Phenylethanoid Glycosides from *Globularia trichosantha*

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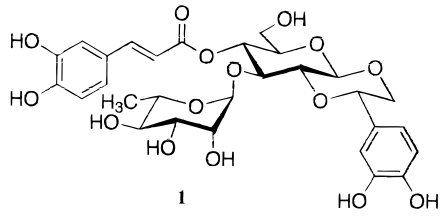
Five phenylethanoid glycosides, crenatoside (= oraposide) (**1**), verbascoside (= acteoside) (**2**), trichosanthoside A (**3**), rossicaside A (**4**), and trichosanthoside B (**5**), were isolated from the aerial parts of *Globularia trichosantha*. Compounds **3** and **5** are new natural compounds, and their structures were established as 3,4-dihydroxy-*β*-phenylethoxy-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-*β*-D-glucopyranoside and 3,4-dihydroxy-*β*-phenylethoxy-*O*-[*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-[*β*-D-xylopyranosyl-(1→6)]-4-*O*-caffeoyl-*β*-D-glucopyranoside, respectively. The structures of all compounds were established by spectral evidence. Compounds **1**−**5** also demonstrated scavenging properties toward the 2,2-diphenyl-1-picrylhydrazyl radical in TLC autographic assays.

The aqueous extract from *Globularia alypum* has been reported to have significant activity against leukemia P-388 and was found to be rich in carbohydrates, sugar alcohols, and phenolic compounds.[1] The same plant has previously been investigated for its iridoid glucosides.[2−5] There exist several reports on the aryl, iridoid, and lignan glycosides from other *Globularia* species: *G. cordifolia*,[6,7] *G. nudicaulis*, and *G. nana*.[8] Among the phenolic compounds, flavone derivatives such as apigenin, luteolin, and 6-hydroxyluteolin have been reported from *Globularia* species.[9−13] In the flora of Turkey, the genus *Globularia* is represented by eight species.[14] *G. alypum* is used as diuretic, laxative, carminative, and tonic in Turkey.[15] Because there is no report on phenylethanoid glycosides, we now describe the isolation and structure elucidation of such glycosides from *G. trichosantha*.

Compounds **1**−**5** were obtained as amorphous powders, whose UV spectra (see Experimental Section) indicated their polyphenolic nature. Their IR spectra showed absorption bands typical for hydroxyls; α,*β*-unsaturated esters; olefinic double bonds; and aromatic rings (see Experimental Section). The structures of compounds **1**, **2**, and **4** were established as crenatoside (= oraposide),[16,17] verbascoside (= acteoside,[18] and rossicaside A,[19] respectively, on the basis of their 1D and 2D NMR spectral properties and FABMS or ESIMS data.

The FABMS of compound **3** exhibited a pseudomolecular ion $[M + Na]^+$ at *m*/*z* 779, compatible with the molecular formula $C_{34}H_{44}O_{19}$, and in good agreement with the observation of one methyl, four methylene, 22 methine, and seven quaternary carbon resonances in its ^{13}C NMR spectrum (Table 1). The ^1H NMR spectrum of **3** (see Table 1) exhibited the characteristic signals belonging to (*E*)-caffeic acid and 3,4-dihydroxyphenylethanol moieties: protons of aromatic rings (2 × ABX systems), two *trans*-olefinic protons (AB system, $J_{AB} = 15.9$ Hz), *β*-methylene at *δ* 2.81 (2H, m), and two nonequivalent protons at *δ* 4.06 and 3.72 (each 1H, m) of the side-chain of the aglycon moiety. Additionally, three anomeric proton resonances appeared at *δ* 5.26 (d, *J* = 1.7 Hz, H-1″ of a α-rhamnose), 4.40 (d, *J* = 7.7 Hz, H-1‴ of a *β*-xylose), and 4.39 (d, *J* = 7.9 Hz, H-1′ of *β*-glucose), indicating its triglycosidic structure. Moreover, the secondary methyl signal at *δ* 1.16 (d, *J* = 6.2 Hz) supported the presence of a rhamnose unit in **3**. The complete assignments of all proton and carbon resonances were based on the results of $^1H-^1H$ COSY, long-range TOCSY, $^1H-^{13}C$ HMQC, and HMBC experiments. The caffeoyl group was positioned at C-4′ of the glucose on the basis of the strong deshielding of H-4′ signal of the glucose unit (*δ* 4.94, t, *J* = 9.5 Hz). The carbon resonances assigned to the *β*-xylose unit showed no unusual chemical shifts, suggesting its terminal position. These results indicated that the structure of **3** is closely related to those of phlinoside B[20] and arenarioside[21] (= forsythoside F)[22], which contain the same molecular subunits. The proton and carbon resonances assigned to the trisaccharidic sugar moiety suggested, however, the presence of different glycosidic links in the sugar chain of compound **3**. A $^1H-^{13}C$ HMQC experiment allowed the unambiguous assignment of all the carbon resonances of the sugar units, and the HMBC experiment permitted the determination of all of the relevant interfragmental connectivities. Thus, cross-peaks were observed between the anomeric proton of glucose (*δ* 4.39, H-1′) and C-α (*δ* 72.19) of the phenylethyl alcohol, H-4′ (*δ* 4.94) of glucose and the carbonyl carbon



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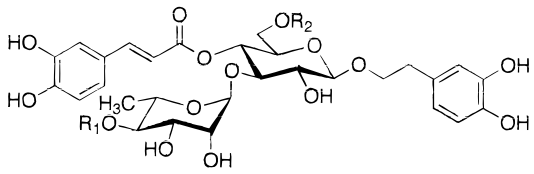

2 R_1 = H, R_2 = H
3 R_1 = β-D-xylopyranose, R_2 = H
4 R_1 = β-D-glucopyranose, R_2 = H
5 R_1 = β-D-xylopyranose, R_2 = β-D-xylopyranose

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Table 1. ^{13}C and ^{1}H NMR Spectral Data for Trichosanthosides A (**3**) and B (**5**) (CD_3OD, ^{13}C, 100.6 MHz; ^{1}H, 400.13 MHz)[a]

		3		**5**[b]	
H/C atom		δC	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)
Aglycon					
1	C	131.40		131.39	
2	CH	117.08	6.71 d (2.0)	117.09	6.72 d (2.0)
3	C	146.08		146.07	
4	C	144.63		144.64	
5	CH	116.27	6.69 d (8.0)	116.29	6.69 d (8.0)
6	CH	121.24	6.58 dd (8.0, 2.0)	121.27	6.59 dd (8.0, 2.0)
α	CH_2	72.19	4.06 m, 3.72 m	72.39	4.05 m, 3.71[c]
β	CH_2	36.53	2.81 m	36.54	2.81 br t (7.1)
Glc					
1′	CH	104.13	4.39 d (7.9)	104.12	4.40 d (7.8)
2′	CH	76.29	3.42 dd (7.9, 9.1)	76.22	3.40 dd (7.8, 9.0)
3′	CH	80.63	3.85 t (9.1)	80.55	3.84 t (9.1)
4′	CH	70.41	4.94 t (9.5)	70.38	4.99 t (9.7)
5′	CH	75.91	3.54 m	74.84	3.21 m
6′	CH_2	62.27	3.68[c]	69.27	3.88 dd (11.5, 1.8)
			3.60[c]		3.61 dd (11.5, 5.8)
Rha					
1″	CH	102.33	5.26 d (1.7)	102.34	5.25 d (1.7)
2″	CH	72.09	3.94 dd (1.7, 3.2)	72.07	3.93 dd (1.7, 3.2)
3″	CH	72.26	3.78 dd (3.2, 9.5)	72.18	3.77 dd (3.2, 9.5)
4″	CH	83.62	3.46 t (9.5)	83.62	3.45[c]
5″	CH	68.69	3.65[c]	68.71	3.66 dq (9.5, 6.2)
6″	CH_2	18.39	1.16 d (6.2)	18.37	1.15 d (6.2)
Xyl (→Rha)					
1‴	CH	106.50	4.40 d (7.7)	106.50	4.39 d (7.7)
2‴	CH	75.70	3.02 dd (7.7, 9.0)	75.69	3.00 dd (7.7, 9.0)
3‴	CH	78.02	3.25 t (9.0)	78.03	3.25 t (9.0)
4‴	CH	70.94	3.37 m	70.93	3.36 m
5‴	CH_2	67.02	3.74[c]	67.02	3.74[c]
			3.09 dd (11.2, 10.4)		3.07 dd (11.6, 10.4)
Xyl (→Glc)[b]					
(1⁗)	CH			105.25	4.26 d (7.4)
(2⁗)	CH			74.72	3.21 dd (7.4, 9.0)
(3⁗)	CH			77.50	3.31 t (9.0)
(4⁗)	CH			71.07	3.48 m
(5⁗)	CH_2			66.86	3.85[c]
					3.18 dd (11.3, 10.3)
Caffeoyl[b]					
1⁗ (⁗′)	C	127.64		127.60	
2⁗ (⁗′)	CH	115.34	7.08 d (2.0)	115.34	7.08 d (2.0)
3⁗ (⁗′)	C	146.74		146.76	
4⁗ (⁗′)	C	149.75		149.83	
5⁗ (⁗′)	CH	116.50	6.80 d (8.3)	116.49	6.80 d (8.2)
6⁗ (⁗′)	CH	123.36	6.97 dd (8.3, 2.0)	123.40	6.98 dd (8.2, 2.0)
α′	CH	114.53	6.26 d (15.9)	114.43	6.26 d (15.8)
β′	CH	148.19	7.59 d (15.9)	148.42	7.60 d (15.8)
C=O	C	168.11		168.41	

[a] Assignments confirmed by TOCSY, long-range COSY, DQF−COSY, HMQC, and HMBC experiments. [b] For compound **5**. [c] Signal patterns are unclear due to overlapping.

resonance (δ 168.11) of the acyl moiety, H-3′ of glucose (δ 3.85) and the anomeric carbon of the rhamnose unit (δ 102.3, C-1″), and H-4″ of rhamnose (δ 3.46) and the anomeric carbon of the xylose unit (106.5, C-1‴). Therefore, the structure of **3** was established as 3,4-dihydroxy-*β*-phenylethoxy-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-*β*-D-glucopyranoside, for which the trivial name trichosanthoside A is proposed.

Compound **5** was isolated as an amorphous powder, with the molecular formula $C_{39}H_{52}O_{23}$ as determined by negative ion ESIMS and ^{1}H and ^{13}C NMR data (Table 1). The ESIMS of **5** exhibited a pseudomolecular ion at *m*/*z* 887 $[M - H]^-$, which was 132 mass units higher than that of **3**, suggesting the presence of an additional pentose unit in **5**. This assumption was supported by the ^{1}H NMR spectrum of **5**, revealing the resonances of four anomeric protons: δ 5.25 (d, $J = 1.7$ Hz), 4.40 (d, $J = 7.8$ Hz), 4.39 (d, $J = 7.7$ Hz), and 4.26 (d, $J = 7.4$ Hz), consistent with the presence of an α-L-rhamnose, a *β*-D-glucose, and two *β*-D-xylose units, respectively. The proton and carbon chemical shifts due to the acyl and aglycon moieties were in good agreement with those of **3**, indicating similar substructures. All structural assignments were substantiated by the results obtained from the 2D shift-correlated $^{1}H-^{1}H$ COSY, long-range TOCSY, $^{1}H-^{13}C$ HMQC, and HMBC spectra of **5**. The ^{13}C NMR spectral data confirmed the tetraglycosidic sugar chain in **5**, exhibiting four anomeric carbon resonances at δ 106.50, 105.25, 104.12, and 102.34, which show correlations with the anomeric protons of two xylose units, glucose and rhamnose, respectively. The proton and the carbon chemical shifts due to the two xylose units indicated that both have to be in terminal positions. The remaining carbon signals arising from the glucose and rhamnose moieties indicated the glucose unit to be glycosylated at C-3′ (δ 80.55) and C-6′ (δ 69.27) and the rhamnose unit at C-4″ (δ 83.62). Because the H-4′ signal of the glucose unit was found to be shifted toward higher frequency (δ 4.99, t, $J = 9.7$ Hz) due to the acylation, the caffeoyl group was positioned at C-4′ of the glucose as in **3**. Finally, all connectivities within **5** were proven by an

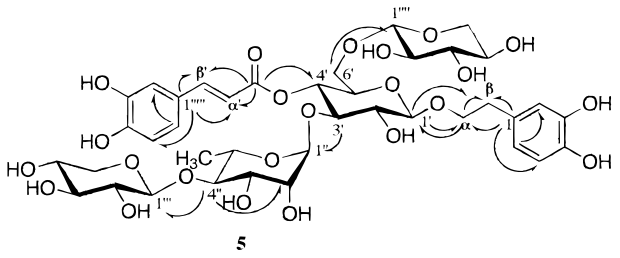

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Figure 1. Selected heteronuclear multiple bond correlations (HMBC) for trichosanthoside B (**5**). Arrows point from carbon to proton.

HMBC experiment, where correlations between H-1′ (δ 4.40) of the glucose unit and the α-C atom (δ 72.39) of the phenethyl moiety, H-1″ (δ 5.25) of the rhamnose and the C-3′ (δ 80.55) of the glucose unit, H-4′ (δ 4.99) of the glucose and the carbonyl carbon (δ 168.41) of the caffeoyl moiety, H-1‴ (δ 4.39) of the first xylose and the C-4″ (δ 83.62) of the rhamnose unit, and H-1⁗ of the second xylose and the C-6′ (δ 69.27) of the glucose unit were observed. Additional significant long-range correlations confirming the proposed structure are shown in Figure 1. Consequently, the structure of compound **5** was established as 3,4-dihydroxy-*β*-phenylethoxy-*O*-[*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-[*β*-D-xylopyranosyl-(1→6)]-4-*O*-caffeoyl-*β*-D-glucopyranoside, for which the trivial name trichosanthoside B is proposed.

Trichosanthoside B (**5**) is only the third example of a tetrasaccharidic phenylethanoid glycoside; the two previous examples are magnolioside C[23] and ballotetroside.[24] This study is also the first report on the isolation of phenylethanoid glycosides from *Globularia* species.[25] *Globularia* species (Globulariaceae) have also been found to contain a rare glycoside, crenatoside (= oraposide, 1),[16,17] which has an ether linkage between a glucose and a phenethyl moiety in addition to a glycosidic linkage. This compound has been reported for its significant pharmacological activities such as antigonadotropic and analgesic activities, aldose reductase inhibition, and a protective effect on cultured keratinocyte cells attacked by free oxygen radicals. The latter was also supported in this study. Compounds **2**−**5** were found to have significant antioxidant properties, based on experiments with 2,2-diphenyl-1-picrylhydrazyl (DPPH), which indicated their ability to efficiently scavenge free radicals.[26,27]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin−Elmer 141 polarimeter using a sodium lamp operating at 589 nm. UV spectra were performed on a Shimadzu UV-160A spectrophotometer. IR spectra were measured on a Perkin−Elmer 2000 FT-IR spectrometer in KBr pellets. NMR measurements in CD_3OD at room temperature were performed on a Bruker AVANCE 400 spectrometer operating at 400.13 and 100.6 MHz for ^1H and ^{13}C, respectively, using the XWIN NMR software package for data acquisition and processing.

The $^1H-^1H$ DQF−COSY,[28] long-range COSY,[29] TOCSY,[30] $^1H-^{13}C$ HMQC,[31] and HMBC,[32] experiments were recorded by employing conventional pulse sequences. FABMS were recorded in a 3-NOBA matrix in the positive ion mode on a ZAB−SEQ instrument, and ESIMS were recorded in the negative ion mode on a Finnigan TSQ 7000 instrument. For radical-scavenging TLC autographic assay, DPPH (Fluka) was used as spray reagent.[27]

Plant Material. *Globularia trichosantha* Fisch. & Mey. (Globulariaceae) was collected from Sivas, between Akdagmadeni and Yildizeli, Central Anatolia, Turkey, in June 1998, at a height of about 1800 m. Voucher specimens (98−001) have been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation. The air-dried, aerial parts (stems, leaves, and flowers, 100 g) of *G. trichosantha* were extracted with MeOH (500 mL × 2) at 45 °C. The combined methanolic extracts were evaporated to dryness in vacuo (30 g, yield 30%). The methanolic extracts were dissolved in H_2O and partitioned with $CHCl_3$. The H_2O phase was lyophilized (25.5 g), and subjected to vacuum liquid chromatography (VLC) using reversed-phase material (Sepralyte 40 μm, 150 g), employing H_2O (200 mL), H_2O−MeOH (95:5, 90:10, 85:15, 80:20, 75:25; 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90; the volume of each solvent mixture 100 mL), and MeOH (200 mL) as eluents to yield 16 fractions (fraction volume: 100 mL), which were combined into eight main fractions, A−H; fractions A (6.591 g), B (3.316 g), C (1.784 g), D (1.242 g), E (2.86 g), F (5.73 g), G (2.59 g), and H (79 mg). Fraction E, eluted with 20−30% MeOH in H_2O, was subjected to MPLC using reversed-phase material (LiChroprep C_{18}; column dimensions 2.6 × 40 cm), and H_2O (250 mL), H_2O−MeOH mixtures with increasing amount of MeOH in H_2O (10−80%, 1800 mL), and MeOH (200 mL) as solvents to yield three fractions, E_1 (195 mg), E_2 (780 mg), and E_3 (534 mg). Fraction E_3 was rechromatographed on a Si gel (50 g) column eluting with $CHCl_3$−MeOH−H_2O (61:32:7; 600 mL) to give compounds **1** (20 mg), **2** (17 mg), **3** (58 mg), and a crude fraction of compound **5** (40 mg). This last was subjected to MPLC through the reversed-phase material [(LiChroprep C_{18}; column dimensions 1.5 × 40 cm) using H_2O−*i*-PrOH mixtures with increasing volumes of *i*-PrOH in H_2O (5%, 100 mL; 10%, 100 mL; 15%, 100 mL; and 20%, 100 mL) to afford pure **5** (12 mg). Repeated chromatographies of the fraction E_2 (780 mg) on Si gel columns using CH_2Cl_2−MeOH−H_2O mixtures (61:32:7; 70:30:3 and 80:20:2, respectively) to yield compounds **2** (107 mg, from the first column chromatography) and **4** (5 mg, from the third column chromatography), respectively.

Crenatoside (= oraposide, 1): $[\alpha]_D$ −58° (*c* 0.6, MeOH); UV (MeOH) λ_{max} 332, 288, 252 sh, 234 sh, and 221 nm; IR (KBr) ν_{max} 3500 (OH), 1700 (α,*β*-unsaturated ester), 1640 (olefinic C=C), and 1610, 1540, 1520 cm^{-1} (aromatic ring); ^1H NMR (400 MHz, CD_3OD), and ^{13}C NMR (100 MHz, CD_3OD) data superimposable with those reported in the literature;[16,17] FABMS *m/z* 645 $[M + Na]^+$.

Verbascoside (= acteoside, 2): $[\alpha]_D$ −75° (*c* 0.3, MeOH); UV (MeOH) λ_{max} 332, 290, 248 sh, 235 sh, and 220 nm; IR (KBr) ν_{max} 3500 (OH), 1700 (α,*β*-unsaturated ester), 1631 (olefinic C=C), and 1604, 1526 cm^{-1} (aromatic ring); ^1H NMR (400 MHz, CD_3OD), and ^{13}C NMR (100 MHz, CD_3OD) data superimposable with those reported in the literature.[18]

Trichosanthoside A (3): $[\alpha]_D$ −110° (*c* 0.45, H_2O); UV (MeOH) λ_{max} 334, 290, 248 sh, 233 sh, and 220 nm; IR (KBr) ν_{max} 3390 (OH), 1698 (α,*β*-unsaturated ester), 1631 (olefinic C=C), and 1614, 1525 cm^{-1} (aromatic ring); ^1H NMR (400 MHz, CD_3OD), and ^{13}C NMR (100 MHz, CD_3OD), see Table 1; FABMS *m/z* 779 $[M + Na]^+$.

Rossicaside A (4): $[\alpha]_D^{20}$ −60° (*c* 0.26, MeOH); UV (MeOH) λ_{max} 330, 287, 233 sh, and 220 nm; IR (KBr) ν_{max} 3400 (OH), 1697 (α,*β*-unsaturated ester), 1635 (olefinic C=C), and 1605, 1525 cm^{-1} (aromatic ring); ^1H NMR (400 MHz, CD_3OD), and ^{13}C NMR (100 MHz, CD_3OD) data superimposable with those reported in the literature;[19] negative ion ESIMS *m/z* 785 $[M - H]^-$.

Trichosanthoside B (5): $[\alpha]_D^{20}$ −127° (*c* 0.33, H_2O); UV (MeOH) λ_{max} 334, 288, 249 sh, 233 sh, and 220 nm; IR (KBr) ν_{max} 3396 (OH), 1698 (α,*β*-unsaturated ester), 1631 (olefinic C=C), and 1605, 1558, 1524 cm^{-1} (aromatic ring); ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1; negative ion ESIMS *m/z* 887 $[M - H]^-$.

Reduction of DPPH Radical. Methanolic solutions (0.1%) of compounds **1**−**5** were chromatographed on a Si gel plate using $CHCl_3$−MeOH−H_2O (61:32:7). After drying, TLC plates were sprayed with a 0.2% DPPH (Fluka) solution in MeOH. Compounds showing a yellow-on-purple spot were regarded as antioxidant.[27]

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