11. Globularifolin, A New Acyl Iridoid Glucoside from *Globularia cordifolia*[1])

by **Ratan K. Chaudhuri** and **Otto Sticher**

Eidgenössische Technische Hochschule, Pharmazeutisches Institut, ETH-Zentrum, CH-8092 Zürich

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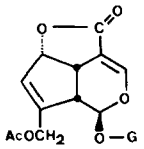
Summary

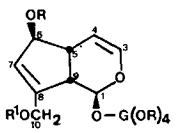
Six iridoid glucosides have been isolated from *Globularia cordifolia* by the combination of open column – and high performance liquid chromatography. The structure of the new compound, named globularifolin, and the identity of the others have been established by chemical transformations and spectral evidence of the compounds and their derivatives.

Introduction. – In connection with our interest on the iridoids of Globularia [1] [2], we investigated the water-soluble constituents of *Globularia cordifolia* L., which is distributed in the central and southern part of Europe. Previous investigations on this species are limited to the identification (thin layer (TLC.) and partition (PC.) chromatography) of asperuloside **1** [3], aucubin **2** [4] and an unknown acyl iridoid [3], which on alkaline hydrolysis afforded aucubin **2** (TLC.). The isolation and characterization of the iridoids of *G. cordifolia* are described in this paper.

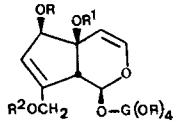
Results and discussion. – From the aerial part of *G. cordifolia*, six iridoid glucosides were isolated in quantities sufficient for their complete characterization. Chemical transformation, derivatization, spectral (UV., IR., ^{1}H- and ^{13}C-NMR.,



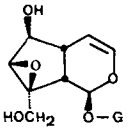
1 Asperuloside



2 Aucubin R = R^1 = H
3 Melampyroside
R = H, R^1 = Benzoyl



4 Monomelittoside
R = R^1 = R^2 = H
6 Globularifolin
R = R^1 = H; R^2 = Benzoyl
7 Globularifolin pentaacetate
R = Ac, R^1 = H, R^2 = Benzoyl



5 Catalpol

G = β-D-Glucose

[1]) Part 3 in the series 'Glycosides of *Globulariaceae*'. For parts 1 and 2 see [1] [2].

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mass) properties and comparison with reference samples, where available, established their identity as aucubin (**2**), melampyroside (**3**), monomelittoside (**4**), catalpol (**5**) and asperuloside (**1**). The structure elucidation of the only new iridoid glucoside, named globularifolin (**6**), is described here.

Globularifolin (**6**) is an amorphous powder, $[\alpha]_D^{20} = -122{,}8°$ ($c = 1.18$, MeOH), with a molecular formula $C_{22}H_{26}O_{11}$. It responds to usual colour reactions for the iridoid-type compounds. On hydrolysis with emulsin, **6** affords glucose and the aglucone, the nature of which is not determined in this study.

Compound **6** shows λ_{max} at 229 and 274 nm in UV. which indicates the presence of a benzoyl chromophore in the molecule. It shows prominent IR. peaks for hydroxyl groups, ester carbonyl, enolic double bond and aromatic ring. Acetylation of **6** at room temperature with acetic anhydride and pyridine provides a pentaacetate **7**, in which one hydroxyl group remained unaffected (IR. and ^{1}H-NMR.). This confirms the presence of six hydroxyl groups in **6**, one of which is tertiary.

The ^{1}H-NMR. (100 MHz, CD_3OD) spectrum of **6** shows signals due to aromatic protons at δ 8.12–7.98 (2 H) and 7.60–7.32 (3 H), three olefinic protons at δ 6.26, ~4.9 and 5.90 and a methylene oxy group at δ 4.94. The first two olefinic signals appear as doublets whereas the latter as triplet (from double doublet) indicating a 5-hydroxylated iridoid in which the cyclopentane ring has a double bond between C(7) and C(8) [5].

The above data quickly led to the supposition that the compound **6** is a benzoyl ester of monomelittoside (**4**) [5]. This has subsequently been confirmed by the alkaline hydrolysis of **6** which afforded benzoic acid and **4**, identical with monomelittoside [5].

Further confirmation of the proposed structure (**6**) (including the site of acylation) for globularifolin is obtained from its ^{13}C-NMR. spectrum which shows, apart from the signals due to the benzoyl part, signals corresponding to 15 carbon atoms and is consistent with an iridoid glucoside structure [6] [7]. Comparison of the ^{13}C-NMR. spectrum of **6** with **4** shows a downfield shift at C(10) and an upfield shift at C(8), thereby locating the benzoyl group at C(10) in **6** [7]. Additionally, a γ-effect (+2.32 ppm) of the acyl substituent at C(10) exerted on C(7) in **6** is also observed. Comparison of this data with that of the related iridoids [7], *i.e.* having a C(7), C(8) – double bond, also show this γ-effect. This could be rationalized on the basis of the C(7),C(8)-bond polarization toward C(8) by the acyl group at C(10). A strong upfield shift (γ-effect, ~4 ppm), is brought about by HO–C(5) group into C(1) on going from **2** to **3** or **4** to **6**. This effect, however, is not observed in the case of iridoids having a C(7),C(8)-epoxy ring or a saturated cyclopentane ring [7].

This is the first demonstration of the occurrence of an acyl derivative of monomelittoside, despite the large abundance of **4** in nature [8]. The iridoid constituents of the two *Globularia* species investigated so far in our laboratory have some distinctive chemical characteristics worthy of mention. All the acylated iridoids of *G. alypum* [1] [2] are catalpol (**5**) or catapol derivatives (corresponding *trans*-diols) and the acyl part is cinnamic acid, whereas in *G. cordifolia,* these are aucubin (**2**) or aucubin derived iridoids (*e.g.* **4**) acylated with benzoic acid. It is

striking to note that the site of acylation in all the iridoids of *Globularia* is at C(10). Asperuloside (**1**) is completely absent in *G. alypum*. These observations are certainly of considerable biogenetic and chemotaxonomic interest [8] and warrant further detailed investigation of the other *Globularia* species.

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Experimental Part

General. Melting points were determined on a *Mettler* FP5 apparatus. UV. spectra [λ_{max} ($\log\varepsilon$)] were determined in spectral MeOH *(Merck)* on a *Perkin-Elmer* 550 spectrometer. IR. spectra (cm^{-1}) were determined on a *Perkin-Elmer* 257 instrument in KBr pellets or in CCl_4. ^1H- and ^{13}C-NMR. spectra (δ ppm, J Hz) were obtained at 100 MHz using a *Varian* HA-100 spectrometer and at 25.2 MHz in *Fourier* transform mode using a *Varian* XL-100-12 spectrometer, respectively, using tetramethyl silane as an internal standard. (MS. (*m/z*) were recorded with a *Hitachi-Perkin-Elmer* RMU 6M spectrometer. Silica gel 60 (70–230 mesh, *Merck*) and neutral alumina (*Woelm* N, Act. I) were used for column chromatography. Silica gel 60 F_{254} *(Merck)* prepared plates were used for TLC. Spots were detected by UV. fluorescence and/or spraying with vanillin-H_2SO_4 followed by heating at 100° for 5–10 min. A *Waters* Assoc. HPLC. model ALC 201 was used throughout. A *Waters* Assoc. M-6000 pump was used as the solvent delivery system and U6K septumless injector. The system was equipped with a *Perkin-Elmer* spectrophotometer (LC 55) with a variable wave length detector and a *Waters* LC-25 microcell. For the analytical and the semi-preparative HPLC. work μ-Bondpak C_{18} columns (30 cm × 4 mm, ID and 30 cm × 8 mm ID), respectively, were used. For the preparative work a *Waters*-Prep LC/500 System equipped with a reversed phase column, and different proportion of MeOH/H_2O was used. Abbreviations: LC. = liquid chromatography, RT. = room temperature.

Extraction and purification. Fresh above ground[2]) parts of *Globularia cordifolia* (1.5 kg) collected from the Walensee area, Switzerland, in May 1978, were cut into small pieces and extracted with petroleum ether (3 × 3 l) and then with MeOH (3 × 3 l) at 40°. The MeOH extract was concentrated to dryness and the residue was taken up in H_2O. The water-soluble portion was passed through a pre-washed (H_2O) column of neutral Al_2O_3 (500 g), eluting with H_2O. The aqueous eluate was concentrated and lyophilized when the crude iridoid (82.1 g) was obtained. A portion of the mixture (30 g) was chromatographed over silica gel (400 g, 65 × 4.3 cm), eluting with CH_2Cl_2/MeOH/H_2O [80:20:2 (3 l), 70:30:3 (2 l), 60:40:4 (2 l)] and six fractions, **A** (1.6 g), **B** (3.1 g), **C** (4.2 g), **D** (1.4 g), **E** (2.1 g) and **F** (9.1 g) were collected.

Asperuloside (**1**) and *Melampyroside* (**3**). Fr. **A** (1 g), which was subjected to preparative LC. [MeOH/H_2O 45:5; flow rate 100 ml/min], gave **1** (40 mg), m.p. 129–131°, $[\alpha]_D^{20} = -184.1°$ ($c = 0.71$, MeOH) and **3** (352 mg), m.p. 107–110°, $[\alpha]_D^{20} = -83.9°$ ($c = 0.56$, MeOH). The identity of **1** and **3** was established by direct comparison (TLC., HPLC., ^1H- and ^{13}C-NMR.) with authentic asperuloside [5] and melampyroside [9] [10], respectively. Melampyroside (**3**) was also present (HPLC.) in Fr. **B** as a major constituent.

Globularifolin (**6**). A part (2 g) of Fr. **C** on prep. LC. (MeOH/H_2O 45:55; flow rate 100 ml/min) gave pure **6** (1.2 g) $[\alpha]_D^{20} = -122.8$ ($c = 1.18$, MeOH). – UV. (CH_3OH): 229 (4.1) and 274 (2.9). – IR. (KBr): ~3340 (br. OH), 1720 (ester carbonyl), 1655 (C=C), 1602, 1585 and 1454 (aromatic ring). – ^1H-NMR. (CD_3OD): 8.12–7.98 (2 ar. H); 7.60–7.32 (3 ar. H); 6.26 (*d*, $J = 6$, 1H, H–C(3)); 5.90 (*t* × *d* × *d*, $J = 1.6$ and 1.2, 1H, H–C(4)); 5.76 (*d*, $J = 3.8$, 1H, H–C(1)); 4.98 (br. *s*, 2H, 2H–C(10)); ~4.9 (*d*, $J = 6$, 1H, H–C(4)); 4.58 (*d*, $J = 7.4$, 1H, H–C(1′)); 4.16 (br. *s*, 1H, H–C(6)); ~3.18 (partly merged inside the solvent signal, H–C(9)).

Globularifolin pentaacetate (**7**). Acetylation of **6** (100 mg) was effected with acetic anhydride and pyridine at RT. Usual processing gave an amorphus powder. $[\alpha]_D^{20} = -138.8$ ($c = 0.67$, $CHCl_3$). – IR. (CCl_4): ~3500 (OH, br.), ~1735 (ester carbonyl, br.). – ^1H-NMR. ($CDCl_3$): 8.1–7.95 (2 ar. H); 7.56–7.36 (3 ar. H); 6.18 (*d*, $J = 6.2$, 1H, H–C(3)); 5.90 (unresolved *t* × *d* × *d*, 1H, H–C(7)); 5.70 (*d*, $J = 2$, 1H, H–C(1)); 5.13 (*d*, $J = 6.2$, 1H, H–C(4)); 4.88 (br. *s*, 2H, 2H–C(10)); 4.72 (*d*, $J = 8$, 1H, H–C(1′)); 3.44 (br. *s*, 1H, H–C(9)); 3.01 (*s*, 1H, HO–C(8)); 2.08–1.95 (5 $OCOCH_3$). – MS.: 676 (M^+, no peak), fragment ion peaks at 494 (2, $M^+ - (105 + 59 + 18)$), 345 (3, $M^+ - 331$); due to the

[2]) Slightly contaminated with roots.

Table. ^{13}C-*NMR. spectral data of iridoids*

C-Atoms	**2**	**3**	**4**	**6**
1	97.22	97.83	93.59	93.66
3	141.49	141.64	142.38	142.28
4	105.72	105.51	108.40	108.25
5	46.07	46.04	72.83	72.64
6	82.74	82.66	80.45	80.20
7	130.27	132.59	127.67	130.47
8	147.95	142.41	148.29	142.57
9	47.89	48.39	53.63	54.06
10	61.32	63.98	60.84	63.23
1′	99.90	100.08	99.40	99.49
2′	74.81	74.72	74.36	74.15
3′	78.07[a]	77.99[a]	78.19[a]	77.80[a]
4′	71.44	71.27	71.57	71.30
5′	77.77[a]	77.72[a]	77.35[a]	77.15[a]
6′	62.60	62.61	62.64	62.57
1″	–	131.02	–	130.76
2″	–	130.54[b]	–	130.47[b]
3″	–	129.59[b]	–	129.47[b]
4″	–	134.39	–	134.25
5″	–	129.59[b]	–	129.47[b]
6″	–	130.54[b]	–	130.47[b]
CO	–	167.71	–	167.24

Values with same superscript in the vertical column are interchangeable.

glucose part at 331 (48), 289, 271 (7), 229, 211, 187, 169 (100), 127 (23), 109 (98) and due to the benzoyl part at 105 (98) and 77 (31).

Hydrolysis of Globularifolin (**6**). A solution of 125 mg of **6** in 20 ml of methanolic 0.1N NaOH was kept overnight, then neutralized with 0.1N HCl. The solvent was evaporated, and the residue on chromatography over silica gel using CH_2Cl_2/MeOH/H_2O 70:30:3 gave benzoic acid (TLC., UV.) and monomelittoside (**4**) (87 mg), $[\alpha]_D^{20} = -169.7$ ($c = 0.83$, MeOH). – ^{1}H-NMR. [5] and ^{13}C-NMR. *(Table)*.

Catalpol (**5**) and *Aucubin* (**2**) were identified in Fr. **D** by analytical HPLC. [11] and aucubin (**2**) was isolated (610 mg) by prep. LC. [11] from a part of Fr. **E** (1 g). The compound was identical with an authentic specimen.

Monomelittoside (**4**). A part (1 g) of Fr. **F** on prep. LC. [MeOH/H_2O 10:90; 100 ml/min] gave **4** (68 mg), $[\alpha]_D^{20} = -169.7°$ ($c = 0.83$, MeOH). – ^{1}H-NMR. [5] and ^{13}C-NMR. *(Table)*.

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