Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 64 (2003) 1295–1301
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Acylated flavone glycosides from *Veronica*

Dirk C. Albach[a], Renée J. Grayer[b], Søren Rosendal Jensen[c], Fevzi Özgökce[d], Nigel C. Veitch[b,*]

[a]*Department of Higher Plant Systematics, Institute of Botany, University of Vienna, Austria*
[b]*Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK*
[c]*Department of Chemistry, The Technical University of Denmark, DK-2800 Lyngby, Denmark*
[d]*Department of Biology, Faculty of Science and Arts, Yüzüncü Yil University, 65080 Van, Turkey*

Received 11 June 2003; received in revised form 11 August 2003

Abstract

A survey of the flavonoid glycosides of selected taxa in the genus *Veronica* yielded two new acylated 5,6,7,3′,4′-pentahydroxyflavone (6-hydroxyluteolin) glycosides and two unusual allose-containing acylated 5,7,8,4′-tetrahydroxyflavone (isoscutellarein) glycosides. The new compounds were isolated from *V. liwanensis* and *V. longifolia* and identified using NMR spectroscopy as 6-hydroxyluteolin 4′-methyl ether 7-*O*-α-rhamnopyranosyl(1‴→2″)[6″-*O*-acetyl-β-glucopyranoside] and 6-hydroxyluteolin 7-*O*-(6″-*O*-(*E*)-caffeoyl)-β-glucopyranoside, respectively. Isoscutellarein 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside was obtained from both *V. intercedens* and *V. orientalis* and its 4′-methyl ether from *V. orientalis* only. Complete ^{1}H and ^{13}C NMR spectral assignments are presented for both isoscutellarein glycosides. Two iridoid glucosides new to the genus *Veronica* (melittoside and globularifolin) were also isolated from *V. intercedens*.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: *Veronica liwanensis*; *V. longifolia*; *V. intercedens*; *V. orientalis*; Scrophulariaceae; Plantaginaceae; Flavonoids; Acylated flavone glycosides; Iridoids

1. Introduction

Veronica L. (family Scrophulariaceae; Plantaginaceae *sensu* Angiosperm Phylogeny Group, 1998) is a large and almost cosmopolitan genus of annual and perennial herbs with its main centres of diversity located in Central and Southern Europe and Turkey. The phytochemistry of the genus has been studied extensively with many species surveyed for their iridoid (Grayer-Barkmeijer, 1973, 1979; Taskova et al., 2002a) and flavonoid constituents (Grayer-Barkmeijer, 1978, 1979; Tomás-Barberán et al., 1988). The flavonoids are mainly flavone glycosides, often with additional hydroxyl substitution at C-6 or C-8 of the A-ring. Acylation of the sugars is another characteristic feature of some of the glycosides (Grayer-Barkmeijer, 1979; Chari et al., 1981). A recent systematic study of *Veronica* based on sequence data from the internal transcribed spacer region of nuclear ribosomal DNA has renewed interest in the taxonomy of this genus (Albach and Chase, 2001) and prompted further investigation into the phytochemistry of some key taxa. During the course of the latter work, two new and two known acylated flavone glycosides were found together with some known iridoid glucosides. The new compounds, 6-hydroxyluteolin 4′-methyl ether 7-*O*-α-rhamnopyranosyl(1‴→2″)[6″-*O*-acetyl-β-glucopyranoside] and 6-hydroxyluteolin 7-*O*-(6″-*O*-(*E*)-caffeoyl)-β-glucopyranoside were obtained from *Veronica liwanensis* C. Koch and *V. longifolia* L., respectively. The known compound, isoscutellarein 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside, was obtained from both *V. intercedens* Bornm. and *V. orientalis* Wender. The latter species also yielded the corresponding 4′-methyl ether. This paper describes the isolation and spectroscopic characterisation of these unusual flavone glycosides and their potential value as chemosystematic markers within the genus. The first occurrence of 5-oxygenated iridoid glucosides in the genus *Veronica* is also reported.

* Corresponding author. Tel.: +44-20-8332-5312; fax: +44-20-8332-5310.
E-mail address: n.veitch@kew.org (N.C. Veitch).

0031-9422/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2003.08.012

2. Results and discussion

Column chromatography of an ethanol extract of leaves of *V. liwanensis* yielded **1** as a yellow solid. The UV spectrum of this compound was typical of that of a luteolin (5,7,3′,4′-tetrahydroxyflavone) derivative and the presence of a short wavelength band at 283 nm indicated that the C-6 position might be hydroxylated (Harborne and Williams, 1971). These preliminary observations were confirmed by extensive analysis of both one- and two-dimensional ^{1}H and ^{13}C NMR spectra. The aromatic region of the ^{1}H NMR spectrum of **1** contained a characteristic resonance for H-3 of a flavone at δ_H 6.76 (1H, *s*, δ_C 103.1 by HSQC), an assignment confirmed by long-range connectivities to δ_C 182.1 (C-4), 163.8 (C-2), 123.1 (C-1′) and 105.5 (C-10) in the HMBC spectrum. Likewise a second singlet resonance in the aromatic region at δ_H 6.94 (1H, δ_C 93.8 by HSQC) was assigned to H-8 on the basis of long-range connectivities to δ_C 151.2 (C-7), 149.0 (C-9), 130.7 (C-6) and 105.5 (C-10). Three spin–spin coupled multiplets at δ 7.44 (1H, *d*, $J=2.3$ Hz, H-2′), 7.11 (1H, *d*, $J=8.7$ Hz, H-5′) and 7.54 (1H, *dd*, $J=8.6$, 2.3 Hz, H-6′) comprised the remaining aromatic resonances and were characteristic of a 3′,4′-*O*,*O*-disubstituted B-ring of a flavonoid. A ROE connectivity detected between the protons of the single methoxyl resonance at δ_H 3.88 (3H, *s*, δ_C 55.7) and H-5′ of the B-ring indicated the presence of a methyl ether at C-4′. This was confirmed by the long-range connectivities observed in the HMBC spectrum from δ_H 3.88 (4′-OCH_3), 7.44 (H-2′) and 7.54 (H-6′) to δ_C 151.1 (C-4′). A downfield-shifted exchangeable resonance at δ 12.69 (1H, *s*) showing long-range connectivities to δ_C 146.8 (C-5), 130.7 (C-6) and 105.5 (C-10) was assigned to the 5-OH proton. Thus the aglycone component of **1** was confirmed to be 6-hydroxyluteolin 4′-methyl ether (5,6,7,3′-tetrahydroxy-4′-methoxyflavone). The remaining resonances in the ^{1}H NMR spectrum of **1** were those of two sugars with anomeric protons at δ 5.36 (1H, *d*, $J=7.6$ Hz) and 5.17 (1H, *d*, $J=1.4$ Hz), an acetyl group at δ 1.94 (3H, *s*) and a methyl group at δ 1.09 (3H, *d*, $J=6.1$ Hz). The ^{1}H resonances of each sugar were assigned from the COSY spectrum using the anomeric proton resonances as starting points. In this case the resonances of the sugar protons were sufficiently dispersed for the complete spin–spin coupling pattern to be traced sequentially for both sugars. The corresponding ^{13}C resonances were obtained from the HSQC spectrum. These chemical shift and coupling constant data (Table 1) indicated that the sugars represented by the anomeric protons at δ 5.36 and 5.17 were β-glucopyranose and α-rhamnopyranose, respectively (Markham et al., 1982; Markham and Geiger, 1994). The anomeric configurations of the sugars were assigned from the values of the $^{3}J_{H\text{-}1,H\text{-}2}$ coupling constants (Markham and Geiger, 1994). A long-range connectivity from δ 5.36 to C-7 in the HMBC spectrum and a ROE connectivity between the same proton and H-8 confirmed the site of attachment of the glycoside to be C-7 and the primary sugar as β-Glc. Further long-range connectivities in the HMBC spectrum from the anomeric proton of α-Rha to C-2″ of β-Glc and from H-2″ of β-Glc to the anomeric carbon of α-Rha confirmed that the sugars were (1‴→2″) linked. The downfield-shifts of the 6″-CH_2 protons of β-Glc to δ 4.32 and 4.06 and of C-6″ of β-Glc to δ_C 63.2 indicated that the acetyl group was linked to C-6″ of this sugar. This was confirmed by the long-range connectivity observed from δ 4.06 (6″-CH_2 of β-Glc) to the carbonyl of the acetyl group at δ_C 169.9. Thus compound **1** was determined to be 6-hydroxyluteolin 4′-methyl ether 7-*O*-α-rhamnopyranosyl(1‴→2″)[6″-*O*-acetyl-β-glucopyranoside], a new flavone glycoside (Fig. 1). The molecular formula of $C_{30}H_{34}O_{17}$ determined for **1** by high-resolution MS was consistent with this conclusion (see Section 3.4).

Compound **2** was a minor component of aqueous methanol extracts of the inflorescences of *V. longifolia* and was purified by preparative PC and semi-preparative HPLC to give a pale yellow solid. The UV spectrum of this compound had a characteristic short wavelength maximum at 288 nm suggesting that it was also a flavone with a hydroxyl group at C-6 (Harborne and Williams, 1971). Analysis of the aromatic region of the ^{1}H NMR spectrum of **2** confirmed that the compound was a 6-hydroxyluteolin derivative, with characteristic

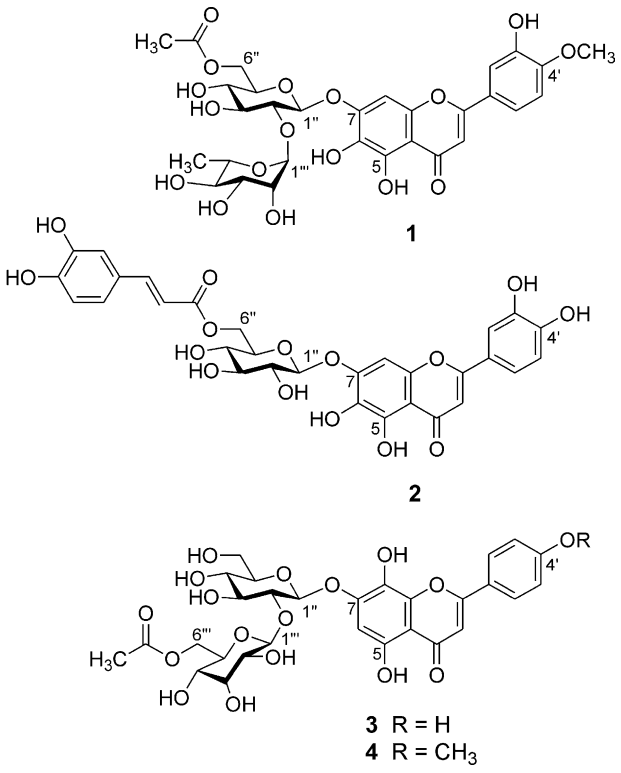


Fig. 1. Acylated flavone glycosides from *Veronica* species.

Table 1
^{1}H NMR spectral assignments and coupling constant data for the acylated flavone glycosides **1–4** (δ in DMSO-d_6 at 37 °C)

	Atom	1	2	3	4
	3	6.76 *s*	6.61 *s*	6.80 *s*	6.89 *s*
	6			6.70 *s*	6.71 *s*
	8	6.94 *s*	6.91 *s*		
	2′	7.44 *d* (2.3)	7.38 *d* (2.3)	7.97 *d* (8.9)	8.09 *d* (8.9)
	3′			6.95 *d* (8.9)	7.14 *d* (8.9)
	5′	7.11 *d* (8.7)	6.83 *d* (8.8)	6.95 *d* (8.9)	7.14 *d* (8.9)
	6′	7.54 *dd* (8.6, 2.3)	7.38 *dd* (8.8, 2.3)	7.97 *d* (8.9)	8.09 *d* (8.9)
	OCH_3	3.88 *s*			3.88 *s*
	5-OH	12.69 *s*	12.74 *br s*	12.33 *s*	12.28 *s*
Glc	1″	5.36 *d* (7.6)	5.12 *d* (7.1)	5.05 *d* (7.5)	5.06 *d* (7.6)
	2″	3.65 *dd* (9.5, 7.6)	3.41 *m*	3.60 *m*	3.60 *m*
	3″	3.52 *m*	3.40 *m*	3.52 *m*	3.52 *m*
	4″	3.25 *m*	3.32 *m*	3.27 *m*	3.27 *m*
	5″	3.75 *m*	3.83 *m*	3.46 *m*	3.46 *m*
	6″	4.32 *dd* (11.9, 1.9)	4.44 *dd* (11.9, 2.1)	3.75 *dd* (11.5, 1.5)	3.76 *dd* (11.5, 1.5)
		4.06 *dd* (12.0, 7.0)	4.26 *dd* (11.9, 6.1)	3.52 *m*	3.52 *m*
Rha	1‴	5.17 *d* (1.4)			
	2‴	3.74 *m*			
	3‴	3.40 *dd* (9.4, 3.2)			
	4‴	3.17 *m*			
	5‴	3.72 *m*			
	6‴	1.09 *d* (6.1)			
All	1‴			4.93 *d* (8.0)	4.93 *d* (8.0)
	2‴			3.27 *dd* (8.0, 2.6)	3.27 *dd* (8.0, 3.0)
	3‴			3.93 '*t*' (2.6)	3.93 '*t*' (2.7)
	4‴			3.43 *dd* (10.0, 2.8)	3.42 *dd* (10.0, 2.7)
	5‴			3.88 *ddd* (10.0, 4.8, 2.3)	3.88 *m*
	6‴			4.10 *dd* (12.0, 2.3)	4.10 *dd* (12.0, 2.3)
				4.04 *dd* (12.0, 4.8)	4.03 *dd* (12.0, 4.9)
OAc		1.94 *s*		1.88 *s*	1.88 *s*
Caffeoyl	α		6.20 *d* (15.9)		
	β		7.40 *d* (15.9)		
	2		6.90 *d* (2.0)		
	5		6.56 *d* (8.3)		
	6		6.74 *dd* (8.3, 2.0)		

resonances for H-3 at δ 6.61 (1H, *s*, δ_C 101.8 by HSQC), H-8 at δ 6.91 (1H, *s*, δ_C 93.2), H-2′ at δ 7.38 (1H, *d*, $J = 2.3$ Hz), H-5′ at δ 6.83 (1H, *d*, $J = 8.8$ Hz) and H-6′ at δ 7.38 (1H, *dd*, $J = 8.8$, 2.3 Hz). The ^{13}C resonance of C-6 at δ 130.3 (assigned by a long-range connectivity from H-8 in the HMBC spectrum) was consistent with a hydroxyl group at this position as determined for compound **1** (Table 1). The remaining resonances in the aromatic region were those of an (*E*)-caffeoyl group, with three spin–spin coupled multiplets at δ 6.90 (1H, *d*, $J = 2.0$ Hz, H-2), 6.56 (1H, *d*, $J = 8.3$ Hz, H-5) and 6.74 (1H, *dd*, $J = 8.3$, 2.0 Hz, H-6) and characteristic α- and β-H resonances at δ 6.20 (1H, *d*, $J = 15.9$ Hz) and 7.40 (1H, *d*, $J = 15.9$ Hz), respectively. The identity of the acyl group was confirmed by complete assignment of its ^{1}H and ^{13}C resonances from COSY, HSQC and HMBC data (Tables 1 and 2). The final group of resonances in the ^{1}H NMR spectrum of **2** were those of a sugar with an anomeric proton at δ 5.12 (1H, *d*, $J = 7.1$ Hz). The NOE detected between this anomeric proton and H-8 confirmed that the sugar was attached to C-7 of the aglycone. Assignment of the remaining ^{1}H and ^{13}C resonances of the sugar and the value of the $^3J_{H\text{-}1,H\text{-}2}$ coupling constant indicated that the sugar was β-glucopyranose (Tables 1 and 2). Of particular interest were the downfield-shifted resonances of 6″-CH_2 (δ 4.44 and 4.26) and C-6″ (δ_C 62.9) which allowed the site of acylation to be identified as C-6″. The structure of **2** was therefore determined to be 6-hydroxyluteolin 7-*O*-(6″-*O*-(*E*)-caffeoyl)-β-glucopyranoside, a new flavone glycoside (Fig. 1). This compound was not particularly stable in solution, with 1D ^{1}H NMR spectra acquired 48 h after dissolution in DMSO-d_6 showing evidence of deterioration. The NMR data on which the structural determi-

nation of **2** was based were acquired immediately after sample preparation.

Compounds **3** and **4** were obtained as yellow solids by column chromatography of ethanol extracts of *V. intercedens* (**3** only) and *V. orientalis* (**3** and **4**). Their structures were determined by NMR spectroscopy to be those of the allose-containing acylated flavone glycosides, isoscutellarein 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside and isoscutellarein 4′-methyl ether 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside, respectively (Fig. 1). Isoscutellarein 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside (**3**) was first characterised by Lenherr et al. (1984a) as a constituent of the leaves of *Stachys recta* L.. Subsequent reports describe its isolation from *Stachys anisochila* Vis. & Panc. (Lenherr and Mabry, 1987), *Stachys aegyptiaca* Pers. (El-Ansari et al., 1991), *Sideritis hyssopifolia* L. (Rodríguez-Lyon et al., 2000), *Sideritis javalambrensis* Pau (Rios et al., 1992), *Veronica didyma* Tenore (= *V. polita* Fries) (Wang et al., 1995) and *Veronica multifida* L. (Ozipek et al., 2002). The compound has also been detected in *Galeopsis* (Tomás-Barberán et al., 1991) and some other species of *Veronica* (Grayer-Barkmeijer, 1979; Tomás-Barberán et al., 1988). The ^{13}C NMR spectrum of **3** obtained in the present study was identical to that published by Lenherr and Mabry (1987). However the assignments of C-5, C-6, C-7 and Glc C-1″ required revision according to correlations observed in HSQC and HMBC experiments (Table 2). ^{1}H NMR assignments of **3** (Table 1) were in good agreement with published data (Lenherr and Mabry, 1987) but indicated that the original assignment for allose 6‴-CH_2 should be revised. The multiplet structure and coupling constants for the allose protons (Table 1) were obtained either directly from the 1D ^{1}H NMR spectrum (H-1‴, H-4‴, H-5‴ and 6‴-CH_2) or using selective 1D TOCSY experiments (H-2‴ and H-3‴). The site of acetylation was confirmed as All C-6‴ from the characteristic downfield shifts of 6‴-CH_2 and C-6‴ and the connectivity in the HMBC spectrum from 6‴-CH_2 to the acetyl carbonyl atom (δ_C 170.1). HMBC data also supported the previous determination of the interglycosidic linkage and the site of glycosylation. A complete set of ^{1}H and ^{13}C NMR spectral assignments for **3** in CD_3OD has also been published (Rodríguez-Lyon et al., 2000). The less common 4′-methyl ether of **3**, isoscutellarein 4′-methyl ether 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside (**4**), was described first from the whole plant of *Veronica filiformis* Sm. (Grayer-Barkmeijer, 1979; Chari et al., 1981) and later from *Stachys recta* (Lenherr et al., 1984a) and *Sideritis javalambrensis* (Rios et al., 1992). Although the ^{13}C NMR spectrum of **4** obtained in the present study was identical to that published by Lenherr et al. (1984a), some of the original assignments (made only on the basis of chemical shift comparisons with related compounds) required revision. The correct assignments for C-5, C-6, C-7, Glc C-1″, All C-2‴ and All C-3‴ of **4** are given in Table 2 and were confirmed by correlations observed in 2D NMR experiments. Similarly the ^{1}H NMR spectral assignments given previously for the

Table 2
^{13}C NMR spectral assignments for acylated flavone 7-*O*-glycosides **1**–**4** (δ in DMSO-d_6 at 37 °C)

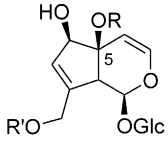
	Atom	1	2[a]	3	4
	2	163.8	164.3	164.0	163.6
	3	103.1	101.8	102.6	103.3
	4	182.1	182.0	182.2	182.3
	5	146.8	n.d.	152.1	152.1
	6	130.7	130.3	99.5	99.5
	7	151.2	151.1	150.4	150.5
	8	93.8	93.2	127.5	127.5
	9	149.0	148.9	143.7	143.7
	10	105.5	105.7	105.6	105.6
	1′	123.1	121.0	121.1	122.8
	2′	113.0	112.7	128.5	128.3
	3′	146.7	145.9	115.9	114.5
	4′	151.1	150.3	161.3	162.4
	5′	112.1	115.5	115.9	114.5
	6′	118.5	118.5	128.5	128.3
	OCH_3	55.7			55.5
Glc	1″	97.7	100.2	100.2	100.1
	2″	77.1	72.7	82.5	82.4
	3″	76.8	75.3	75.5	75.5
	4″	69.8	69.5	69.3	69.2
	5″	73.7	73.5	77.1	77.1
	6″	63.2	62.9	60.5	60.5
Rha	1‴	100.5			
	2‴	70.3			
	3‴	70.3			
	4‴	72.0			
	5‴	68.6			
	6‴	17.8			
All	1‴			102.5	102.4
	2‴			71.4	71.4
	3‴			70.7	70.7
	4‴			66.8	66.8
	5‴			71.5	71.4
	6‴			63.5	63.5
OAc		20.4		20.3	20.3
		169.9		170.1	170.1
Caffeoyl	α		112.9		
	β		145.0		
	1		125.1		
	2		114.7		
	3		145.5		
	4		148.3		
	5		115.2		
	6		120.2		
	CO		166.4		

[a] Chemical shift values determined from the indirectly detected dimension in HSQC and HMBC experiments (n.d. = not determined by long-range correlation).

sugars of **4** required revision and a complete set is given in Table 1 for the first time. The site of acetylation, the location of the methyl ether group, the interglycosidic linkage and the site of glycosylation were all confirmed by HMBC data to be as described in the original studies (Chari et al., 1981; Lenherr et al., 1984a).

The distribution of the acylated flavone glycosides **2–4** in the genus *Veronica* is of interest from a taxonomic viewpoint. In particular, 6-hydroxyluteolin glycosides acylated with phenolic acids are characteristic of the subgenus Pseudolysimachion (Opiz) M.M.Mart.Ort., Albach & M.A. Fisch. (Albach and Grayer, unpublished results). This subgenus includes *V. longifolia*, the source of the new 6-hydroxyluteolin glycoside **2**, which is acylated with caffeic acid. Species in this subgenus have been treated as a separate genus (*Pseudolysimachion* Opiz) by some authors. The unusual allose-containing 8-hydroxyflavone glycosides **3** and **4** were found previously (Grayer-Barkmeijer, 1979; Tomás-Barberán et al., 1988; Wang et al., 1995; Ozipek et al., 2002) in only two groups of species of *Veronica* within subgenera Pocilla (Dumort.) M.M.Mart.Ort., Albach & M.A. Fisch. and Pentasepalae (Benth.) M.M.Mart.Ort., Albach & M.A. Fisch. to which *V. intercedens* and *V. orientalis* also belong. Although these two groups of species have been considered to be taxonomically distinct based on different life histories (Römpp, 1928), the most recent studies using DNA sequence data show that all species of *Veronica* producing 8-hydroxyflavone glycosides are in fact closely related (Albach, 2002). The presence of **3** in *V. intercedens* and both **3** and **4** in *V. orientalis* is therefore consistent with these results.

In addition to the flavone glycosides described above, some iridoid glucosides were also isolated (see Section 3.3), including the 5-oxygenated derivatives melittoside and globularifolin from *V. intercedens* (Fig. 2). The discovery of these compounds is of special interest as they are new to *Veronica* and no 5-oxygenated iridoids have been reported previously from the genus (Taskova et al., 2002a). Globularifolin has been reported only once before as a constituent of *Globularia cordifolia* L. (Plantaginaceae *sensu* Angiosperm Phylogeny Group, 1998) (Chaudhuri and Sticher, 1980). Melittoside is also a fairly rare constituent in iridoid-bearing plants, but has been found in several species of the closely related *Plantago* (Rønsted et al., in press; Taskova et al., 2002b) and in some Lamiaceae taxa, notably three species of *Stachys*, *S. grandidentata* Lindl. (Muñoz et al., 2001), *S. sieboldii* Miq. (Miyase et al., 1990) and *S. recta* (Lenherr at al., 1984b).



Melittoside R = Glc, R' = H
Globularifolin R = H, R' = PhCO

Fig. 2. 5-Oxygenated iridoid glucosides of *Veronica intercedens*.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 at 37 °C on Bruker 400 MHz or Varian 500 MHz instruments. Standard pulse sequences and parameters were used for the experiments. Chemical shift references were obtained from the solvent resonances of DMSO-d_6 at δ_H 2.50 and δ_C 39.5, relative to TMS. High resolution ESI-MS (positive mode) were obtained on a Bruker Apex II instrument with an internal calibrant. Positive ion APCI-MS were obtained using a quadrupole ion-trap instrument (Finnigan LCQ) as described previously (Grayer et al., 2000). Analytical and semi-preparative HPLC were carried out using a Waters LC600 pump and a 996 photodiode array detector. A Merck LiChrospher 100RP-18 (250×4.0 mm i.d.; 5 μm particle size) column was used for analytical HPLC with a flow rate of 1 ml/min. The column temperature was maintained at 30 °C.

3.2. Plant material

V. liwanensis was collected in the Lesser Caucasus, Georgia (voucher specimen *Schneeweiss 7792*, WU), *V. intercedens* and *V. orientalis* were collected in Pelli Dagi, Prov. Bitlis, Turkey (voucher specimens *Albach 666* and *Albach 716*, respectively, WU and VANF) and *V. longifolia* was grown in cultivation in the garden of one of the authors (R.J.G.), in Berkshire, England (voucher specimen *LEP 20391*, L).

3.3. Isolation of glycosides

Dried plant material (aerial parts) of *V. liwanensis* (4.8 g), *V. intercedens* (1.8 g) and *V. orientalis* (14.0 g) was extracted separately by blending in EtOH (150 ml). The mixture was brought to the boiling point and then left to stand at room temperature for 3 days. After filtering, each extract was taken to dryness and partitioned in H_2O:Et_2O (25 ml each); the aq. layer was conc. to give the crude extract (0.65 g, ca. 0.2 g and 1.11 g, respectively). Each extract was processed by preparative chromatography using a Merck Lobar RP-18 column size B. The initial eluant was H_2O followed by 10:1, 4:1, 2:1, 5:4 and 4:5 H_2O:MeOH mixtures and finally MeOH. The flavone glycosides **1**, **3** and **4** were eluted as fractions with 4:5 H_2O:MeOH as eluant in each case. Compound **1** (15 mg) was obtained from *V. liwanensis*, which also yielded catalpol (20 mg) and verproside (ca.

40 mg); similarly *V. intercedens* yielded **3** (20 mg) as well as melittoside (ca. 5 mg) and globularifolin (ca. 5 mg); finally both **3** (54 mg) and **4** (14 mg) were obtained from *V. orientalis* together with fractions comprising mainly aucubin (60 mg), verminoside (170 mg) and a mixture of catalpol esters (70 mg). The iridoid glucosides were identified by their NMR spectra and comparison with spectra of standards or with values given in the literature.

Compound **2** was obtained from freeze-dried inflorescences (15 g) of *V. longifolia*. These were cut into small pieces and extracted in 250 ml boiling aq. MeOH (80%) for 5 min. After steeping for 2 h at room temperature the extract was decanted and the plant material reextracted in 250 ml aq. MeOH (80%) for 12 h. The two extracts were combined, filtered and evaporated under reduced pressure to near-dryness. The resulting ppt. was redissolved in 10 ml aq. MeOH and subjected to prep. PC using H_2O as solvent. This step separated sugars, iridoids and caffeic acid derivatives (high R_f) from flavonoids (R_f close to zero). The components of the flavonoid fraction were separated using a Merck LiChrospher 100RP-18 (250×10 mm i.d.; 5 μm particle size) column with a flow rate of 4.5 ml/min coupled to diode-array detection and the purification of **2** was monitored from its distinctive UV spectrum (λ_{max} 288, 339 nm). This semi-preparative HPLC step was performed twice using gradient programmes with MeOH and H_2O as the mobile phases to yield **2** as a pale yellow solid (2.7 mg).

3.4. 6-Hydroxyluteolin 4′-methyl ether 7-O-α-rhamnopyranosyl(1‴→2″)[6″-O-acetyl-β-glucopyranoside] (1)

Yellow solid (MeOH); UV (MeOH) λ_{max} nm: 283, 344; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; HRESIMS *m/z*: 689.1690 $[M+Na]^+$ (calc. for $C_{30}H_{34}O_{17}Na$, 689.1688).

3.5. 6-Hydroxyluteolin 7-O-(6″-O-(E)-caffeoyl)-β-glucopyranoside (2)

Pale yellow solid (MeOH); UV (MeOH) λ_{max} nm: 288, 339; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; APCI-MS (positive mode) *m/z*: 627 $[M+H]^+$, 465 $[(M–162)+H]^+$, 303 $[aglycone+H]^+$.

3.6. Isoscutellarein 7-O-(6‴-O-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside (3)

Yellow solid (MeOH); UV (MeOH) λ_{max} nm: 276, 306, 327; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

3.7. Isoscutellarein 4′-methyl ether 7-O-(6‴-O-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside (4)

Yellow solid (MeOH); UV (MeOH) λ_{max} nm: 277, 307, 325; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

Acknowledgements

We thank the Studienstiftung des deutschen Volkes for a doctoral scholarship to D.C.A. Access to high-field NMR facilities (500 MHz) was kindly provided by the Medical Research Council Biomedical NMR Centre, National Institute for Medical Research, Mill Hill, London, UK. APCI-MS data were provided by Dr Geoffrey Kite (Royal Botanic Gardens, Kew) and high-resolution MS data by chemiSPEC (North of England Business and Innovation Centre, Sunderland Enterprise Park East, Sunderland, UK).

References

Albach, D.C., 2002. Biosystematics of *Veronica*. PhD Thesis, University of Vienna.

Albach, D.C., Chase, M.W., 2001. Paraphyly of *Veronica* (Veroniceae; Scrophulariaceae): evidence from the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA. J. Plant Res. 114, 9–18.

Angiosperm Phylogeny Group, 1998. An ordinal classification for the families of flowering plants. Ann. Missouri Bot. Gard. 85, 531–553.

Chari, V.M., Grayer-Barkmeijer, R.J., Harborne, J.B., Österdahl, B.-G., 1981. An acylated allose-containing 8-hydroxyflavone glycoside from *Veronica filiformis*. Phytochemistry 20, 1977–1979.

Chaudhuri, R.K., Sticher, O., 1980. Glycosides of Globulariaceae. 3. Globularifolin, a new acyl iridoid glucoside from *Globularia cordifolia*. Helv. Chim. Acta 63, 117–120.

El-Ansari, M.A., Barron, D., Abdalla, M.F., Saleh, N.A.M., Le Quéré, J.L., 1991. Flavonoid constituents of *Stachys aegyptiaca*. Phytochemistry 30, 1169–1173.

Grayer, R.J., Kite, G.C., Abou-Zaid, M., Archer, L.J., 2000. The application of atmospheric pressure chemical ionization liquid chromatography-mass spectrometry in the chemotaxonomic study of flavonoids: characterization of flavonoids from *Ocimum gratissimum* var. *gratissimum*. Phytochem. Anal. 11, 257–267.

Grayer-Barkmeijer, R.J., 1973. A chemotaxonomic study of *Veronica*: iridoid glucosides. Biochem. Syst. 1, 101–110.

Grayer-Barkmeijer, R.J., 1978. Flavonoids in *Parahebe* and *Veronica*: a chemotaxonomic study. Biochem. Syst. Ecol. 6, 131–137.

Grayer-Barkmeijer, R.J., 1979. Chemosystematic Investigations in *Veronica* L. (Scrophulariaceae) and Related Genera. PhD Thesis, University of Leiden.

Harborne, J.B., Williams, C.A., 1971. 6-Hydroxyluteolin and scutellarein as phyletic markers in higher plants. Phytochemistry 10, 367–368.

Lenherr, A., Mabry, T.J., 1987. Acetylated allose-containing flavonoid glucosides from *Stachys anisochila*. Phytochemistry 26, 1185–1188.

Lenherr, A., Lahloub, M.F., Sticher, O., 1984a. Three flavonoid glycosides containing acetylated allose from *Stachys recta*. Phytochemistry 23, 2343–2345.

Lenherr, A., Meier, B., Sticher, O., 1984b. Modern HPLC as a tool for chemotaxonomical investigations: iridoids and acetylated flavonoids in the group of *Stachys recta*. Planta Med. 84, 403–409.

Markham, K.R., Chari, V.M., Mabry, T.J., 1982. Carbon-13 NMR spectroscopy of flavonoids. In: Harborne, J.B., Mabry, T.J. (Eds.), The Flavonoids. Advances in Research. Chapman and Hall, London, pp. 19–134.

Markham, K.R., Geiger, H., 1994. ^{1}H NMR spectroscopy of flavonoids and their glycosides in DMSO-d_6. In: Harborne, J.B. (Ed.), The Flavonoids. Advances in Research since 1986. Chapman and Hall, London, pp. 441–497.

Miyase, T., Ueno, A., Kitani, T., Kobayashi, H., Kawahara, Y.,

Yamahara, J., 1990. Studies on *Stachys sieboldii* Miq. I. Isolation and structures of new glycosides. Yakugaku Zasshi 110, 652–657.

Muñoz, O., Peña, R.C., Montenegro, G., 2001. Iridoids from *Stachys grandidentata* (Labiatae). Z. Naturforsch. 56c, 902–903.

Ozipek, M., Saracoglu, I., Ogihara, Y., Çaliş, İ., 2002. Nuatigenin-type steroidal saponins from *Veronica fuhsii* and *V. multifida*. Z. Naturforsch. 57c, 603–608.

Rios, J.L., Mañez, S., Paya, M., Alcaraz, M.J., 1992. Antioxidant activity of flavonoids from *Sideritis javalambrensis*. Phytochemistry 31, 1947–1950.

Rodríguez-Lyon, M.L., Díaz-Lanza, A.M., Bernabé, M., Villaescusa-Castillo, L., 2000. Flavone glycosides containing acetylated sugars from *Sideritis hyssopifolia*. Magn. Reson. Chem. 38, 684–687.

Römpp, H., 1928. Die Verwandtschaftsverhältnisse in der Gattung *Veronica*. Repet. Spec. Nov. Regni Veg. Beih. 50, 11–71.

Rønsted, N., Franzyk, H., Mølgaard, P., Jaroszewski, J., Jensen, S.R. Chemotaxonomy and evolution of *Plantago*. Pl. Syst. Evol. (in press).

Taskova, R., Peev, D., Handjieva, N., 2002a. Iridoid glucosides of the genus *Veronica* s.l. and their systematic significance. Pl. Syst. Evol. 231, 1–17.

Taskova, R., Evstatieva, L., Handjieva, N., Popov, S., 2002b. Iridoid patterns of genus *Plantago* L. and their systematic significance. Z. Naturforsch. 57c, 42–50.

Tomás-Barberán, F.A., Grayer-Barkmeijer, R.J., Gil, M.I., Harborne, J.B., 1988. Distribution of 6-hydroxy-, 6-methoxy- and 8-hydroxy-flavone glycosides in the Labiatae, the Scrophulariaceae and related families. Phytochemistry 27, 2631–2645.

Tomás-Barberán, F.A., Gil, M.I., Ferreres, F., Tomás-Lorente, F., 1991. Correlations between flavonoid composition and infrageneric taxonomy of some European *Galeopsis* species. Phytochemistry 30, 3311–3314.

Wang, C.Z., Zhong, J.J., Liao, J.C., 1995. Flavonoid and iridoid glycosides from *Veronica didyma* Tenore. Ind. J. Chem. 34B, 914–916.