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A new phenylethanoid triglycoside in *Veronica beccabunga* L

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ABSTRACT

Besides the expected iridoid glucosides aucubin and catalpol as well as three known esters of the latter, *Veronica beccabunga* (brooklime) was shown to contain five carboxylated iridoid glucosides, namely gardoside, mussaenosidic acid, 8-epiloganic acid, arborescosidic acid and alpinoside. In addition to these compounds, the plant contained salidroside and a previously unknown caffeoyl phenylethanoid glycoside (CPG) which we have named chionoside J. The structure was elucidated mainly by 1D and 2D NMR spectroscopy to be 2″-(β-glucopyranosyl)-plantamajoside. The distribution of plantamajoside and its derivatives as well as that of carbocyclic iridoids with an 8,9-double bond is briefly discussed, and it is noted that such compounds are mainly confined to the tribe Veroniceae of the Plantaginaceae.

1. Introduction

Plants of the genus *Veronica* (Speedwell, Plantaginaceae) have traditionally been considered members of the family Scrophulariaceae; however, the genus has recently been transferred to the Plantaginaceae on the basis of DNA sequence investigations (Olmstead et al., 2001; Albach et al., 2005). The chemistry of *Veronica* has been investigated intensively and the genus usually contains the iridoid glucosides aucubin (**6**) and catalpol (**7**) together with a variety of aromatic esters of the latter (Grayer-Barkmeijer, 1979; Taskova et al., 2004; Jensen et al., 2005). In connection with an ongoing work on sequestration of plant secondary metabolites in phytophagous insects (Opitz et al., 2010), we undertook a chemical investigation of *Veronica beccabunga* L. (brooklime). This species has previously been investigated using chromatographic methods (Grayer-Barkmeijer, 1979; Taskova et al., 2004; Crisan et al., 2010), but apparently no isolation of the glycosidic compounds has been reported so far.

2. Materials and methods

2.1. General

Chromatography was performed on a Merck Lobar RP-18 column (size B) eluting with H_2O–MeOH mixtures (1:0 to 1:1); compounds are listed in order of elution; the amount of mannitol (**1**) was estimated from the ^{13}C NMR spectrum of the crude sugar fraction. NMR spectra were recorded on a Varian Unity Inova-500 MHz (^1H) or Mercury-300 MHz (^{13}C) instrument in MeOH-d_4 using the solvent peak (δ 3.30 or 49.0) as the internal standard. 2D DQF-COSY, gHSQC, HMBC and NOESY spectra were acquired using standard pulse sequences. LC-HR ESIMS was performed on an Agilent HP 1100 Liquid Chromatograph

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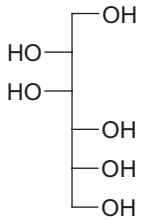
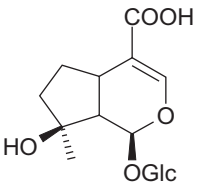
equipped with a BDS-C18 reversed phase column running a water–acetonitrile (50 ppm TFA in water) gradient. The LC was coupled to an LCT of a TOF MS (Micromass, Manchester, UK) operated in the positive electrospray ion mode using 5-leucineenkephalin as lock mass.

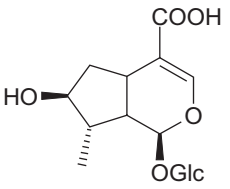
The known compounds isolated were identified by their NMR data: mannitol (**1**), (Bock and Pedersen, 1983); iridoids **2**–**7** and salidroside (**8**) with authentic samples (Rønsted et al., 2000); veronicoside (**7a**) (Sticher and Afifi-Yazar, 1979a); verproside (**7b**) (Afifi-Yazar and Sticher, 1980); amphicoside (**7c**) (Kapoor et al., 1971); verminoside (**7d**) (Sticher and Afifi-Yazar, 1979b).



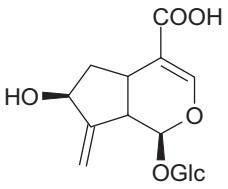
1; Mannitol



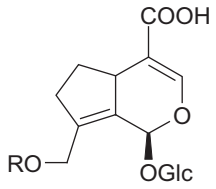
2; Mussaenosidic acid



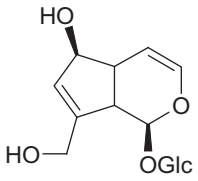
3; 8-Epiloganic acid



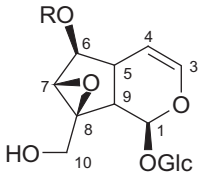
4; Gardoside



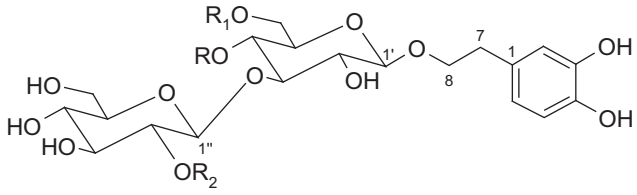
5; R=H; Arborescosidic acid
5a; R=Acetyl; Alpinoside



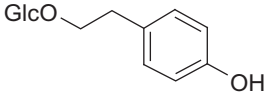
6; Aucubin



7; R=H; Catalpol
7a; R=Benzoyl; Veronicoside
7b; R=3,4-di-OH-Benzoyl; Verproside
7c; R=Vanilloyl; Amphicoside
7d; R=Caffeoyl; Verminoside



9; R=Caffeoyl; R_1=H; R_2=Glc; Chionoside J
10; R=H; R_1=Feruloyl; R_2=Glc; Scroside C
11; R=H; R_1=Caffeoyl; R_2=Glc; Isochionoside J
12; R=H; R_1=Caffeoyl; R_2=H; Plantamajoside



8; Salidroside

2.2. Plant material

V. beccabunga was grown from seeds (IPEN no. DE-0-B-2250905) from the Botanical Garden and Botanical Museum of Berlin-Dahlem, Germany, where a voucher specimen (B 10 0342111) is deposited at the Herbarium B. The seeds were sown once and subsequently propagated by cuttings which were harvested in March 2009.

2.3. Work-up

Dry foliage (21 g) was homogenized with boiling EtOH (150 ml) and filtered. The concentrated extracts were partitioned in Et_2O–H_2O, after which the aq. phase was dried to give 2.1 g of crude extract; chromatography of an aliquot (1.2 g, after dissolving in 10% acetic acid) gave a sugar fraction (650 mg, with *ca.* 50% mannitol (**1**)); catalpol (**7**; 10 mg); aucubin (**6**; 30 mg); a 3:1 mixt. of gardoside and mussaenosidic acid (**2** and **3**; 10 mg); a 1:1 mixt. of epiloganic acid and salidroside (**3** and **8**; 50 mg); arborescosidic acid (**5**; 50 mg); verproside (**7b**; 70 mg); a fract. A containing **7b**, alpinoside (**5a**) and an

unknown (**9**) in the proportion 2:1:3 (50 mg); a 1:1 mixt. of amphicoside and verminoside (**7c** and **7d**; 240 mg); verminoside (**7d**; 50 mg); and veronicoside (**7a**; 40 mg).

2.4. Rechromatography of fract. A

Chromatography of Fraction A on a Merck HiBar column (250-25) packed with LiChrosorb RP-18 gave a fraction (11 mg) containing the pure **9**.

2.5. Chionoside J (**9**)

Isolated as a glass: $[\alpha]_D^{20} = -6^\circ$ (MeOH; *c* 1.2); ^1H and ^{13}C NMR in Table 1; LC-HR ESIMS *m/z*: 825.2403 $[M + Na]^+$; ($C_{35}H_{46}NaO_{21}$ requires 825.2429).

Table 1
^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra of **9** and model compounds in CD_3OD.

Atom	Chionioside J (**9**)		Scroside C (**10**)[a]	Isochionoside J (**11**)[b]		Plantamajoside (**12**)[c]
	^1H – δ (mult. Hz)[d]	^{13}C	^{13}C	^1H – δ (mult. Hz)[d]	^{13}C	^{13}C
Agluc						
1		131.5	132.3		131.4	131.5
2	6.71 (d, 1.9)	117.2	117.6	6.68 (d, 2.0)	117.2	117.2
3		146.1	148.8		146.1	145.8
4		144.6	147.2		144.6	144.2
5	6.67 (d 8.0)	116.3	112.7	6.62 (d, 8.0)	116.3	116.5
6	6.56 (dd, 1.9, 8.0)	121.3	120.1	6.53 (dd, 2.0, 8.0)	121.3	121.5
7	2.78 2H (m)	36.5	36.1	2.77 (2H, m)	36.7	36.3
8	3.72/4.04 (m/m)	72.2	71.1	3.72, 3.95 (m/m)	72.3	72.2
Central Glc						
1′	4.45 (d 8.0)	103.3	103.2	4.43 (d, 7.0)	103.5	103.7
2′	3.54 (obsc)	74.6	74.4	3.50 obsc.	73.8	75.7
3′	3.88 (t 9.1)	85.6	86.2	3.50 obsc.	89.9	83.8
4′	4.93 (t 9.3)	70.8	70.3	3.50 obsc.	70.1	70.7
5′	3.53 (obsc)	75.9	76.4	3.59 obsc.	75.0	75.5
6′	3.53 (obsc)	62.3	62.6	4.33 (dd, 5.9, 11.8)	64.5	62.1
	3.63 (br. d 10)			4.50 (dd, 1.5, 11.8)		
3′-Glc (inner)						
1″	4.62 (d 7.2)	104.1	104.6	4.62 (d, 7.8)	104.0	104.8
2″	3.36 (obsc)	84.5	85.3	3.50 obsc.	85.1	74.8
3″	3.55 (obsc)	76.6	77.6	3.39 (t, 9.3)	77.6	77.3
4″	3.27 (obsc)	70.8	71.2	3.35 obsc.	71.0	71.0
5″	3.14 (m)	77.5	78.1	3.35 obsc.	78.0	77.6
6″	3.47 (dd, 4.9, 11.9)	62.3	62.2	3.63 (dd, 5.7, 12.0)	62.4	62.1
	3.68 (obsc)			3.87 (br. d, 11.5)		
2″-Glc (outer)						
1‴	4.64 (d 7.2)	105.7	106.4	4.61 (d, 7.7)	106.4	
2‴	3.25 (obsc)	76.1	76.7	3.27 (dd 7.7, 9.2)	76.2	
3‴	3.36 (obsc)	77.6	77.6	3.38 obsc.	77.6	
4‴	3.37 (obsc)	70.9	71.2	3.35 obsc.	71.2	
5‴	3.36 (obsc)	78.6	78.1	3.36 obsc.	78.8	
6‴	3.75 (obsc)	62.2	62.2	3.73 (dd, 4.6, 12.0)	62.3	
	3.92 (br. d 11.9)			3.91(dd, 1.8, 12.0)		
4′-Acyl	4′-Caffeoyl		4′-Feruloyl	4′-Caffeoyl		4′-Caffeoyl
1⁗		127.6	126.8		127.6	127.6
2⁗	7.06 (d, 1.7)	115.3	111.5	7.03 (d, 2.0)	114.8	115.0
3⁗		146.8	149.1		146.8	146.5
4⁗		149.7	151.1		149.6	149.4
5⁗	6.78 (d 8.2)	116.6	116.7	6.76 (d, 8.2)	116.5	116.7
6⁗	6.98 (dd, 1.7, 8.2)	123.2	124.0	6.89 (dd, 2.0, 8.2)	123.2	123.3
α⁗	6.33 (d 15.9)	115.1	115.9	6.28 (d, 15.9)	115.0	147.6
β⁗	7.58 (d 15.9)	147.6	146.0	7.55 (d, 15.9)	147.2	115.3
CO⁗		168.5	167.4		169.1	168.7
			55.9			

[a] Data from Li et al., 1998 (solvent pyridine).
[b] Data from Taskova et al., 2010.
[c] Data from Maggi et al., 2009.
[d] obsc = obscured.

3. Results and discussion

3.1. Isolated compounds

The dry plant material was briefly boiled with ethanol and after extraction, the water-soluble part of the extract was subjected to reverse phase column chromatography and the isolated compounds were characterized by their NMR spectra (see Section 2.1). Mannitol (**1**) was the main carbohydrate present. The following iridoid acids were isolated: mussaenosidic acid (**2**), 8-epiloganic acid (**3**), gardoside (**4**) and arborescosidic acid (**5**) as well as its acetyl ester alpinoside (**5a**). Furthermore we found the expected aucubin (**6**), as well as a little catalpol (**7**) in addition to the catalpol esters (**7a**–**7d**). Aside from the iridoid glucosides we also isolated salidroside (**8**) and a new caffeoyl phenylethanoid glycoside (CPG) (**9**).

Compound **9** was isolated as a colourless glass, $[\alpha]_D^{20} = -6^\circ$. The molecular formula was $C_{35}H_{46}O_{21}$as deduced from the quasimolecular ion obtained by LC-HR ESIMS (observed *m*/*z* 825.2403 $[M + Na]^+$). The NMR spectroscopic data (Table 1) could be assigned by interpretation of the 1D and 2D (DQF-COSY, gHSQC and gHMBC) spectra. Thus, the ^{13}C NMR spectrum of **9** exhibited the expected 35 signals; by comparison with model compounds, eight of these could be assigned to a 3,4-dihydroxyphenylethyl group and another set of nine resonances could be allocated to a caffeoyl ester moiety. The remaining 18 signals were surmised to belong to three hexoaldoside units since three of them were found in the 103–106 ppm region of the spectrum, the shift values signifying anomeric carbon atoms, while the remaining 15 signals were found between 62 and 86 ppm. One anomeric signal at δ_C 103.3 could be assigned as C-1′ since it exhibited a cross-peak with H-8 (δ_H 3.72) of the 3,4-dihydroxyphenylethyl group in the HMBC spectrum. This allowed us to assign H-1′ and using the COSY and the HSQC spectra, we could likewise assign the remaining proton and carbon signals arising from a central β-glucopyranosyl moiety (see Table 1), taking in account also the visible ^1H coupling pattern. The position of the caffeoyl ester group at the C-4′ carbon atom was seen both from the low field absorption of H-4′ (δ_H 4.93), and from the presence of an HMBC correlation between H-4′ and the carbon atom of the CO′′′′-group of the caffeoyl moiety. The low field resonance shift of the signals from C-3′ (δ_C 85.6) and H-3′ (δ_H 3.88), showed that this was the linkage position to a second glycosyl unit. Another HMBC correlation from H-3′ to C-1″ (δ_C 104.1), made it possible to identify the anomeric carbon of this second (inner) glycosyl unit, and as above, the 2D NMR spectral data now allowed to assign the remaining resonances from this hexopyranosyl unit. Also this unit had a carbon signal with a low field resonance, namely C-2″ (δ_C 84.5), and a cross-peak in the HMBC spectrum from the corresponding proton, H-2″ (δ_H 3.36) to the last unassigned anomeric carbon atom C-1‴ (δ_C 105.7) confirmed that the third (outer) hexopyranosyl moiety was indeed sited at this position. Comparison of $\delta_{C‴}$ together with the remaining five unassigned carbon signals with model compounds (**10** and **11**, Table 1) confirmed that these belonged to another β-glucopyranosyl moiety. Furthermore, comparison of the carbon NMR data assigned to the second (inner) hexopyranosyl unit with the model compound **10**, similarly confirmed that this also had to be a β-glucopyranosyl moiety. In conclusion, compound **9** is β-glucopyranosyl-(1→2)-β-glucopyranosyl-(1→3)-calceolarioside A or 2″-(β-glucopyranosyl)-plantamajoside, and we have named it chionoside J, since the isomeric compound **11** from *Veronica thomsonii* (Buchanan) Cheeseman has previously been named isochionoside J (Taskova et al., 2010).

The above structural elucidation has mainly been based on the similarity of the ^{13}C NMR data. In contrast, when considering the ^1H NMR data, we see that the shift values for the H-2″ to H-6″ are much more dissimilar in chionoside J (**9**) and its iso-form (**11**). This must be ascribed to the difference in shielding effect of the aromatic ring of the ester moiety, changing from the 4′- to the 6′-position. Such an effect is apparently not important for the ^{13}C chemical shifts.

3.2. Chemotaxonomy

Caffeoyl phenylethanoid glycosides (CPGs) are very common in the plant order Lamiales. However, CPGs with a 3′-O-glucosyl substituent (*i.e.* derivatives of plantamajoside, **12**) have almost exclusively been reported from Plantaginaceae and mainly from *Plantago* (Rønsted et al., 2000) and from *Veronica* and its allies in Veroniceae (Taskova et al., 2006). The only exceptions are **12** and purpureaside B from cell-cultures of *Rehmannia glutinosa* Libosch, Rehmanniaceae (Shoyama et al., 1986); artselaeroside B from *Pedicularis artselaeri* Maxim., Orobanchaceae (Su et al., 1998); **12** has also been reported from three species of Gesneriaceae, namely *Aeschynanthus speciosus* Hook (Jensen, 1996), *Chirita longgangensis* W.T. Wang (Wang et al., 2005) and *Chirita eburnea* Hance (Chen et al., 2010), and finally, it has been found in *Mimulus cardinalis* Douglas ex Benth., Phrymaceae (Jensen, unpubl). Another kind of compound of special taxonomic interest is the iridoid glucosides with an 8,9-double bond, such as **5** and **5a**. We have previously noted (Albach et al., 2005) that compounds with this structural feature are solely reported from genera within the tribe Veroniceae of the Plantaginaceae, namely *Globularia*, *Paederota*, *Plantago*, *Veronica* and *Wulfenia*. Since this, such compounds have furthermore been found in *Erinus* (Taskova et al., 2005), and more recently also in *Lagotis* (Jensen et al., 2009).

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