Two New Phenylpropanoid Glycosides from the Leaves and Flowers of *Erica arborea*

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Two new phenylpropanoid glucosides, 1,2-*erythro*-1-(3,4,5-trimethoxyphenyl)-2-(*β*-D-glucopyranosyloxy)propan-1,3-diol (**1**) and 7,8-*threo*-2′,8-epoxysyringylglycerol-7-*O*-*α*-D-glucopyranoside (ericarboside; **2**) have been isolated together with four known compounds 2′,7-epoxysyringylglycerol-8-*O*-*β*-D-glucopyranoside (ficuscarpanoside B; **3**), benzylrutinoside (hydrangeifolin; **4**), phenethylrutinoside (**5**), and caffeic acid from the BuOH soluble part of the MeOH extract of the leaves and flowers of *E. arborea* L. Final purification of the compounds was achieved on a reversed-phase HPLC. Their structures have been elucidated by extensive 1D- and 2D-NMR, and mass spectroscopic techniques.

Introduction. – The genus *Erica* (Ericaceae), comprising *ca.* 100 species, is distributed in Europe, the Middle East, and Africa [1]. *Erica arborea* L., commonly known as 'tree heath', 'briar root', or 'funda', is native to a number of countries in Africa, Temperate Asia, and Europe and also naturalized in the British Isles, Australia, and New Zealand [2][3]. *Erica arborea* is a shrub or small evergreen tree with a height of one to seven meters, and produces numerous small white flowers [4]. In Turkey, this species is widely distributed throughout West and North Anatolia and the Mediterranean basin, and its leaves and flowers have been used as diuretic, urinary antiseptic, diet tea, and laxative [5]. A phytochemical study on *E. arborea* growing in Turkey was reported by our group, leading to the isolation of (−)-epicatechin and quercitrin [6]. We further reported the isolation and identification of two flavone glycosides tricetin 4′-*O*-*α*-L-rhamnopyranoside and isorhamnetin 3-*O*-*α*-L-rhamnopyranoside from the leaves of *Erica arborea*, purchased from a local market in Canakkale (western Anatolia) [7]. A study on the volatile constituents of the flowers was reported by another group [8]. From other *Erica* species, monoterpenes and condensed tannins have been previously reported [9][10], as well as flavonoids [11][12]. In continuation

of our studies on *E. arborea*, we now report the structure elucidation of two new phenylpropanoid glucosides, 1,2-*erythro*-1-(3,4,5-trimethoxyphenyl)-2-(*β*-D-glucopyranosyloxy)propan-1,3-diol[1]) (**1**) and 7,8-*threo*-2′,8-epoxysyringylglycerol-7-*O*-*α*-D-glucopyranoside[1]) (ericarboside) (**2**), along with four known compounds.

Results and Discussion. – The phytochemical investigation of the BuOH extract of *Erica arborea* led to the characterization of three phenylpropanoid glucosides, including the new compounds **1** and **2**, and the known 2′,7-epoxysyringylglycerol-8-*O*-*β*-D-glucopyranoside (**3**). In addition, two known rutinosides hydrangeifolin (**4**) and phenethylrutinoside (**5**), and caffeic acid were isolated (*Fig. 1*).

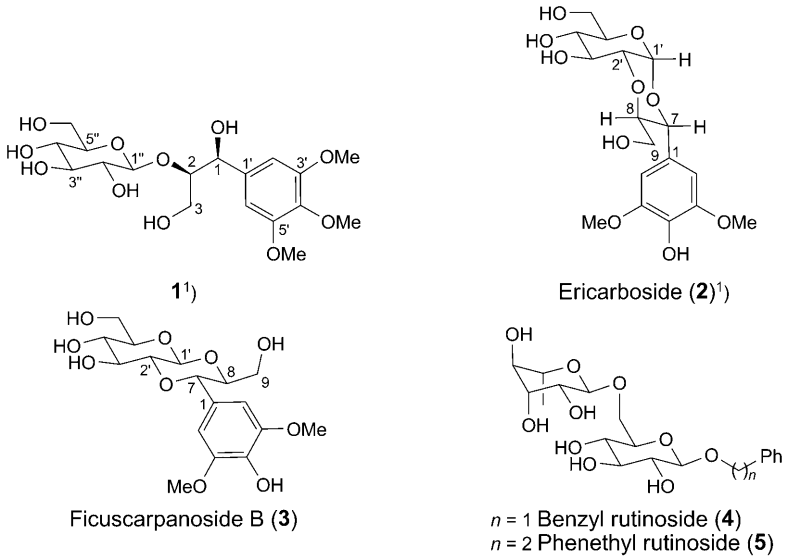

Fig. 1. *The isolated compounds from* Erica arborea

HR-FAB-MS (negative-ion mode) of compound **1** exhibited an $[M-H]^-$ *quasi*-molecular ion at *m*/*z* 419.1548, consistent with the formula $C_{18}H_{28}O_{11}$ (calc. 419.1553) with five degrees of unsaturation. The presence of a hexose sugar was inferred from a fragment ion at *m*/*z* 241.10794 ($[M-Glc]^-$), consistent with the formula $C_{12}H_{17}O_5^-$ in HR-FAB-MS (negative-ion mode) spectrum (calc. 241.1076). The EI-MS showed the fragment ion $[C_{10}H_{13}O_4]^+$ at *m*/*z* 197 as main peak for the $(MeO)_3C_6H_2CHOH$ moiety.

In the ^{1}H-NMR spectrum of compound **1** (*Table*), a *singlet* signal for two H-atoms in aromatic region at *δ*(H) 6.72 assigned to H–C(2′) and H–C(6′)[1]) showed the presence of a 3,4,5-trisubstituted phenyl moiety. These substituents were three MeO groups, resonating at *δ*(H) 3.74 and 3.84 (2 MeO). The ^{1}H- and ^{13}C-NMR data (*Table*) suggested the presence of a propanetriol moiety attached to this phenyl ring by the observation of the signals at *δ*(H) 4.84 (*δ*(C) 75.2), *δ*(H) 3.95 (*δ*(C) 85.3), *δ*(H) 3.62 and 3.67 (*δ*(C) 62.7), corresponding to H–C(1), H–C(2), and H–C(3),

[1]) Arbitrary numbering. For systematic names, see *Exper. Part.*

respectively. The coupling constants of the H–C(2) signal at δ(H) 3.95 (br. *dd*, J = 4.0, 7.4) were indicative for the *erythro* configuration at H–C(1) and H–C(2) [13]. Thus, J couplings of H–C(2) were found to be 4.0 Hz with one of the HO–CH_2 H-atoms H_a–C(3), and 7.4 Hz with H–C(1), while the other HO–CH_2 H-atom H_b–C(3) almost did not couple with H–C(2), and appeared as a broadened *doublet* showing only geminal coupling (9.5 Hz). This finding was verified by *Dreiding* model studies. The ^{1}H- and ^{13}C-NMR spectra also showed the presence of a hexose sugar which was identified as β-D-glucopyranose[2]) by characteristic resonances for the anomeric H-atom at δ(H) 4.37 (J(1″,2″) = 7.5) and other H-atoms. The linkage of the glucose moiety to C(2) was deduced by an HMBC experiment (*Fig. 2*) which showed a correlation between C(2) at δ(C) 85.3 and the anomeric H-atom at δ(H) 4.37 for H–C(1″). In addition, a ^3J correlation from the O-bearing CH group H–C(2) at δ(H) 3.95 to the anomeric C-atom at δ(C) C(1″) 103.8 was observed. Another important HMBC correlation was observed between H–C(2′) at δ(H) 6.72 and C(1) at δ(C) 75.2 assigning the linkage position of the phenyl moiety to C(1). All the spectral data assigned the structure of **1** to be a new compound, 1,2-*erythro*-1-(3,4,5-trimethoxyphenyl)-2-(β-D-glucopyranosyloxy)propan-1,3-diol[1]). The 7-*O*- and 9-*O*-glucosides of the related aglycone (7*S*,8*R*)-syringylglycerol were previously isolated from *Ficus microcarpa* [14] and *Hyssopus officinalis*, respectively [15].

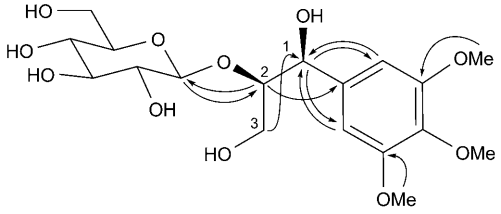

Fig. 2. *Selected HMBC* (H → C) *interactions of compound* **1**[1])

Compound **2** showed similar signals to those of compound **1**. In the ^{1}H-NMR spectrum (*Table*) there was a *singlet* signal of two H-atoms at δ(H) 6.75 indicating the presence of a trisubstituted phenyl moiety of similar structure to that of compound **1**. However, in the ^{1}H-NMR spectrum, only one MeO signal corresponding to six H-atoms (2 MeO groups) was observed. The ^{13}C-NMR spectrum showed 17 C-atom signals (*Table*) confirming the lack of one MeO C-atom compared to compound **1**. The signal at δ(C) 135.3 for C(4)[1]) in the ^{13}C-NMR spectrum and HR-EI-MS results revealed that the third substituent should be a OH group at the C(4) position of the phenyl moiety. The HR-EI-MS showed the fragment ion $[C_9H_{11}O_4]^+$ at m/z 183.0657 as the base peak for $(MeO)_2(OH)C_6H_2CHOH$ confirming the presence of the OH group. Moreover, the *singlet* at δ(H) 6.75 for H–C(2) and H–C(6) showed an HMBC (*Fig. 3*) with the quaternary C-atom C(4) at δ(C) 135.3, confirming the position of the OH group to be at C(4). Another important HMBC, shown by H–C(2) and H–C(6), was with C(7) at δ(C) 76.2 in the dioxane ring as well as three-bond correlations from H–C(7) at δ(H) 4.46 to both C(2) and to C(6) were observed.

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[2]) The configuration of the glucose residue was tentatively assigned as D from biogenetic considerations.

Table. *[1]H- and [13]C-NMR Data for Compounds* **1** *and* **2** (400 and 100 MHz, resp., in CD_3OD; δ in ppm, J in Hz)

1[1])				**2**[1])			
	δ(H)	δ(C)	HMBC		δ(H)	δ(C)	HMBC
C(1′)	–	138.3		C(1)	–	132.8	
H–C(2′,6′)	6.72 (*s*)	105.2	C(1), C(4′)	H–C(2,6)	6.75 (*s*)	105.3	C(7), C(4)
C(3′,5′)	–	154.2		C(3,5)	–	153.8	
C(4′)	–	139.9		C(4)	–	135.3	
H–C(1)	4.84[a])	75.2	C(2′), C(6′), C(3)	H–C(7)	4.46 (*d*, J = 7.5)	76.2	C(2), C(6), C(9)
H–C(2)	3.95 (*dd*, J = 4.0, 7.4)	85.3	C(1′), C(1″)	H–C(8)	3.92–3.95 (*m*)	83.1	C(1), C(1′)
H_a–C(3)	3.67 (*dd*, J = 4.0, 9.5)	62.7	C(1)	H_a–C(9)	3.70–3.74 (*m*)	62.5	C(7)
H_b–C(3)	3.62 (br. *d*, J = 9.5)		C(1)	H_b–C(9)	3.44–3.46 (*m*)		C(7)
H–C(1″)	4.37 (*d*, J = 7.5)	103.8	C(2), C(3″), C(5″)	H–C(1′)	5.10 (*d*, J = 3.5)	93.9	C(7), C(3′), C(5′)
H–C(2″)	3.15 (*t*, J = 7.5)	74.4	C(4″)	H–C(2′)	3.35–3.40 (*m*)	73.8	C(4′), C(8)
H–C(3″)	3.38–3.40 (*m*)	77.8	C(1″), C(5″)	H–C(3′)	3.62–3.67 (*m*)	74.8	C(5′)
H–C(4″)	3.27 (*dd*, J = 8.8, 3.1)	71.4	C(2″), C(6″)	H–C(4′)	3.28–3.32 (*m*)	71.6	C(2′), C(6′)
H–C(5″)	3.35–3.37 (*m*)	77.8	C(3″)	H–C(5′)	3.78–3.82 (*m*)	72.9	C(1′), C(3′)
H_a–C(6″)	3.80–3.86 (*m*)	62.4	C(4″)	H_a–C(6′)	3.71 (*dd*, J = 11.6, 3.0)	62.7	C(4′)
H_b–C(6″)	3.62 (*dd*, J = 10.0, 4.1)		C(4″)	H_b–C(6′)	3.84 (br. *d*, J = 11.6)		C(4′)
3′,5′-MeO	3.84 (*s*)	56.4	C(3′), C(5′)	3,5-MeO	3.77 (*s*)	56.7	C(3), C(5)
4′-MeO	3.74 (*s*)	61.1	C(4′)				

[a]) Overlapped with H_2O signal.

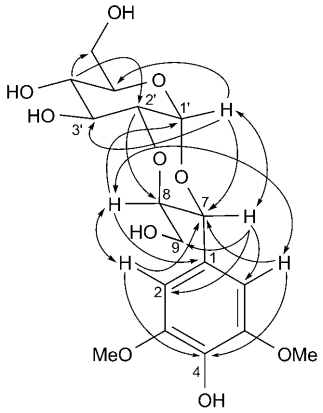

Fig. 3. *Selected HMBC* (H → C) *and NOESY* (H ↔ H) *correlations for compound* **2**[1])

In the ^{1}H-NMR spectrum of **2**, compared to that of compound **1**, the anomeric H-atom was observed more downfield at δ(H) 5.10 (*d*, J = 3.5) for H–C(1′). It was deduced to be a doubly O-bearing CH group based on an HMQC experiment by the

observation of a correlation with the ^{13}C-NMR signal at δ(C) 93.9. The small J value of anomeric H-atom indicated the α-configuration of the glucose moiety. A three-bond HMBC (*Fig. 3*) between H–C(1′) and C(7) was observed which indicated a linkage through an O-atom between C(7) and C(1′). A significant HMBC, observed between H–C(2′) and C(8) (see *Fig. 3*), also confirmed the linkage of glucose to the aglycone *via* a dioxane ring.

The HR-EI-MS data supported the structure by exhibiting a molecular ion peak at m/z 388.1387, which is consistent with the formula $C_{17}H_{24}O_{10}$, corresponding to six degrees of unsaturation originating from an aromatic ring, a pyranoside moiety, and the presence of an additional ring.

The NOESY spectrum indicated correlations between H–C(7) and H–C(1′) of Glc as well as between H–C(8) and H–C(2,6), revealing the relative configuration of H–C(7) and H–C(8) as β and α, respectively (*Fig. 3*). In the ^{1}H-NMR spectrum, a coupling constant of $J = 7.5$ Hz between H–C(7) and H–C(8), as well as *Dreiding* model studies exhibited a chair configuration of the dioxane ring which indicated the *threo* form at C(7) and C(8), both H–C(7) and H–C(8) being axial. Therefore, the structure of compound **2** was elucidated as the new compound 7,8-*threo*-2′,8-epoxysyringylglycerol-8-*O*-α-D-glucopyranoside[1]). The structure is very similar to fissistigmoside which has previously been isolated from *Fissistigma polyanthum*. The only difference is the presence of a *cis*-fused ring system in compound **2** instead of *trans*-fused in fissistigmoside [16].

Compound **3** exhibited very similar signals to those of **2** in the ^{1}H- and ^{13}C-NMR spectra. The most important difference originated from the β-configuration of the sugar moiety which was revealed by a *doublet* signal with a coupling constant of 7.8 Hz for the anomeric H-atom at δ(H) 4.45. There was no HMBC between H–C(1′) and C(7) when compared to compound **2**. On the other hand, H–C(1′) showed a correlation with C(8), indicating the linkage between C(1′) and C(8). Since H–C(2′) showed a correlation with C(7), this compound was identified as 2′,7-epoxysyringylglycerol-8-*O*-β-D-glucopyranoside, which was isolated earlier from *Ficus microcarpa* and named as ficuscarpanoside B (cremanthodioside) [14].

The structures of the two rutinosides have been identified as benzylrutinoside (hydrangeifolin; **4**) and phenethylrutinoside (**5**). Compounds **4** and **5** were first reported from *Margyricarpus setosus* and *Premna subscandes*, respectively. The structures were confirmed by comparison with the reported data [17][18].

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Experimental Part

General. Thin-layer chromatography (TLC): precoated silica gel plates (*DC-Alugram 60 UV$_{254}$* of *E. Merck*), coloration by spraying $Ce(SO_4)_2$. Column chromatography (CC): *Polyamide-6 DF* (*Riedel-de Haen AG*) and silica gel (SiO_2; *E. Merck*, 230–400 mesh); polyclar (polyvinylpolypyrrolidon) from *Aldrich* (Milwaukii, WI), and was used without purification. Recycling prep. HPLC (*LC-908W, Analytical Industry Co. Ltd.*, Japan) was used for final purifications with *YMC-Pack J'sphere ODS L-80*

and *H-80* columns (8 nm pore size, 4 μm particle size, 150 × 4.6 mm), Japan. Optical rotations: *JASCO DIP-360* digital polarimeter, in MeOH. UV Spectra: *Shimadzu UV240* spectrophotometer. IR Spectra: *Vector 22*, *Bruker* spectrophotometer on KBr pellets. ^{1}H- and ^{13}C-NMR, HMQC, and HMBC spectra: *Bruker AV-400* spectrometer, at 400 (^{1}H) and 100 MHz (^{13}C); chemical shifts reported in δ [ppm]; TMS was used as internal standard; coupling constants (J) in Hz. EI- and HR-EI-MS: *JEOL JMS 600 H* mass spectrometer. FAB- and HR-FAB-MS spectra: *JEOL JMS-AX505WA* instrument.

Plant Material. Leaves and flowers of *Erica arborea* L. (Ericaceae) were collected from Kazdağı, Çanakkale (Dardanelles), Turkey, April 2002. A voucher specimen was deposited (COMU-370) with the Dardanel Herbarium Biology Department of Faculty of Science and Arts, Çanakkale Onsekiz Mart University, Turkey.

Extraction and Isolation. Dried and powdered leaves and white flowers of *E. arborea* L. (1002 g) were macerated in MeOH (3 × 3 l) for a week at r.t. The extract was filtered and concentrated under reduced pressure. The concentrated extract (271 g) was suspended in H_2O (1 l), and then successively partitioned with AcOEt (4 × 250 ml) and BuOH (4 × 250 ml). The BuOH extract (20 g) was fractionated on a polyclar column with the mixtures of CH_2Cl_2/MeOH and MeOH/H_2O to obtain 4 fractions (*Frs. A – D*).

Frs. A – C were subjected to chromatography on polyamide. *Fr. A* was separated by polyamide column by using MeOH/CH_2Cl_2 (0:1 – 2:8). Subfractions of *Fr. A* were applied to RP-HPLC (*ODS H-80* column; MeOH/H_2O, 1:1; flow rate 4 ml/min) to yield compounds **4** (7 mg) and **5** (5 mg). *Fr. B* was also separated on a polyamide column by using $CHCl_3$/MeOH (1:0 – 1:1). Subsequently, the first subfraction of *Fr. B* was purified on an *L-80* column by RP-HPLC, and compound **1** (4 mg) was isolated with MeOH/H_2O (1:2; flow rate 4 ml/min). The second subfraction of *Fr. B* was also subjected to HPLC on the *L-80* column and eluted with the same solvent system (MeOH/H_2O 1:2; flow rate 4 ml/min) to yield compounds **2** (4 mg) and **3** (5 mg). Caffeic acid (3 mg) was isolated from *Fr. C* by using MeOH/CH_2Cl_2 (0:1 – 4:6) as elution system on a polyamide column.

*(1*S*,2*R*)-1,3-Dihydroxy-1-(3,4,5-trimethoxyphenyl)propan-2-yl β-*D*-Glucopyranoside*; **1**). Colorless gum. $[\alpha]_D^{25} = -18$ ($c = 0.3$, MeOH). UV (MeOH): 205 (3.95), 227 (3.24), 262 (2.33). IR (KBr): 3388, 1605, 1507, 1415, 1245, 1165, 1050. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 420 (5, $[C_{18}H_{28}O_{11}]^+$), 197 (100, $[(MeO)_3C_6H_2CHOH]^+$), 147 (70, $[C_{10}H_{13}O_4]^+$), 105 (90), 85 (80, [propanetriol ($C_3H_6O_3$)]$^+$). FAB-MS (neg.; matrix: glycerol): 419 ($[M-H]^-$), 389 ($[C_{17}H_{25}O_{10}]^-$), 327 ($[M-3\,MeO]^-$). HR-FAB-MS (neg.): 419.1548 ($[M-H]^-$, $C_{18}H_{27}O_{11}^-$; calc. 419.1553), 241.1079 ($[M-Glc]^-$, $C_{12}H_{17}O_5^-$; calc. 241.1076).

*(2*R*,3*R*,4a*S*,6*R*,7*S*,8*S*,8a*R*)-Hexahydro-3-(4-hydroxy-3,5-dimethoxyphenyl)-2,6-bis(hydroxymethyl)-4a*H*-pyrano[2,3-*b*][1,4]dioxine-7,8-diol*; **2**). Colorless gum. $[\alpha]_D^{25} = +20$ ($c = 0.18$, MeOH). UV (MeOH): 208 (3.75), 232 (3.45), 272 (2.49), 2.92 (sh). IR (KBr): 3400, 1625, 1530, 1420, 1250, 1160, 1055. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 388 (8, M^+), 226 (18, $[C_{11}H_{14}O_5]^+$), 183 (100, $[C_9H_{11}O_4]^+$), 153 (10, $[C_8H_9O_3]^+$), 85 (80, [propanetriol ($C_3H_6O_3$)]$^+$). FAB-MS (neg.; matrix: glycerol): 387 ($[M-H]^-$), 225 ($[C_{11}H_{14}O_5]^+$), 183 ($[C_9H_{11}O_4]^+$). HR-EI-MS: 388.1387 ($C_{17}H_{24}O_{10}^+$; calc. 388.1369), 183.0657 ($C_9H_{11}O_4^+$; calc. 183.0657).

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