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Phenolic Compounds from *Globularia cordifolia*

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From the methanolic extract of the underground parts of *Globularia cordifolia*, a new neolignan diglycoside, dehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**1**) was isolated along with a known neolignan glycoside, dehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside (**2**). In addition, 2 flavone glycosides, chrysoeriol 7-*O*-β-D-allopyranosyl-(1→2)-β-D-glucopyranoside (**3**), stachyspinoside (**4**) and 5 phenylethanoid glycosides, verbascoside (**5**), isoverbascoside (**6**), leucoseptoside A (**7**), martynoside (**8**) and rossicaside A (**9**) were obtained and characterized. The structures of the isolates were established by 1D and 2D NMR spectroscopy in combination with IR, UV and MS analysis.

Key Words: *Globularia cordifolia*, Globulariaceae, neolignan glycosides, dehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, flavonoid glycosides, phenylethanoid glycosides.

Introduction

In the flora of Turkey, the genus *Globularia* L. (Globulariaceae) is represented by 9 species[1,2] , some of which are traditionally used as diuretics, laxatives, carminatives and tonics and for the treatment of hemorrhoids[3,4]. In our previous paper, we isolated iridoid and bisiridoid glycosides from *G. cordifolia*[5]. Further investigation on the underground parts of this plant yielded neolignan, flavonoid and phenylethanoid glycosides. We now present the isolation and the structure elucidation of a new neolignan diglucoside, dehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**1**) as well as a neolignan glucoside (**2**), 2 flavone glycosides (**3**-**4**) and 5 phenylethanoid glycosides (**5**-**9**) from the underground parts of *G. cordifolia*.

Experimental

General experimental procedures: UV spectra were recorded on a Shimadzu UV-160A spectrophotometer. IR spectra (KBr) were measured on a Perkin Elmer 2000 FT-IR spectrometer. Bruker AMX 600

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instruments (600 MHz for ^{1}H and 150 MHz for ^{13}C) with the XWIN NMR software package were used to acquire NMR data. Positive-mode ESIMS were recorded on a Finnigan TSQ 7000 instrument. TLC analyses were carried out on silica gel 60 F_{254} precoated plates (Merck, Darmstadt, Germany), and detection was performed with 1% vanillin/H_2SO_4. For medium-pressure liquid chromatographic (MPLC) separations, a Lewa M5 pump, a LKB 17000 Minirac fraction collector, a Rheodyne injector, and Büchi columns (column dimensions 2.6 x 46 cm and 1.8 x 35 cm) were used. Silica gel 60 (0.063-0.200 mm; Merck, Darmstadt, Germany) was utilized for open column chromatography (CC). LiChroprep C-18 (Merck) material was used for vacuum liquid chromatography (VLC) and MPLC.

Plant Material: *Globularia cordifolia* L. (Globulariaceae) was collected from Kastamonu, Pınarbaşı, northern Anatolia, Turkey, in June 2001. A voucher specimen (HUEF 01002) is deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation: The air-dried and powdered underground parts of *G. cordifolia* (220 g) were extracted twice with MeOH (2 x 1.5 L) at 45 °C. The combined methanolic extracts were evaporated to dryness (22 g, yield 10%). The crude extract was dissolved in H_2O and partitioned against $CHCl_3$. The lyophilized H_2O phase (18.750 g) was fractionated over LiChroprep C-18 (VLC). The employment of H_2O, H_2O-MeOH mixtures with increasing ratio of MeOH in H_2O (10-90% , MeOH) and MeOH afforded 9 main fractions, A-I. Fraction D (1.890 g) was separated by C_{18}-MPLC using 5% to 60% MeOH in H_2O as an eluent to give 4 fractions, D_1-D_4. Purification of fraction D_4 (680 mg) by Si gel CC (CH_2Cl_2-MeOH-H_2O, 70:30:3 to 61:32:7 v/v/v) furnished verbascoside (**5**, 8 mg) and rossicaside A (**9**, 33 mg). Fraction F (2.900 g) was likewise subjected to C_{18}-MPLC using stepwise gradients of MeOH (10% -60%) in H_2O to yield 4 main fractions, F_1-F_4. Fraction F_2 (384 mg) was applied to a Si gel column eluting with $CHCl_3$-MeOH-H_2O mixtures (85:15:1 to 61:32:7, v/v/v) to yield the crude fraction of compound **1**. Dehydrodiconiferyl alcohol 9-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**1**, 3 mg) was purified by using Si gel CC (EtOAc-MeOH-H_2O, 100:10:5, v/v/v). Repeated chromatography of fraction F_3 (450 mg) on a Si gel column ($CHCl_3$-MeOH-H_2O, 90:10:1 to 70:30:3 v/v/v) gave 3 fractions, F_{3a}-F_{3c} and isoverbascoside (**6**, 20 mg). Fraction F_{3b} (73 mg) was rechromatographed over Si gel eluting with EtOAc-MeOH-H_2O (100:8:4 v/v/v) mixture to afford leucosceptoside A (**7**, 33 mg) and dehydrodiconiferyl alcohol 9-*O*-*β*-D-glucopyranoside (**2**, 8 mg). Fraction G (4.128 g) was also subjected to C_{18}-MPLC using stepwise gradients of MeOH in H_2O (10% -70% MeOH) to give 5 main fractions, G_1-G_5. Fraction G_4 (433 mg) was similarly separated by C_{18}-MPLC using 35% to 55% MeOH in H_2O as an eluent to afford 2 fractions, G_{4a} and G_{4b}. Fraction G_{4b} (200 mg) was rechromatographed over Si gel eluting with $CHCl_3$-MeOH-H_2O (85:15:1 to 61:32:7 v/v/v) to yield stachyspinoside (**4**, 7 mg) and impure martynoside. Purification of martynoside (**8**, 3 mg) was achieved by Si gel CC (EtOAc-MeOH-H_2O, 100:5:3 v/v/v). Fraction G_5 (890 mg) was subjected to a Si gel column to afford 3 fractions, G_{5a}-G_{5c}. Repeated chromatography of fraction G_{5b} (37 mg) on a Si gel column ($CHCl_3$-MeOH-H_2O, 70:30:3 v/v/v) gave pure chrysoeriol 7-*O*-*β*-D-allopyranosyl-(1→2)-*β*-D-glucopyranoside (**3**, 3 mg).

Dehydrodiconiferyl alcohol 9-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (1): Amorphous powder; ESIMS m/z: 705 $[M+Na]^+$ ($C_{32}H_{43}O_{16}$); UV λ_{max} (MeOH, nm): 222, 278, 340; IR ν_{max} (KBr, cm^{-1}): 3403 (OH), 1609, 1560, 1459 (aromatic ring); ^{1}H NMR (600 MHz, CD_3OD): Table 1; ^{13}C NMR (CD_3OD, 150 MHz): Table 1.

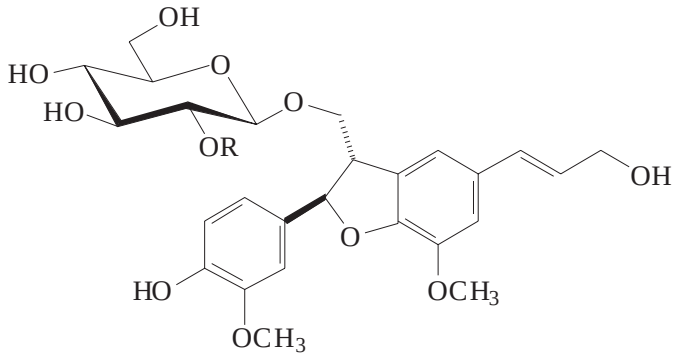
Table 1. The ^{13}C and ^{1}H NMR spectroscopic data for **1** (CD_3OD,^{13}C: 150 MHz; ^{1}H: 600 MHz)[a].

C/H		δ_C ppm	δ_H ppm, J (Hz)
1	C	134.3	
2	CH	109.8	7.00 d (1.2)
3	C	149.1	
4	C	147.1	
5	CH	115.6	6.80 d (7.5)
6	CH	119.2	6.91 dd (7.5, 1.2)
7	CH	88.3	5.70 d (7.0)
8	CH	52.4	3.70 m
9	CH_2	71.1	4.22 dd (10.5, 6.5)
			3.88 dd (10.5, 7.5)
3-OCH_3	CH_3	55.7	3.84 s
1'	C	132.5	
2'	CH	111.3	6.96 d (1.2)
3'	C	145.6	
4'	C	149.0	
5'	C	134.8	
6'	CH	116.3	7.04 d (1.2)
7'	CH	131.8	6.55 d (16.0)
8'	CH	127.4	6.23 dt (16.0, 5.9)
9'	CH_2	63.0	4.22 d (5.9)
3'-OCH_3	CH_3	56.1	3.91 s
1"	CH	102.5	4.52 d (7.5)
2"	CH	81.9	3.56 dd (7.5, 9.0)
3"	CH	77.0	3.57 t (9.0)
4"	CH	70.6	3.33 t (9.0)
5"	CH	77.3	3.30 m
6"	CH_2	62.0	3.90 dd (12.0, 2.0)
			3.71 dd (12.0, 4.5)
1"'	CH	104.6	4.64 d (7.5)
2"'	CH	75.6	3.21 dd (7.5, 9.0)
3"'	CH	77.3	3.32 t (9.0)
4"'	CH	70.7	3.33 t (9.0)
5"'	CH	77.3	3.13 m
6"'	CH_2	61.8	3.71 dd (12.0, 2.0)
			3.57 dd (12.0, 4.5)

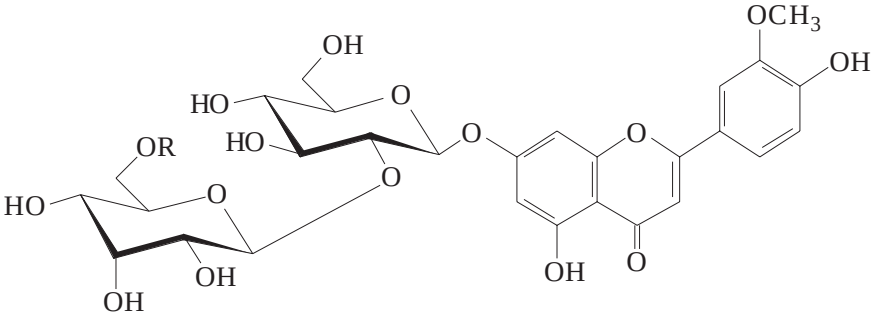
[a]All proton and carbon assingments are based on 1D TOCSY and 2D NMR (COSY, HSQC and HMBC).

Results and Discussion

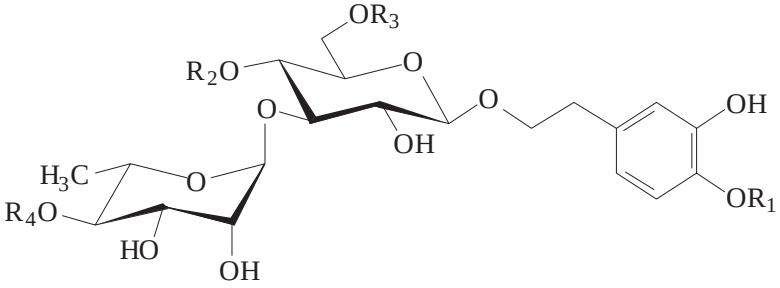
Compound **1** was obtained as an amorphous powder. The molecular formula was established as $C_{32}H_{43}O_{16}$ on the basis of the pseudomolecular ion appearing in the positive ESIMS (m/z 705 $[M + Na]^+$) and ^{13}C NMR data (see Table 1). The UV spectrum showed maxima at 222, 278 and 340 nm. The IR spectrum suggested the presence of hydroxyl (3403 cm^{-1}) and aromatic (1609, 1560, 1459 cm^{-1}) functionalities. The ^{1}H NMR spectrum (see Table 1) showed 5 aromatic proton signals. Of these, the proton resonances at δ_H 7.00 (d, J= 1.2 Hz; H-2), 6.91 (dd, J= 7.5, 1.2 Hz; H-6) and 6.80 (d, J= 7.5 Hz; H-5) were observed as an



1 R = β-D-Glc; **2** R = H



3 R = H; **4** R = Ac



5 $R_1 = R_3 = R_4$ = H, R_2 = *trans*-caffeoyl
6 $R_1 = R_2 = R_4$ = H, R_3 = *trans*-caffeoyl
7 $R_1 = R_3 = R_4$ = H, R_2 = *trans*-feruloyl
8 $R_1 = CH_3$, $R_3 = R_4$ = H, R_2 = *trans*-feruloyl
9 $R_1 = R_3$ = H, R_2 = *trans*-caffeoyl, R_4 = β-D-Glc

Figure 1. Phenolic compounds (**1**-**9**) from *G. cordifolia*.

ABX system, suggesting the presence of a trisubstituted aromatic moiety within **1**. Furthermore, the ^{1}H NMR spectrum of **1** displayed 2 *trans* olefinic proton signals at δ_H 6.55 (d, J= 16.0 Hz) and 6.23 (dt, J= 16.0, 5.9 Hz), which appeared as an AB part of an ABX_2 system and 2 aromatic methoxyl singlets at δ_H 3.91 and 3.84. In addition, 2 anomeric proton signals at δ_H 4.64 (d, J= 7.5 Hz) and 4.52 (d, J= 7.5 Hz) indicated its diglycosidic structure. Assignments for all proton and carbon resonances were achieved by 1D TOCSY, COSY and HSQC experiments, which indicated both sugars are β-glucopyranose. The appearance of a downfield signal at δ_C 81.9 for C-2" resonances of the glucose unit and the long range

correlations between this carbon and the anomeric proton of the terminal glucose (δ_H 4.64) in the HMBC spectrum (see Figure 2), revealed the presence of a (1→2)-glycosidic linkage between 2 glucose moieties. Apart from 12 signals due to 2 β-glucose units and 2 OMe signals, the ^{13}C NMR spectrum of **1** contained 18 carbon atoms consistent with a lignan structure. From a detailed inspection of all proton and carbon data associated with the interpretation of the 1D and 2D NMR experiments, compound **1** was predicted to be a dehydrodiconiferyl alcohol type neolignan diglucoside. Accordingly, the signal at δ_H 5.70 (d, J= 7.0 Hz) was ascribed to H-7 of the benzofuran ring. H-7 correlated with a methine proton at δ_H 3.70 (H-8), which in turn showed additional coupling with the oxymethylene protons (δ_H 4.22 and 3.88; H_2-9) in the COSY spectrum. H-7, H-8 and H_2-9 were observed at the same spin system in the 1D TOCSY spectrum. On the other hand, the other oxymethylene protons at δ_H 4.22 (H_2-9') are at the same spin system with the 2 *trans* olefinic protons of the hydroxypropenyl side chain of the neolignan skeleton in the 1D TOCSY spectrum. The glycosidic linkage was determined to be at C-9 due to the downfield shift of the C-9 (δ_C 71.1) and the HMBC cross-peak between this carbon and the anomeric proton of the inner glucose (H-1", δ_H 4.52). Although the stereochemistry at C-7 and C-8 could not be established with the available data, but the configuration of H-7 and H-8 was able to be confirmed as *trans* from the large coupling constant ($J_{7,8}$ = 7.0 Hz) in the ^{1}H NMR spectrum[6,7]. Consequently, the structure of **1** was elucidated as dehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, which has not yet been reported.

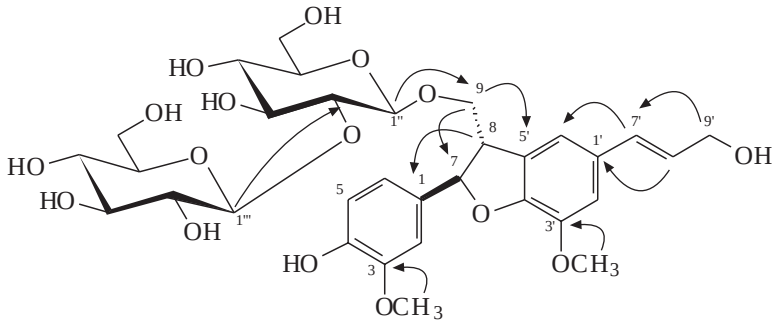


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Figure 2. Selected HMBC correlations for **1**.

In addition to this compound, a known neolignan glycoside, dehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside (**2**)[8], along with flavone glycosides, chrysoeriol 7-*O*-β-D-allopyranosyl-(1→2)-β-D-glucopyranoside (**3**)[9], stachyspinoside (**4**)[10] and the phenylethanoid glycosides verbascoside (**5**)[11], isoverbascoside (**6**)[12,13], leucosceptoside A (**7**)[14], martynoside (**8**)[15] and rossicaside A (**9**)[16] were also isolated and identified by comparison of their spectroscopic (NMR and MS) data with those published in the literature. This study is also the first report on the isolation of neolignan glycosides from the genus *Globularia*.

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