Iridoids from *Globularia dumulosa*

Hasan Kirmizibekmez[a], Pinar Akbay[b], Otto Sticher[b], and İhsan Çalış[a,*]

[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) Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

* Author for correspondence and reprint requests

Z. Naturforsch. **58 c**, 181–186 (2003); received October 15/October 31, 2002

Two new iridoids, 10-*O*-benzoylglobularigenin (**1**) and dumuloside (**2**) were isolated from the aerial parts of *Globularia dumulosa* together with seven known iridoid glucosides, davisioside (**3**), aucubin (**4**), melampyroside (**5**), catalpol (**6**), 10-*O*-benzoylcatalpol (**7**), alpinoside (**8**) and deacetylalpinoside (**9**). Three phenylethanoid glycosides, verbascoside, decaffeoylverbascoside, leucosceptoside A and three flavone glucosides, pectolinarigenin 7-*O*-β-D-glucopyranoside, nepetin 7-*O*-β-D-glucopyranoside, demethoxycentaureidin 7-*O*-β-D-glucopyranoside were also isolated and characterized. The structure elucidation of the isolated compounds was performed by spectroscopic (UV, IR, HR-MALDIMS, 1D- and 2D NMR) methods.

Key words: Globularia dumulosa, Iridoids, Phenylethanoid Glycosides, Flavone Glycosides

Introduction

In the flora of Turkey, the genus *Globularia* (Globulariaceae) is represented by nine species (Edmondson, 1982; Duman, 2001). In Anatolian folk medicine, *G. alypum* is used as diuretic, laxative, carminative and tonic (Baytop, 1984), whereas *G. trichosantha* is utilized for the treatment of hemorrhoids (Sezik *et al.*, 1991). Our previous studies have resulted in the isolation of phenylethanoid and iridoid glycosides from *G. trichosantha* (Calis *et al.*, 1999, 2001) and *G. davisiana* (Calis *et al.*, 2002a) and sugar esters along with iridoid and phenylethanoid glycosides from *G. orientalis* (Calis *et al.*, 2002b). In the course of an investigation of *Globularia* species growing in Turkey, we have now investigated an endemic species, *G. dumulosa* O. Schwarz. In this paper we report the isolation and structure elucidation of two new iridoids, 10-*O*-benzoylglobularigenin (**1**) and dumuloside (**2**) obtained from the aerial parts of *G. dumulosa*.

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. A Bruker AMX 300 instrument (300 MHz for ^{1}H and 75.5 MHz for ^{13}C) with XWIN NMR software package was used to acquire NMR data. Positive mode HR-MALDIMS were recorded on a 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 17.000 Minirac fraction collector, a Rheodyne injector, and a Büchi column (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) and vacuum liquid chromatography (VLC). LiChroprep C-18 (Merck) material was used for MPLC and VLC. Sephadex LH-20 (Fluka) was also used for further separations.

Plant material

Globularia dumulosa O. Schwarz. (Globulariaceae) was collected from Denizli, Acipayam, Southwest Anatolia, Turkey, in July 2001. Voucher specimens (HUEF 01006) have been deposited at the herbarium of the Department of the Pharma-

0939–5075/2003/0300–0181 $ 06.00

cognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and isolation

The air-dried and powdered aerial parts of *G. dumulosa* (170 g) were extracted twice with MeOH (2 × 800 ml) at 45° C. The combined methanolic extracts were evaporated to dryness *in vacuo* (52.5 g, yield 30%). The crude extract was dissolved in H_2O and partitioned against $CHCl_3$. An aliquot (34 g) of the lyophilized H_2O phase (40.1 g) was fractionated over LiChroprep C-18 (VLC). Employment of H_2O, H_2O-MeOH mixtures with increasing amount of MeOH in H_2O (5–80%, MeOH) and MeOH afforded nine main fractions, A-I. Fraction B (7.9 g) was subjected to SiO_2-VLC using a $CHCl_3$-MeOH-H_2O gradient system (90:10:1 to 50:50:5 v/v/v) to yield four fractions, B_1–B_4. Fraction B_2 (323 mg) was rechromatographed on silica CC ($CHCl_3$-MeOH-H_2O, 90:10:1 to 80:20:1 v/v/v) to give two fractions, B_{2a} and B_{2b}. Purification of fr. B_{2a} (87 mg) by Sephadex LH-20 CC using MeOH furnished demethoxycentaureidin 7-*O*-β-D-glucopyranoside (3 mg). Fraction B_4 (2.5 g) was subjected to C_{18} medium pressure liquid chromatography (C_{18}-MPLC) employing increasing amount of MeOH in H_2O (0–40%) to afford catalpol (**6**, 46 mg), aucubin (**4**, 114 mg), decaffeoylverbascoside (7 mg) and a crude fraction of deacetylalpinoside. The latter was further applied to a Sephadex LH-20 column using MeOH as eluent to give pure **9** (3 mg). Fraction D (2.950 g) was similarly separated by C_{18}-MPLC using 10 to 60% MeOH in H_2O as eluents to give five fractions, D_1–D_5. Fraction D_2 (449 mg) was rechromatographed on silica CC ($CHCl_3$-MeOH-H_2O, 80:20:1 to 70:30:3 v/v/v) to obtain 10-*O*-benzoylcatalpol (**7**, 11 mg) and an additional fraction D_{2b}. Purification of fr. D_{2b} (200 mg) by C_{18}-MPLC (15–35% MeOH) furnished alpinoside (**8**, 7 mg) and verbascoside (142 mg). Fraction D_3 (281 mg) was applied to a Si gel column eluting with $CHCl_3$-MeOH-H_2O mixture (70:30:3 v/v/v) to give dumuloside (**2**, 28 mg) and additional fraction D_{3b}. Repeated chromatography of fr. D_{3b} (64 mg) on a Sephadex LH-20 column using MeOH as eluent yielded nepetin 7-*O*-β-D-glucopyranoside (4 mg). Fraction D_5 (117 mg) was purified by silica CC using gradient $CHCl_3$-MeOH-H_2O mixtures (85:15:0 to 70:30:3 v/v/v) to obtain 10-*O*-benzoylglobularigenin (**1**, 6 mg) and melampyroside (**5**, 59 mg). Fraction F (4.610 g) was likewise subjected to C_{18}-MPLC using stepwise gradients of MeOH (20–60%) in H_2O to yield five main fractions, F_1-F_5. Repeated chromatography of fraction F_2 (875 mg) using the similar method (C_{18}-MPLC; 20–55% MeOH) gave verbascoside (38 mg) and fractions F_{2b}–F_{2d}. Fraction F_{2c} (158 mg) was rechromatographed over Si gel eluting with $CHCl_3$-MeOH-H_2O (80:20:2 to 70:30:3 v/v/v) to give leucosceptoside A (10 mg) along with impure davisioside. The latter was further applied to a Si gel column employing EtOAc-MeOH-H_2O (100:8:4 v/v/v) mixture to afford pure davisioside (**3**, 29 mg). Fraction H (1.380 g) was also subjected to C_{18}-MPLC using stepwise gradients of MeOH in H_2O (35–75% MeOH) to give melampyroside (**5**, 24 mg) and three main fractions, H_2–H_4. Fraction H_4 (93 mg) was also purified by Sephadex LH-20 CC employing MeOH as eluent to obtain pectolinarigenin 7-*O*-β-D-glucopyranoside (9 mg).

10-*O*-Benzoylglobularigenin (**1**): Amorphous powder; $[\alpha]_D^{20}$ –41° (*c* = 0.1, MeOH); HR-MALDIMS *m/z*: 313.1041 $[M+Na]^+$; UV λ_{max} (MeOH, nm): 207 (sh), 229, 275; IR υ_{max} (KBr, cm^{-1}) 3346 (OH), 1722 (ester C = O), 1457 (aromatic ring); ^{1}H-NMR (300 MHz, CD_3OD): Table I; ^{13}C-NMR (CD_3OD, 75.5 MHz): Table I.

Dumuloside (**2**): Amorphous powder; $[\alpha]_D^{20}$ –70° (*c* = 0.1, MeOH); HR-MALDIMS *m/z*: 473.1416 $[M+Na]^+$; UV λ_{max} (MeOH, nm): 207, 233, 273; IR υ_{max} (KBr, cm^{-1}) 3460 (OH), 1718 (ester C = O), 1654 (C = C-O), 1603, 1559, 1542, 1508 (aromatic ring); ^{1}H-NMR (300 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, $[\alpha]_D$ –41 (*c* 0.1, MeOH). The molecular formula was determined as $C_{16}H_{18}O_5$ by using a combination of positive-ion HR-MALDIMS (*m/z* 313.1041, $[M+Na]^+$) and ^{13}C NMR data (see Table I). The UV spectrum exhibited maxima at 207 (sh), 229 and 275 nm. The IR spectrum showed absorption bands for hydroxyl (3346 cm^{-1}), ester carbonyl (1722 cm^{-1}) and aromatic (1457 cm^{-1}) functionalities. The ^{1}H NMR spectrum (see Table

Table I. The ^{13}C and ^{1}H NMR spectroscopic data and HMBC correlations for **1** (CD_3OD, ^{13}C: 75.5 MHz; ^{1}H: 300 MHz)*.

C/H		δ_C ppm	δ_H ppm, *J* [Hz]	HMBC (H→C)
1	CH	100.0	4.43 d (7.9)	C-3, C-8, C-9
3α	CH_2	63.0	3.63 m	C-1
3β			3.93 m	C-1, C-5
4α	CH_2	25.0	1.83 m	C-3, C-5, C-6
4β			1.76 m	C-3, C-5, C-6
5	CH	48.3	2.37 m	C-1, C-3, C-6, C-9
6	CH	78.2	4.69 br d (7.9)	C-4, C-7, C-8
7	CH	132.5	5.86 br s	C-5, C-8, C-9, C-10
8	C	144.0		
9	CH	50.9	2.58 t (7.5)	C-1, C-5, C-6, C-7, C-8
10	CH_2	64.2	5.01 d (14.8)	C-7, C-8, C-9, C = O
			4.91 d (14.8)	C-7, C-8, C = O
1′	C	131.3		
2′	CH	130.6	8.06 dd (7.4, 1.3)	C = O, C-4′
3′	CH	129.7	7.50 t (7.4)	C-1′, C-5′
4′	CH	134.4	7.63 m	C-2′, C-6′
5′	CH	129.7	7.50 t (7.4)	C-1′, C-3′
6′	CH	130.6	8.06 dd (7.4, 1.3)	C = O, C-4′
C = O	C	167.2		

* All proton and carbon assignments are based on 2D NMR (DQF-COSY, HSQC, HMBC and ROESY).

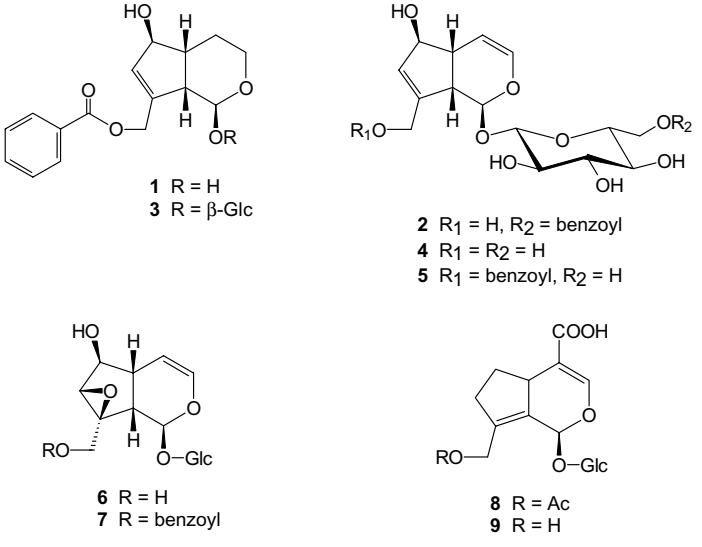

Fig. 1. Iridoids (**1–9**) from *G. dumulosa.*

I) contained signals due to an olefinic proton (δ_H 5.86), an acetal proton (δ_H 4.43), an oxygenated methine proton (δ_H 4.69), two oxymethylenes (δ_H 3.93 and 3.63; δ_H 5.01 and 4.91), two methines (δ_H 2.58, 2.37) and two diastereopic protons of a methylene (δ_H 1.83, 1.76). Additional aromatic proton signals at δ_H 8.06 (2H), 7.63 (1H) and 7.50 (2H) together with the corresponding carbon resonances, supported the presence of a benzoyl moiety. The ^{13}C NMR spectrum of **1** displayed 16 signals, seven of which were ascribed to a benzoic acid. All the remaining carbon resonances indicated that **1** has an iridoid skeleton composed of a cyclopentanopyran ring system with nine carbon atoms. The complete assignments of all proton and carbon resonances were based on DQF-COSY, HSQC and HMBC (see Table I) experiments. Thus, the established NMR data of **1** were similar to those of davisoside (Calis *et al.*, 2002a), which was previously isolated from *G. davisiana*, except the absence of any sugar signal in the NMR spectra of compound **1**. In the HMBC spectrum the expected long-range couplings for the iridoid skeleton were observed. The downfield shifts for

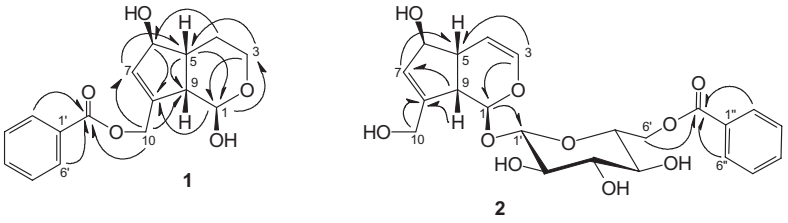

Fig. 2. Selected HMBC Correlations for **1** and **2**.

H_2-10 (δ_H 5.01 and 4.91) signals and the significant ^{1}H-^{13}C HMBC cross-peak between H_2-10 and the carbonyl carbon (δ_C 167.2) of the benzoic acid indicated the site of acylation. To prove the relative stereochemistry of the chiral centers in **1**, a 2D-ROESY experiment was performed. ROe cross-peaks of significant intensity between H-9/H-5 and H-9/H-4β indicated that these protons lie on the same side (β) of the molecule. Contrary, prominent ROe correlations were observed between H-1/H-3α, H-1/H-6 and H-4α/H-6. Therefore, the secondary alcohol functions at C-1 and C-6 had to be in the β position. These correlations also confirmed the *cis* fusion of the cyclopentan and pyran rings as expected. Consequently, the structure of compound **1** was determined as a non-glycosidic iridoid with a saturated $\Delta^{3,4}$. We propose the trivial name 10-*O*-benzoylglobularigenin for this compound.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D$ −70 (*c* 0.1, MeOH). The molecular formula was determined to be $C_{22}H_{26}O_{10}$ by positive-ion HR-MALDIMS (*m/z* 473.1416, $[M+Na]^+$) and ^{13}C NMR data (see Table II). Compound **2** exhibited UV maxima at 207, 233 and 273 nm. The IR spectrum showed absorption bands at 3460 (br OH), 1718 (ester) 1654 (C = C-O) and 1603, 1559, 1542, 1508 cm^{-1} (aromatic ring). Analysis of the ^{1}H NMR spectrum (see Table II) revealed **2** to be an iridoid glycoside with an acyl moiety. The olefinic proton signals at δ_H 5.63 (H-7), 6.30 (H-3), 5.08 (H-4) and oxymethine signal at δ_H 4.25 (H-6) indicated that the structure of the aglycone is like that of aucubin. The anomeric proton resonance at δ_H 4.70 (d, *J* = 7.8 Hz) together with the signals in the region 3.26–4.62 suggested the presence of a β-glucopyranosyl unit. Additional aromatic proton signals at δ_H 8.00 (2H), 7.61 (1H) and 7.48

Table II. The ^{13}C and ^{1}H NMR spectroscopic data and HMBC correlations for **2** (CD_3OD, ^{13}C: 75.5 MHz; ^{1}H: 300 MHz)*.

C/H		δ_C ppm	δ_H ppm, *J* [Hz]	HMBC (H→C)
1	CH	98.4	4.73 d (7.7)	C-1′
3	CH	141.7	6.30 dd (6.1, 1.8)	C-1, C-4, C-5
4	CH	105.6	5.08 dd (6.1, 4.0)	C-3
5	CH	46.7	2.59 m	
6	CH	83.0	4.25†	
7	CH	130.7	5.63 d (1.0)	C-5, C-9
8	C	147.7		
9	CH	47.7	2.84 t (7.7)	C-1, C-7, C-8
10	CH_2	61.5	4.23 d (15.4)	
			4.11 d (15.4)	C-8
1′	CH	100.1	4.70 d (7.8)	C-1
2′	CH	74.8	3.26 dd (7.8, 8.6)	C-3′
3′	CH	77.8	3.39†	C-4′
4′	CH	72.0	3.38†	C-3′
5′	CH	75.6	3.60 m	C-3′, C-4′
6′	CH_2	65.1	4.62 dd (11.8, 2.3)	
			4.47 dd (11.8, 6.8)	C = O
1″	C	131.3		
2″	CH	130.6	8.00 dd (7.5, 1.3)	C = O, C-4″, C-6″
3″	CH	129.6	7.48 t (7.5)	C-1″, C-5″
4″	CH	134.4	7.61 m	C-2″, C-6″
5″	CH	129.6	7.48 t (7.5)	C-1″, C-3″
6″	CH	130.6	8.00 dd (7.5, 1.3)	C = O, C-2″, C-4″
C = O	C	167.8		

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

(2H) and the corresponding carbon resonances were typical of a benzoyl moiety. The complete assignments of all proton and carbon resonances were based on the DQF-COSY, HSQC and HMBC (see Table II) experiments. Thus, compound **2** was established as benzoyl derivative of aucubin. The proton signals assigned to H_2-6′ (δ_H 4.62 and 4.47) of the glucose unit were found to be shifted downfield due to acylation. The HMBC correlations between H-6′ (δ_H 4.47) of the glucose and the carbonyl carbon (δ_C 167.8) of the benzoic acid suggested C-6′ to be site of benzoylation. Consequently, the structure of compound **2** was determined as 6′-*O*-benzoylaucubin. For this new compound we propose the trivial name dumuloside.

Besides these new compounds, seven known iridoid glucosides, davisioside (**3**) (Calis *et al.*, 2002a), aucubin (**4**) (Bianco *et al.*, 1983), melampyroside (**5**) (Chaudhuri and Sticher, 1980), catalpol (**6**) (Chaudhuri and Sticher, 1981), 10-*O*-benzoylcatalpol (**7**) (Foderaro and Stermitz, 1992), alpinoside (**8**) (Jensen *et al.*, 1996), deacetylalpinoside (= arborescosidic acid) (**9**) (Calis *et al.*, 2001; Ronsted *et al.*, 2000), three known phenylethanoid glycosides, verbascoside (Sticher and Lahloub, 1982), decaffeoylverbascoside (Burger *et al.*, 1987), leucosceptoside A (Calis *et al.*, 1988) and three flavone glucosides, pectolinarigenin 7-*O*-β-D-glucopyranoside (Merfort, 1988), nepetin 7-*O*-β-D-glucopyranoside (Agrawal, 1989), demethoxycentaureidin 7-*O*-β-D-glucopyranoside (Yuldashev *et al.*, 1996) were also isolated and identified by comparison of their spectral data with published values.

10-*O*-benzoylglobularigenin (**1**), and davisioside (**3**) represent a rare iridoid skeleton lacking the double bond between C-3 and C-4. 10-*O*-benzoylglobularigenin (**1**) is also the first non-glycosidic iridoid isolated from the genus *Globularia.* Among the isolated compouds, 8,9-unsaturated iridoids like alpinoside (**8**) and deacetylalpinoside (**9**) are very rare. These compounds have only been found in some species of *Veronica* and *Plantago* before. So these compounds may play an important role for the relationships between these three genera.

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.

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