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ACYLATED 6-HYDROXYLUTEOLIN DIGLUCOSIDES FROM *GLOBULARIA ELONGATA*

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Key Word Index—*Globularia elongata*; Globulariaceae; 6-hydroxyluteolin 7-sophoroside; 6-hydroxyluteolin 7-6‴-caffeoylsophoroside; 6-hydroxyluteolin 7-6‴-*p*-coumaroylsophoroside; ^{1}H NMR; ^{13}C NMR', FDMS.

Abstract—Three flavonoid aglycones and three glycosides have been isolated from the aerial parts of *Globularia elongata*. The aglycones were identified as apigenin, luteolin and 6-hydroxyluteolin. Structural elucidation of the glycosides employing spectroscopic methods (UV, IR, ^{1}H NMR, ^{13}C NMR, FDMS) revealed 6-hydroxyluteolin 7-sophoroside and two new acylated derivatives, 6-hydroxyluteolin 7-6‴-caffeoylsophoroside and its *p*-coumaroyl analogue.

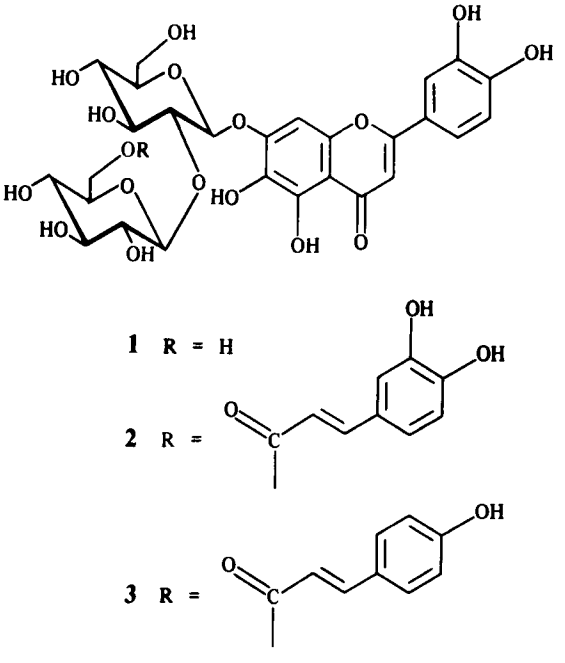
INTRODUCTION

Globularia elongata Hegetschw. et Heer is a perennial herb growing in the mountainous regions of Middle Europe [1, 2]. Previously, the iridoids: monotropein, asperulin, aucubin and catalpol were reported in the leaves of this plant [3]. Glycosides of Globulariaceae have been recently studied by Sticher and co-workers [4–8], but flavonoids have not been investigated. However, in a flavonoid survey of the Tubiflore, Harborne and Williams [9] found 6-hydroxyluteolin in all the *Globularia* species they examined. In a more detailed study of *G. cordifolia* mono- and diglucosides and rhamnoglucosides of 6-hydroxyluteolin and scutellarein and also their methyl ethers were isolated [9]. In this paper the isolation and structural elucidation of the flavonoids from *G. elongata* are described. Two of them (**2** and **3**) appear to be new acylated diglycosides.

RESULTS AND DISCUSSION

From the ether extract of the aerial parts of *G. elongata* three flavones were isolated and identified as apigenin, luteolin and 6-hydroxyluteolin by direct comparison with authentic samples (mp, PC, TLC, UV). The ethyl acetate/butanolic fraction yielded glycosides **1**, **2** and **3**, which were examined by chemical and spectral methods.

Total acid hydrolysis of **1**–**3** all afforded 6-hydroxyluteolin, (coPC, UV, ^{1}H NMR) [9, 10] and glucose (coPC, TLC). UV data of **1**, particularly the position of band II (284 nm), the same for the glycoside and its aglycone, as well as the NMR data (Tables 1, 2) excluded the possibility of 8-hydroxyluteolin being the aglycone (Wessely–Moser rearrangement). The position of attachment of sugars to the aglycone was also determined by UV analysis. λ_{max} at 284 nm (in methanol) is typical of the flavones with a free 6-hydroxyl group [9–11]. The detection of glucosylation at C-7 in the case of 6-oxygenated flavones by the effect of NaOAc on the UV spectrum is known to be unreliable. The spectra recorded with other



shift reagents showed that the 3'-,4'- and 5-hydroxyl groups were free. Thus, the position of glucosylation must be at the 7-hydroxyl. Mild acid hydrolysis liberated 6-hydroxyluteolin 7-glucoside from **1**–**3** as the only monoglucoside. This indicated an additional substituent in **2** and **3** attached to the terminal glucose unit. FDMS of **1** showed a quasimolecular ion at *m/z* 627 $[M+H]^+$ corresponding to the molecular formula $C_{27}H_{30}O_{17}$. Interglycosidic linkage 1 → 2 was established on the basis of ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR of the tetramethylsilyl ether of **1** (Table 1) exhibited the signals of two anomeric glucose protons at 4.68 and 5.56 ppm [11]. In the ^{13}C NMR spectrum (Table 2) the C-2″ signal (inner glucose) was shifted downfield by 7.2 and C-1″ upfield by

Table 1. ^{1}H NMR data of flavonoids **1**–**3** (δ ppm)*

Compound	Sugar								Hydroxycinnamoyl					
	H-2′ H-6′	H-5′	H-8	H-3	H-$1''$	H$_1'''$	H$_2''$ – H$_5''$	2H$_6'''$	H-2	H-3	H-5	H-6	H(α)	H(β)
1	7,37 m	6,85 *d* (9,0)	6,82 *s*	6,33 *s*	5,56 *d* (6,0)	4,68 *d* (7,5)	3,05 –4,03		—	—	—	—	—	—
2	7,45 m	6,99 *d* (9,0)	6,85 *s*	6,32 *s*	5,75 *d* (6,0)	4,80 *d* (7,5)	3,30 – 4,00	4,03 – 4,58	[6,76 –		6,97	(3H)]	6,15 *d* (16,0)	7,46 *d* (16,0)
3	7,45 m	6,90 *d* (9,0)	6,87 *s*	6,33 *s*	5,84 *d* (6,0)	4,75 *d* (7,5)	3,30 – 4,00	4,02 – 4,50	7,20 *d* (9,0)	6,75 *d* (9,0)	6,75 *d* (9,0)	7,20 *d* (9,0)	6,10 *d* (16,0)	7,50 *d* (16,0)

*Numbers in parentheses denote coupling constants in Hz.

Table 2. ^{13}C NMR data of falvonoids **1**–**3** (δ ppm)

Carbon number	Flavonoid skeleton			Carbon number	Sugar			Carbon number	Hydroxycinnamoyl	
	1	**2**	**3**		**1**	**2**	**3**		**2**	**3**
2	164,3	164,3	164,3	1″	100,2	100,2	100,3	1	125,6	125,0
3	103,7	103,9	104,0	2″	81,4	81,8	81,8	2	114,7	129,8
4	182,1	182,1	182,1	3″	76,0	76,0	76,1	3	145,3	115,6
5	146,4	146,4	146,4	4″	69,9	69,8	69,8	4	148,0	159,5
6	130,6	131,0	131,0	5″	76,2	76,1	76,1	5	115,9	115,6
7	150,9	150,7	150,7	6″	60,6	60,7	60,7	6	120,9	129,8
8	94,4	95,0	95,2	1‴	102,5	102,5	102,5	β	144,7	144,3
9	149,0	148,9	148,9	2‴	74,2	74,2	74,2	α	113,9	113,9
10	106,3	106,3	106,3	3‴	75,5	75,5	75,5	C = 0	166,2	166,2
1′	121,7	121,7	121,7	4‴	69,4	69,4	69,5			
2′	113,4	113,4	113,4	5‴	76,6	73,9	73,9			
3′	145,5	145,4	145,5	6‴	60,6	62,7	62,7			
4′	149,5	149,5	149,5							
5′	115,9	116,0	116,0							
6′	118,8	118,9	118,9							

2.3 ppm compared with the chemical shifts for the corresponding carbon atoms of the terminal glucose [12]. Thus the structure of **1** is confirmed as 6-hydroxyluteolin 7-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranoside.

Compounds **2** and **3** showed an infrared spectrum absorption band at 1685 cm^{-1} indicating the ester group conjugated with the double bonds [13, 14]. In the UV spectrum of **2** and **3** band I appeared at a shorter and band II at a longer wavelength compared with the UV spectrum of **1**. Mild alkaline hydrolysis of **2** and **3** gave caffeic acid and *p*-coumaric acid, respectively. Deacylated products have not been obtained probably due to the high alkali sensitivity of 6-hydroxyluteolin [15]. The mass spectrum (FDMS) of **2** exhibited a signal at *m/z* 789 $[M + H]^+$ (base peak) corresponding to the molecular formula $C_{36}H_{36}O_{20}$. Furthermore, structurally significant signals were recorded at *m/z* 510 [caffeoylsophorose + Na]$^+$ and *m/z* 302 [M – 162 – 324] due to the loss of the acylated diglucosyl moiety. FDMS of **3** showed quasimolecular ions at *m/z* 773 $[M + H]^+$, 795 $[M + Na]^+$ and 812 $[M + K]^+$ corresponding to the molecular formula $C_{36}H_{36}O_{19}$. Fragment ions in that case were not obtained. In the ^{1}H NMR spectra of **2** and **3** (Table 1) the signals of eight and nine aromatic protons were observed, respectively, in addition to two characteristic doublets of the olefinic protons (J_{trans} = 16 Hz) for a hydroxycinnamoyl residue. The signals of sugar protons were placed similarly as that in the spectrum of **1**, apart from the C-6‴ two protons, which were shifted downfield to the range 4.02–4.58 ppm. This suggested that the acyl group in **2** and **3** is at the C-6‴ hydroxyl of the glucose molecule [16].

The proposed structures of **2** and **3** were also confirmed by ^{13}C NMR. In comparison with the spectrum of **1** the signal for acylated oxymethine carbon C-6‴ in **2** and **3** was shifted downfield by 2.1 ppm, whereas the adjacent carbon signal (C-5‴) was shifted upfield by 2.7 ppm. Chemical shifts of the remaining carbon atoms of the diglucosyl moiety and flavonoid skeleton were almost identical for **1**, **2** and **3**. The assignment of these signals was aided by the published data [17–19]. Additionally in the ^{13}C NMR spectra of **2** and **3** the signals of nine carbon atoms of a phenylpropanoid residue were observed. Chemical shifts of these signals agreed with the literature data, respectively for caffeic [20] and *p*-coumaric acids [21]. Hence **2** is 6-hydroxyluteolin 7-*O*-β-D-(6‴-caffeoyl)glucopyranosyl (1 → 2)-β-D-glucopyranoside and **3** is 6-hydroxyluteolin 7-*O*-

β-D-(6‴-*p*-coumaroyl)glucopyranosyl(1 → 2)-β-D-glucopyranoside.

6-hydroxyluteolin 7-diglucoside has been earlier reported in ref. [9] from *G. cordifolia*, but interglycosidic linkage was not defined. Recently two 6-hydroxyluteolin diglucosides have been described: the 7-gentiobioside (mp 215–218°) from *Lomatogonium corinthiacum* [22] and the 7-sophoroside (mp 210–214°) from *Frullania jackii* [23]. Since melting point of compound **1** is very different from that of the diglucoside from *F. jackii* and the authors did not report NMR data, the identity of both compounds cannot be ascertained.

Compounds **2** and **3** are described for the first time. Acylated flavonoid glycosides have not been reported previously from the Globulariaceae. However, an acylated glycoside of an 8-hydroxyflavone was previously described by Chari *et al.* [19] from *Veronica* species, a member of Scrophulariaceae which is a related family.

EXPERIMENTAL

Plant material. G. elongata was cultivated in the Garden of Medicinal Plants in Łódź, Poland and originated from the seeds of the Botanical Garden in Würzburg. Aerial parts of the plant were collected in June 1981. A voucher specimen is deposited in the Herbarium of the Department of Pharmacognosy Medical Academy of Łódź, Poland.

General techniques. PC was carried out on the Whatman No 1 paper with BAW (system 1), EtOAc–HCOOH–H_2O (10:2:3) (system 2), HOAc–H_2O–conc HCl (15:82:3) (system 3), HOAc–H_2O–conc HCl (30:10:3) (system 4), HOAc 50% (system 5); TLC on DC-Alufolien Kieselgel 60 (Merck) with EtOH–25% NH_4OH–H_2O (20:1:4) (system 6) and $CHCl_3$–MeOH–HOAc (9:1:0.1) (system 7). Column chromatography was achieved on MN-Polyamid SC6 (grain size 0.05–0.16 mm) with H_2O–MeOH in different concentrations and on cellulose 123 (Schleicher–Schüll) using $CHCl_3$–MeOH–H_2O (16:9:2). UV spectra with the usual shift reagents were made according to standard procedures [15] IR in KBr. ^{1}H NMR spectra of TMSi ethers of compounds **1–3** were recorded in CCl_4 and ^{13}C NMR of underivatized glycosides in DMSO-d_6 both with TMS as int. standard. FDMS spectra were produced on a Varian MAT 711 spectrometer, emitter current 20–22 mA. Peracetates of **1**, **2** and **3** were prepared in the usual manner (Ac_2O/pyridine 24 hr, room temp.).

Isolation and identification of flavonoids. Dried aerial parts (leaves, stalks and flowers) of *G. elongata* (650 g) were extracted successively with petrol, $CHCl_3$, MeOH and 80% MeOH. The combined MeOH and aq. MeOH extracts were evap to 500 ml. The aq. conc. was successively extracted with Et_2O, EtOAc and *n*-BuOH. From the Et_2O extract by chromatography on a polyamide column, using MeOH 70% as a solvent, apigenin (12 mg), luteolin (8 mg) and 6-hydroxyluteolin (3 mg) were obtained and identified by mp, PC (systems 1, 4, 5), TLC (system 7) and UV data [15] compared with the authentic samples. Combined EtOAc and *n*-BuOH extracts (84.0 g) were submitted to CC on polyamide (500 g) to obtain fractions containing **1** (eluent MeOH 20–40%), which were submitted to repeated CC on polyamide. After cryst. from 80% MeOH 120 mg of **1** was obtained. Fractions eluted with MeOH 50–80% yielded a crystalline mixture of **2** and **3** (1.47 g), which was submitted to CC on celulose (600 g) to afford **2** (350 mg) and **3** (190 mg).

6-Hydroxyluteolin 7-sophoroside (**1**). Pale yellow needles from 80% MeOH, mp 299–302°, R_f 0.10 (system 1), 0.23 (system 2), 0.11 (system 3), 0.40 (system 5). UV λ_{max} nm: 255, 284, 346 (MeOH); 262, 392 (NaOMe); 275, 303, 345 sh, 428 ($AlCl_3$); 260, 297, 373 ($AlCl_3$/HCl); 283,354,400 sh (NaOAc); 262,285,360 (NaOAc/H_3BO_3). ^{1}H and ^{13}C NMR data see Tables 1, 2. IR ν cm^{-1}: 3420 (*br*, OH), 1676 (C=O, γ-pyron), 1622, 1578, 1570, 1527, 1510 (–C=C–).

6-Hydroxyluteolin 7-sophoroside peracetate (**1a**). Colourless needles from EtOH 96°, mp 125–127°; ^{1}H NMR ($CDCl_3$) δ ppm: 2.25–2.50 (12 H, OCOMe arom. × 4), 1.90–2.18 (21 H, OCOMe aliph. × 7).

6-Hydroxyluteolin 7-O-(6‴-caffeoyl-)sophoroside (**2**). Pale yellow needles from 80% MeOH, mp 232–234°. R_f 0.16 (system 1), 0.36 (system 2), 0.07 (system 3), 0.37 (system 5), UV λ_{max} nm: 255 sh, 287, 337 (MeOH); 262, 308, 385 (NaOMe); 270, 304, 352, 420 ($AlCl_3$) 240, 298, 362 ($AlCl_3$/HCl); 255 sh, 287, 348 (NaOAc); 259, 289, 352 (NaOAc/H_3BO_3). ^{1}H and ^{13}C NMR data see Tables 1, 2. IR ν cm^{-1}: 3380 (*br*, OH), 1685 (C=O ester), 1655 (C=O γ-pyron), 1625, 1570, 1535, 1523 (–C=C–).

6-Hydroxyluteolin 7-O-(6‴-caffeoyl-)sophoroside peracetate (**2a**). Colourless crystals from 70% MeOH mp 132–135°. ^{1}H NMR ($CDCl_3$) δ ppm: 7.52 (*d*, 16 Hz, H_β); 6.25 (*d*, 16 Hz, H_α), 2.22–2.48 18 H, OCOMe arom. × 6), 1.85–2.20 (18 H OCOMe aliph. × 6).

6-Hydroxyluteolin 7-O-(6‴-p-coumaroyl)sophoroside (**3**). Pale yellow needles from 80% MeOH, mp 220–223° R_f 0.22 (system 1), 0.41 (system 2) 0.12 (system 3), 0.44 (system 5). UV (λ_{max} nm): 255 sh, 291, 320, 345 sh (MeOH); 268, 312 sh, 382 (NaOMe), 274 sh, 303, 422 ($AlCl_3$); 265 sh, 300, 374 ($AlCl_3$/HCl); 292, 319,358 sh (NaOAc); 267 sh, 292, 319, 356 (NaOAc/H_3BO_3). ^{1}H and ^{13}C NMR data see Tables 1,2. IR ν cm^{-1}: 3350 (*br*, OH), 1685 (C=O ester), 1650 (C=O, γ-pyron), 1625, 1600, 1570, 1510, 1495 (–C=C–).

6-Hydroxyluteolin 7-O-(6‴-p-coumaroyl)sophoroside peracetate (**3a**). White amorph powder. ^{1}H NMR ($CDCl_3$) δppm: 7.43 (*d*, 16 Hz,H_β), 6.20 (*d*, 16 Hz, H_α), 2.15–2.50 (15 H, OCOMe arom. × 5), 1.80–2.13 (18 H, OCOMe aliph. × 6).

Acid hydrolysis. (i) Complete acid hydrolysis of **1–3**. 10 mg of the glycoside was refluxed with H_2SO_4 10% for 3 hr. Insoluble ppt. (aglycone) was filtered and recryst. from MeOH–H_2O to give 6-hydroxyluteolin. The filtrate was extracted with Et_2O and aq. layer neutralized with Amberlit (OH^-). In the Et_2O extract from the hydrolysate of **2** and **3** caffeic acid and *p*-coumaric acid, respectively, were identified by coPC and TLC (systems 1, 4, 7). The aq. layer gave glucose as the only sugar, R_f 0.18 (system 1), 0.38 (system 6). (ii) Partial acid hydrolysis of **1–3** by 1 N TFA (trifluoracetic acid). The glycoside (5 mg) was refluxed with 1 N TFA (2 ml) for 15 min. The mixture was evapd. *in vacuo*, solid residue was resolved in 50% MeOH and submitted to PC (system 1) and TLC (system 6). Each hydrolysate showed glucose and sophorose identified by comparison with authentic samples. (iii) Partial acid hydrolysis of **1–3** by 0.1% HCl. The glycoside (5 mg) was resolved in MeOH (2 ml) and 0.2% HCl (2 ml) was added. The mixture was refluxed for 1 hr, cooled and extracted with Et_2O followed by EtOAc. In the Et_2O extract of **2** and **3** caffeic and *p*-coumaric acid were identified, respectively as well as 6-hydroxyluteolin (all in traces). The EtOAc extracts showed (PC, TLC) unhydrolysable glycosides, a hydroxycinnamoyl derivatives of glucose (not examined) and 6-hydroxyluteolin 7-*O*-glucoside, which was obtained by prep. PC and identified comparing Rf values and UV spectra with an authentic sample isolated from *Inula britannica* [24].

Alkaline hydrolysis of **2** *and* **3**. A soln. of the glycoside (5 mg) in KOH 0.5% (5 ml) was allowed to stand at room temp. for 20 min. The reaction mixture was acidified with dil. HCl and extracted with Et_2O. The Et_2O extract was washed with H_2O, evapd. to dryness, resolved in MeOH (0.5 ml) and submitted to PC and

TLC (system 1, 4, 5, 7) when caffeic and *p*-coumaric acid, respectively, were identified.

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