Iridoid and bisiridoid glycosides from Globularia cordifolia

Iridoid and Bisiridoid Glycosides from *Globularia cordifolia*

Hasan Kirmizibekmez[a], Ihsan Çaliş[a,*], Pinar Akbay[b], and Otto Sticher[b]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey. Fax: +90-312-3114777. E-mail: icalis@hacettepe.edu.tr

[b] Department of Applied BioSciences, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH) Zurich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

* Author for correspondence and reprint requests

Z. Naturforsch. **58 c**, 337–341 (2003); received December 19, 2002

From the methanolic extract of the underground parts of *Globularia cordifolia*, a new iridoid glycoside, 5-hydroxydavisioside (**1**) and a new bisiridoid glycoside, globuloside C (**2**) were isolated along with six known iridoid glycosides, aucubin, melampyroside, monomelittoside, globularifolin, alpinoside and asperuloside. The structures of the isolates were established by 1D and 2D NMR spectroscopy in combination with IR, UV and MS analyses.

Key words: Globularia cordifolia, Iridoid and Bisiridoid Glycosides

Introduction

Globularia cordifolia L. (Globulariaceae) is a mat-forming shrublet growing in limestone cliffs in Central and South Europe (Edmondson, 1982). Several phytochemical studies exhibited that the main constituents of *G. cordifolia* were iridoid glycosides (Chaudhuri and Sticher, 1980) and flavonoids (Harborne and Williams, 1971). As a part of our work on the isolation and identification of secondary metabolites from Turkish *Globularia* species, we herein present the isolation and the structure elucidation of a new iridoid glycoside, 5-hydroxydavisioside (**1**) and a new bisiridoid glycoside, globuloside C (**2**) obtained from the underground parts of *G. cordifolia.*

Material and Methods

General experimental procedures

Optical rotations were measured on a Rudolph autopol IV Polarimeter using a sodium lamp operating at 589 nm. 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 300 and DRX 500 instruments (300 and 500 MHz for ^{1}H and 75.5 MHz for ^{13}C) with XWIN NMR software package were used to acquire NMR data. Positive-mode ESIMS were recorded on a Finnigan TSQ 7000 instrument. Positive-mode HR-MALDIMS was recorded on an Ionspec-Ultima-FTMS spectrometer, 2,5-dihydroxybenzoic acid (DHB) as matrix. TLC analyses were carried on silica gel 60 F_{254} precoated plates (Merck, Darmstadt); detection by 1% vanillin/H_2SO_4. For medium-pressure liquid chromatographic separations, a Lewa M5 pump, a LKB 17000 Minirac fraction collector, a Rheodyne injector, and Büchi columns (column dimensions 2.6 × 46 cm, and 1.8 × 35 cm) were used. Silica gel 60 (0.063–0.200 mm; Merck, Darmstadt) was utilized for open column chromatography (CC). LiChroprep C-18 (Merck) material was used for VLC and MPLC.

Plant material

Globularia cordifolia L. (Globulariaceae) was collected from Kastamonu, Pinarbasi, North Anatolia, Turkey, in June 2001. Voucher specimens (HUEF 01002) have been deposited at the herbarium of the Department of the Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and isolation

The air-dried and powdered roots and rhizomes of *G. cordifolia* (220 g) were extracted twice with MeOH (2 × 1.5 l) at 45° C. The combined methanolic extracts were evaporated to dryness *in vacuo* (22 g, yield 10%). The crude extract was dissolved in H_2O and partitioned against $CHCl_3$. The lyophilized H_2O phase (18.75 g) was fractionated over LiChroprep C-18 (VLC). Employment of H_2O, H_2O-MeOH mixtures with increasing

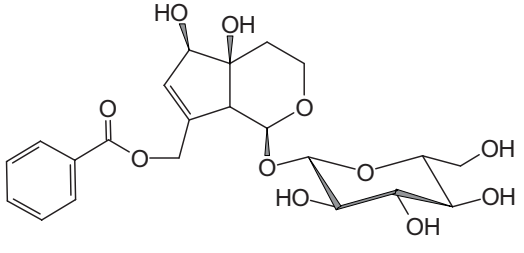
amount of MeOH in H_2O (10–90%, MeOH) and MeOH afforded nine main fractions, A–I. Fraction B (895 mg) was subjected to C_{18} medium pressure liquid chromatography (C_{18}-MPLC) employing increasing amount of MeOH in H_2O (0–50%) to afford fraction B_1, asperuloside (4 mg) and alpinoside (15 mg). Fraction B_1 (99 mg) was rechromatographed on silica CC (CH_2Cl_2-MeOH-H_2O, 70:30:3 v/v/v) to give aucubin (10 mg) and monomelittoside (9 mg). Fraction D (1.890 g) was similarly separated by C_{18}-MPLC using 5 to 60% MeOH in H_2O as eluents to give alpinoside (35 mg) in addition to three fractions, D_2–D_4. Fraction D_3 (82 mg) was applied to a Si gel column eluting with $CHCl_3$-MeOH-H_2O mixture (70:30:3 v/v/v) to give globuloside C (**2**, 10 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 four main fractions, F_1–F_4. 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 three fractions, F_{3a}–F_{3c}. 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 5-hydroxydavisioside (**1**, 9 mg). Fraction G (4.13 g) was also subjected to C_{18}-MPLC using stepwise gradients of MeOH in H_2O (10–70% MeOH) to give five main fractions, G_1–G_5. Fraction G_2 (660 mg) was applied to a Si gel column eluting with CH_2Cl_2-MeOH-H_2O mixture (90:10:1 to 70:30:3 v/v/v) to obtain melampyroside (6 mg) and globularifolin (156 mg).

5-Hydroxydavisioside (**1**): Amorphous powder; $[\alpha]_D^{20}$ – 76° (*c* = 0.1, MeOH); ESIMS *m/z*: 491 $[M+Na]^+$, 507 $[M+K]^+$; UV λ_{max} (MeOH, nm): 231, 275; IR υ_{max} (KBr, cm^{-1}) 3415 (OH), 1739 (ester C=O), 1475, 1438 (aromatic ring); ^{1}H-NMR (500 MHz, CD_3OD): Table I; ^{13}C-NMR (CD_3OD, 125 MHz): Table I.

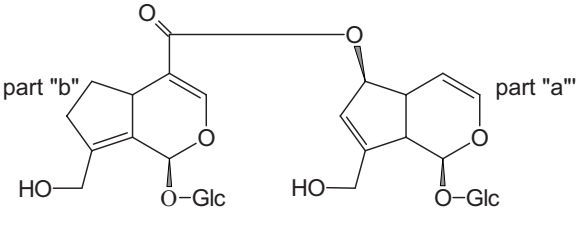
Globuloside C (**2**): Amorphous powder; $[\alpha]_D^{20}$ – 80° (*c* = 0.1, MeOH); HR-MALDIMS *m/z*: 725.2280 $[M+Na]^+$; UV λ_{max} (MeOH, nm): 224; IR υ_{max} (KBr, cm^{-1}) 3397 (OH), 1737 (ester C=O), 1625 (C=C-O); ^{1}H-NMR (500 MHz, CD_3OD): Table II; ^{13}C-NMR (CD_3OD, 75.5 MHz): Table II.

Results and Discussion

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{20}$ – 76° (*c* 0.1, MeOH). Its molecular formula was determined to be $C_{22}H_{28}O_{11}$ on the basis of positive-ion ESIMS (*m/z* 491, $[M+Na]^+$ and 507 $[M+K]^+$) and ^{13}C NMR data (see Table I). The UV spectrum exhibited maxima at 231 and 275 nm. The IR spectrum showed absorption bands for hydroxyl (3415 cm^{-1}), ester carbonyl (1739 cm^{-1}) and aromatic (1475 and 1438 cm^{-1}) functionalities. The ^{1}H NMR spectrum (see Table I) of **1** displayed signals due to an acylated iridoid glycoside. The anomeric proton resonance at δ_H 4.57 (d, *J* = 7.9 Hz) and the signals in the region 3.25–3.80 indicated the presence of a β-glucopyranosyl unit. Additional aromatic proton signals at δ_H 8.07 (2H), 7.62 (1H) and 7.50 (2H) were typical for a benzoyl moiety. The ^{13}C NMR spectrum of **1** exhibited 22 signals, six of which were attributed to a β-glucopyranosyl unit, while seven of which were ascribed to a benzoic acid. All the remaining resonances arising from the aglycone were shown by the DEPT-135 spectrum to consist of two quaternary (2C), four methine (4CH) and three methylene ($3CH_2$) carbons. All of the ^{1}H and ^{13}C chemical shifts of **1** were determined by the assistance of 2D NMR (DQF-COSY, HSQC and HMBC). Thus, the oxymethylene (δ_H 4.15 and 3.50)



1



2

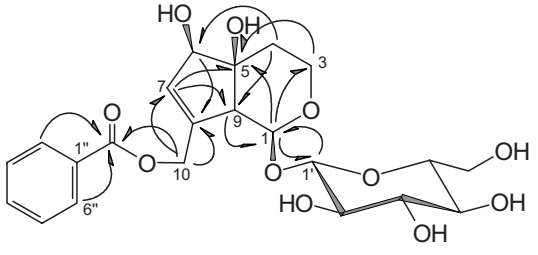
Fig. 1. New iridoids (**1–2**) from *G. cordifolia.*

Table I. The ^{13}C and ^{1}H NMR spectroscopic data and HMBC correlations for **1** (CD_3OD, ^{13}C: 125 MHz; ^{1}H: 500 MHz)*.

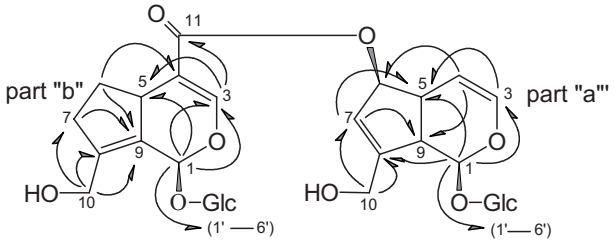
C/H		δ_C ppm	δ_H ppm, *J* [Hz]	HMBC (H→C)
1	CH	96.5	5.26 d (3.4)	C-1′, C-3, C-5, C-8
3	CH_2	58.2	4.15†	C-1, C-5
			3.50 m	C-1, C-4, C-5
4	CH_2	33.3	1.88 m	C-3, C-5, C-6, C-9
			1.62 m	C-3, C-5, C-6, C-9
5	C	77.4		
6	CH	79.3	4.15†	C-4, C-5, C-7, C-8
7	CH	130.3	5.89 br s	C-5, C-6, C-9, C-10
8	C	143.5		
9	CH	53.6	2.81 br s	C-1, C-5, C-8
10	CH_2	63.5	5.00 dd (12.5)	C-7, C-8, C-9, C=O
			4.96 d (12.5)	C-7, C-8, C-9, C=O
1′	CH	99.7	4.57 d (7.9)	C-1, C-2′, C-3′
2′	CH	74.7	3.24 dd (7.9, 9.0)	C-1′, C-4′
3′	CH	77.7	3.37 t (9.0)	C-2′, C-4′, C-5′
4′	CH	71.7	3.27†	C-5′, C-6′
5′	CH	78.1	3.25†	C-3′
6′	CH_2	62.8	3.79 dd (11.9, 1.8)	C-4′
			3.59 dd (11.9, 5.5)	C-4′, C-5′
1″	C	131.2		
2″	CH	130.7	8.07 dd (7.5, 1.5)	C=O, C-4″, C-6″
3″	CH	129.7	7.50 t (7.5)	C-1″, C-4″, C-5″
4″	CH	134.4	7.62 m	C-2″, C-6″
5″	CH	129.7	7.50 t (7.5)	C-1″, C-3″, C-4″
6″	CH	130.7	8.07 dd (7.5, 1.5)	C=O, C-2″, C-4″
C=O	C	167.6		

* All proton and carbon assignments are based on 2D NMR (DQF-COSY, HSQC, HMBC).
† Signal patterns are unclear due to overlapping.

and the methylene (δ_H 1.88 and 1.62) proton signals were ascribed to H_2-3 and H_2-4 respectively, indicating the lacking of the double bond between C-3 and C-4 in the cyclopentane ring. The DQF-COSY spectrum of **1** suggested that H_2-3 and H_2-4 existed as one spin system. The absence of any other homonuclear coupling observed for H_2-4 was indicative of C-5 being fully substituted. On the other hand, the acetal proton (δ_H 5.26) of the iridoid skeleton was coupled with a methine proton at δ_H 2.81 (H-9). No other coupling was observed for H-9 suggested that both C-5 and C-8 were totally substituted. In the HMBC spectrum (see Table I, Fig. 2), a pair of H_2-10 AB doublets at δ_H 5.00 (1H, *J* = 12.5 Hz) and 4.96 (1H, *J* = 12.5 Hz) showed ^{1}H-^{13}C long-range correlations with the carbonyl carbon signal of the benzoyl moiety at δ_C 167.6 suggested C-10(OH) to be site of benzoylation. The cross-peaks between H-1 and C-1′ and *vice versa*, indicated that the β-glucopyranosyl unit was linked to the C-1(OH). The complete NMR data of **1** based on the 2D NMR were closely related to that of davisioside (Calis *et al.*, 2002), except for downfield shift of C-5 (δ_C 77.4) and the absence of H-5 resonances in the NMR spectra of **1**. These data revealed that C-5 position of compound **1** was oxygenated. By the



1



2

Fig. 2. Selected HMBC correlations for **1** and **2**.

above observations, compound **1** was found to be a hydroxylated analogue of davisioside and named as 5-hydroxydavisioside.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{20}$ – 80° (*c* 0.1, MeOH). Its molecular formula was determined to be $C_{31}H_{42}O_{18}$ on the basis of positive-ion HR-MALDIMS (*m/z* 725.2280 $[M+Na]^+$) and ^{13}C NMR data (see Table II). The IR spectrum showed absorption bands at 3397, 1737 and 1625 cm^{-1}, indicating the presence of OH, ester C=O and C=C-O groups respectively. The UV spectrum exhibited a maximum at 224 nm. The ^1H and ^{13}C NMR spectra of **2** clearly indicated its dimeric nature by the duplication of the signals typical of an iridoid glycoside. The signals in the region of δ_H 3.15–4.70 including two anomeric proton resonances at δ_H 4.66 and 4.68 (both d, *J* = 7.8 Hz) supported the presence of two β-glucopyranosyl units in **2**. One-half of the molecule, part "a" was easily assigned to an aucubin-type iridoid due to the olefinic signals at δ_H 6.29 (dd, *J* = 6.2, 1.9 Hz, H-3), 5.03 (dd, *J* = 6.2, 3.6 Hz, H-4) and 5.81 (d, *J* = 1.8 Hz, H-7). The second half, part "b" was indicated to be a C-4 substituted iridoid structure on the basis of the signals at δ_H 7.38 (d, *J* = 1.8 Hz) assigned to H-3 and δ_C 152.3, 114.4 and 168.5, which were ascribed to C-3, C-4 and C-11 respectively. Assignment of all the proton and carbon signals was achieved through a combination of DQF-COSY, HSQC and HMBC (see Table II, Fig. 2) 2D NMR experiments. Thus, it was deduced that part "a" is aucubin while part "b" is deacetylalpinoside (see Table II). The connectivity between parts "a" and "b" was found to be an ester linkage between the C-6(OH) of part "a" (aucubin moiety) and the carboxyl group (C-11) of part "b" (deacetylalpinoside moiety) due to the downfield shift for H-6 (δ_H 5.38) of the part "a". The complete ^1H and ^{13}C NMR data of **2** secured by 2D NMR experiments were almost identical to that of globuloside B (Calis *et al.*, 2001), except for the absence of benzoyl resonances in the spectra of **2**. Additionally, the resonances of 10-oxymethylene (δ_H 4.37 and 4.20; δ_C 61.1) of aucubin part were at higher field than the corresponding resonances of globuloside B, supporting the disappearance of acylation at C-10(OH). Consequently, compound **2** was established as debenzoylglobuloside B and we propose the trivial name globuloside C.

Besides these new compounds, six known iridoid glycosides, aucubin (Bianco *et al.*, 1983), melampy-

Table II. The ^{13}C and ^1H NMR spectroscopic data and HMBC correlations for **2** (CD_3OD, ^{13}C: 75.5 MHz; ^1H: 500 MHz)*.

		Part "a"					Part "b"		
C/H		δ_C ppm	δ_H ppm, *J* [Hz]	HMBC (H→C)	C/H		δ_C ppm	δ_H ppm, *J* [Hz]	HMBC (H→C)
1	CH	96.5	5.14 d (6.5)	C-1′, C-3, C-5	1	CH	92.4	6.37 s	C-1′, C-3, C-5, C-9
3	CH	141.8	6.29 dd (6.2, 1.9)	C-1, C-4, C-5	3	CH	152.3	7.38 d (1.8)	C-1, C-4, C-5, C-11
4	CH	105.0	5.03 dd (6.2, 3.6)	C-3, C-5, C-6, C-9	4	C	114.4		
5	CH	42.4	2.91 m		5	CH	39.1	3.60 m	
6	CH	84.7	5.38 dd (1.8, 3.7)		6	CH_2	32.4	2.56 m	C-5, C-8, C-9
7	CH	126.0	5.81 d (1.8)	C-5, C-6, C-8, C-9				1.45 m	C-4, C-5
8	C	151.6			7	CH_2	34.8	2.50 m	C-8, C-9
9	CH	48.3	3.04 t (6.5)	C-1, C-5, C-7, C-8	8	C	131.5		
10	CH_2	61.1	4.37 d (13.2)	C-7, C-8	9	C	142.7		
			4.20 d (13.2)	C-7, C-8	10	CH_2	59.1	4.27 d (13.9)	C-7, C-8, C-9
								4.20 d (13.9)	C-8, C-9
1′	CH	99.8	4.66 d (7.9)	C-1, C-2′	11	C	168.5		
2′	CH	74.9	3.22 dd (7.9, 9.1)						
3′	CH	78.0	3.38†		1′	CH	100.2	4.68 d (7.9)	C-1, C-3′, C-5′
4′	CH	71.6	3.29†		2′	CH	74.7	3.15 dd (7.9, 9.1)	
5′	CH	78.3	3.30†		3′	CH	77.9	3.38†	
6′	CH_2	62.6	3.88†		4′	CH	71.4	3.29†	
			3.67†		5′	CH	78.3	3.30†	
					6′	CH_2	62.5	3.88†	
								3.67†	

* All proton and carbon assignments are based on 2D NMR (DQF-COSY, HSQC and HMBC).
† Signal patterns are unclear due to overlapping.

roside (Chaudhuri and Sticher, 1980), monomelittoside (Chaudhuri and Sticher, 1980), globularifolin (Chaudhuri and Sticher, 1980), alpinoside (Jensen *et al.*, 1996) and asperuloside (Otsuka *et al.*, 1991) were also isolated and identified by comparison of their spectral data with published values.

5-Hydroxydavisioside (**1**) represents a rare iridoid skeleton lacking the double bond between C-3 and C-4. Globuloside C (**2**) is the third example for dimeric iridoids isolated from the genus *Globularia.* The other examples of this type bisiridoids, globulosides A and B have been isolated from *Globularia trichosantha* (Calis *et al.*, 2001). It is interesting that all these bisiridoids have been obtained from the underground parts of these plants. 5-Substituted iridoids like monomelittoside, globularifolin and 5-hydroxydavisioside (**1**) have only been encountered in *G. cordifolia* among the *Globularia* species up to now. So such compounds might have some taxonomic potential for the title species.

Acknowledgment

The authors thank Prof. Dr. Hayri Duman, Gazi University, Ankara, for authentification of the plant material; Dr. Engelbert Zass, Institute of Organic Chemistry, ETH Zurich, for performing computer-based literature searches; Dr. Walter Amrein and Mr. Rolf Haefliger Institute of Organic Chemistry, ETH Zurich for recording all mass spectra.

Bianco A., Passacantilli P., Polidori G., Nicoletti M., and Messana I. (1983), NMR spectroscopy of epimeric pairs of glucosidic iridoids from Rubiaceae. Gazz. Chim. Ital. **113**, 829–834.

Calis I., Kirmizibekmez H., and Sticher O. (2001), Iridoid glycosides from *Globularia trichosantha*. J. Nat. Prod. **64**, 60–64.

Calis I., Kirmizibekmez H., Tasdemir D., and Ireland C. M. (2002), Iridoid glycosides from *Globularia davisiana*. Chem. Pharm. Bull. **50**, 678–680.

Chaudhuri R. K., and Sticher O. (1980), Globularifolin, a new acyl iridoid glucoside from *Globularia cordifolia*. Helv. Chim. Acta **63**, 117–120.

Edmondson J. R. (1982), In: Flora of Turkey and East Aegean Islands (Davis P. H., ed). University Press. Edinburgh, Vol. 7, pp. 27–31.

Harborne J. B., and Williams C. A. (1971), 6-hydroxyluteolin and scutellarein as phyletic markers in higher plants. Phytochemistry **10**, 367–378.

Jensen S. R., Olsen C. E., Rahn K., and Rasmussen J. H. (1996), Iridoid glucosides in *Plantago alpina* and *P. altissima*. Phytochemistry **42**, 1633–1636.

Otsuka H., Yoshimura K., Yamasaki K., and Cantoria M. C. (1991), Isolation of 10-*O*-acyl iridoid glucosides from a Philippine medicinal plant, *Oldenlandia corymbosa* L. (Rubiaceae). Chem. Pharm. Bull. **39**, 2049–2052.