d)	128.2 (d)	129.2 (d)
6''	130.0 (d)	128.9 (d)	129.9 (d)
7''	146.6 (d)	145.6 (d)	146.5 (d)
8''	118.6 (d)	117.5 (d)	115.9 (d)
9''	168.5 (s)	167.4 (s)	168.5 (s)

[a]δ values are in ppm, and mulitiplicities by DEPT are noted in parentheses. [b]CD_3OD was used as a solvent. [c]$CDCl_3$ was used as a solvent.

respectively. These activities were comparable to Trolox (IC_{50} 269.6 μg/mL).

Experimental Section

Reagents and Instruments. All the solvents used were of analytical grade. Sephadex LH-20 (bead size 25-100 μm) from Pharmacia (Sweden) and silica gel (40-63 μm) from Merck were used for column chromatography. Celite was purchased from Haedong Co. (Korea). A Hitach (L-7100) HPLC with UV detector (L-7200) was used for the semi-preparative isolation of the compounds. The NMR spectra were recorded using a JNM-LA 400 (Jeol) instrument. The chemical shift values are reported in ppm relative to the solvent used. Optical rotations were measured on a Jasco P-1030 automatic polarimeter. The high resolution mass spectra were obtained from the Korea Basic Science Institute Seoul Center.

Plant Material. The leaves and twigs of *E. emarginata* (J-293) were collected at the campus of Jeju National University in August 2006. The plant species was identified by Dr. Gwanpil Song at Biodiversity Research Institute, Jeju Technopark, Jeju. A voucher specimen is deposited the Laboratory of Natural Product Chemistry, Jeju National University.

Isolation Procedures. The leaves and twigs of *E. emarginata* (5.3 kg) were cut into small pieces, and extracted with methanol (45 L) with constant stirring for 24 h at rt. The extraction mixtures were filtered and concentrated under reduced pressure to give a gummy mass (0.48 kg). A portion of the extract (12.3 g) was suspended over water (1 L), and fractionated into *n*-hexane, ethyl acetate (EtOAc) and *n*-butanol. The obtained EtOAc fraction (4.3 g) dissolved in methanol was adsorbed on Celite (120 g) in a beaker. After evaporation of the methanol, the coated Celite solid was packed into a glass column. The resulting Celite column was eluted sequentially with 1 L of each solvent to give *n*-hexane (fr. HX, 8.7 mg), CH_2Cl_2 (fr. DM, 1.1 g), EtOAc (fr. EA, 2.5 g) and methanol (fr. MT, 434.0 mg) fractions. The CH_2Cl_2 fraction (DM, 1.1g) was subjected to silica gel column chromatography (CC) with chloroform/methanol (6/1) to afford eight fractions (frs. DM1 to DM8). Compounds **5** (3.0 mg), **6** (69.2 mg), **7** (4.3 mg) and **2** (29.0 mg) were obtained from fractions DM1, DM4, DM5 and DM6, respectively. Fraction DM8 (241.7 mg) was purified again with silica gel CC using chloroform/methanol (6/1) to afford compound **1** (48.1 mg). The EtOAc fraction (EA, 2.5 g) was subjected to silica gel CC with chloroform/methanol (6/1) to give seven fractions (frs. EA1 to EA8). Fraction EA6 (162.5 mg) was purified by silica gel CC with chloroform/methanol (3/1) to give eight fractions (frs. EA6-1 to EA6-8). Compound **4** (672.7 mg) was obtained from fraction EA5. Fraction EA6-3 (83.1 mg) was purified by reverse phase HPLC with a CH_3CN-H_2O gradient to afford compound **3** (5.0 mg).

Hydrolysis of Compounds 1 and 2. Each sample (1 mg) dissolved in 5% HCl (1 mL) was heated at 90 °C for 2 hr with stirring. Chloroform (5 mL) and water (5 mL) were added to the hydrolyzed reaction mixture. TLC analysis was conducted with the concentrated water phase. The spot was visualized by spraying an anisladehyde-H_2SO_4 agent. The sugar was identified by comparing its R_f value with that of authentic D-glucose (R_f 0.12, CH_2Cl_2:MeOH:H_2O 70:27:3).

Eutigoside D (1): Viscous liquid; $[\alpha]_D^{20}$ –40.6 (*c* 0.06, MeOH); ^1H and ^{13}C NMR data: Tables 1 and 2; HMBC correlations (H→C#); H-2→C-3, C-4, C-7; H-5→C-1, C-3; H-6→C-2, C-4; H-7→C-1, C-2, C-8; H-8→C-1, C-7, C-1'; H-1'→C-5'; H-5'→C-3'; H-6'→C-5', C-9''; H-7''→C-2'', C-8'', C-9''; H-8''→C-1'', C-9''; HR-FAB-MS: *m/z* 446.1577 $[M]^+$ (calcd for $C_{23}H_{26}O_9$ 446.1577).

Eutigoside E (2): Viscous liquid; $[\alpha]_D^{20}$ +3.7 (*c* 0.06, MeOH); ^1H and ^{13}C NMR data: Tables 1 and 2; HMBC correlations (H→C#); H-2→C-1, C-3; H-3→C-1, C-2, C-1'; H-1'→C-3; H-6'→C-9''; H-2''→C-4, C-7; H-7''→C-2'', C-8'', C-9''; H-8'' → C-1'', C-9''; HR-FAB-MS: *m/z* 419.1319 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_9Na$ 419.1318).

Acknowledgments. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0007254).

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