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Chemotaxonomy of Veroniceae and its allies in the Plantaginaceae

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Abstract

In a chemosystematic investigation of tribe Veroniceae (Plantaginaceae), representatives of *Camptoloma*, *Sibthorpia*, *Veronica* subg. *Pentasepalae* and subg. *Hebe*, *Veronicastrum*, *Wulfenia*, and the related *Ellisiophyllum* and *Globularia* were examined for non-flavonoid glycosides. From the 14 species studied, 28 different iridoid glucosides and 10 caffeoyl phenylethanoid glucosides (CPGs), as well as salidroside and arbutin were isolated and characterized by NMR; of these, five compounds were previously unknown. It was found that the representatives of Veroniceae, as well as *Globularia*, were characterized by mannitol, aucubin, catalpol and catalpol esters. Each of the three studied species of *Veronica* subg. *Hebe* contained at least one of the 6-*O*-catalpol esters typical for *Veronica s. str.* (verminoside), supporting the inclusion of *Hebe* in *Veronica*. However, their main constituents were esters of 6-*O*-rhamnopyranosylcatalpol; a CPG, hebeoside (2′-β-xylopyranosyl-verbascoside) was isolated from *V.* (*Hebe*) *salicifolia*. The two species of *Veronicastrum* also contained 6-*O*-rhamnopyranosylcatalpol esters, including the previously unknown 2″,3″- and 3″,4″-dicinnamoyl derivatives and, in contrast to the earlier reports, they lacked 6-*O*-catalpol esters. The main iridoid constituents in the three investigated species of *Wulfenia* were 10-*O*-aucubin and 10-*O*-catalpol esters (isoscrophularioside or globularin) while baldaccioside (10-*O*-cinnamoyl asystasioside E) was isolated from *W. baldaccii*. *Globularia vulgaris* contained 10-*O*-catalpol esters (e.g., globularin) and, in addition, asperuloside together with its benzoyl analogue named besperuloside. The representatives of *Sibthorpia* and *Ellisiophyllum* were almost completely devoid of iridoids; this, however, together with the CPGs present implied a close relationship between the two genera. *Camptoloma lyperiiflorum* lacked hexitols but contained esters of 6-*O*-rhamnopyranosylcatalpol different from those found in Veroniceae but known from *Buddleja*, *Scrophularia* and *Verbascum* (Scrophulariaceae *s. str.*).

Keywords: Chemotaxonomy; Plantaginaceae; Veroniceae; Mannitol; Iridoid glucosides; 6-*O*-Rhamnopyranosylcatalpol esters; Besperuloside; Baldaccioside; CPGs; Hebeoside

1. Introduction

The Scrophulariaceae *s.l.* is a large angiosperm family for which a systematic treatment and subdivision based on morphological grounds have been difficult and led to differences of opinion and taxonomic instability (Bentham, 1846, 1876; von Wettstein, 1891; Pennell, 1935; Melchior, 1964; Thieret, 1967). Much research has contributed to the ongoing efforts to create a phylogenetic classification of Scrophulariaceae using morphological microcharacters (Minkin and Eshbaugh, 1989; Argue, 1993; Bigazzi, 1993), as well as chemical characters including iridoid (Kooiman, 1970; Nicoletti et al., 1988a), flavonoid (Tomás-Barberán et al., 1988) or betaine distribution (Blunden et al., 2003).

In several recent molecular investigations the representatives of Bentham's (1876) tribe Digitaleae formed a strongly supported clade with *Plantago* (Olmstead and Reeves, 1995; Olmstead et al., 2001; Bello et al., 2002; Albach et al., 2005). They were nested in a large clade together with part of Bentham's tribes Antirrhineae, Cheloneae, Gratioleae, Angelonieae, including the small families Globulariaceae, Callitrichaceae and Hippuridaceae. This clade has been recognized as the family Plantaginaceae in the APG classification of the flowering plants (APG, 2003).

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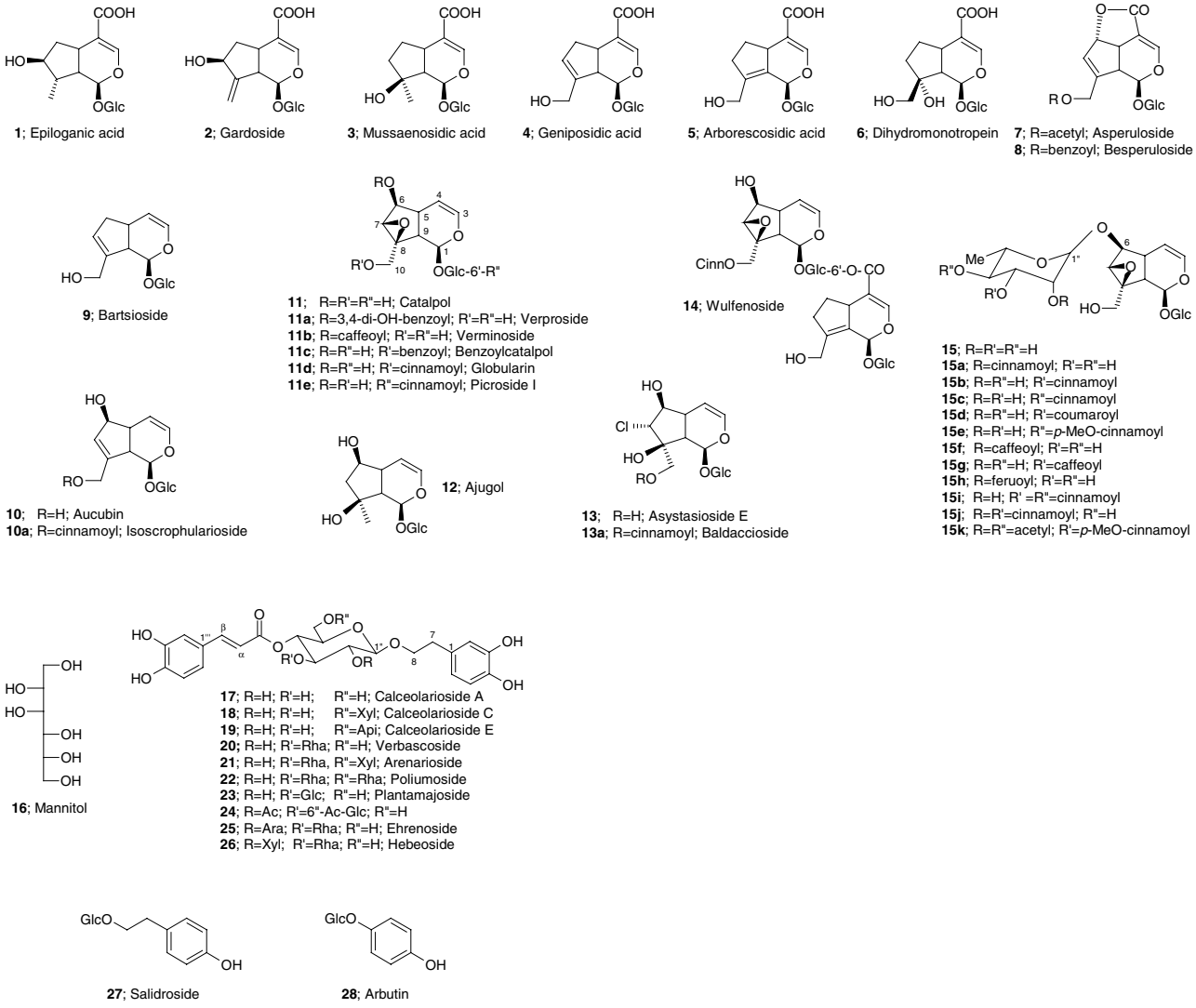


The present study is a chemosystematic investigation of non-flavonoid glycosides in certain tribes of the "new" Plantaginaceae (sensu Albach et al., 2005). The emphasis is on tribe Veroniceae for which the chemical characters are of special interest due to the reported incongruence of molecular data (Albach and Chase, 2004) and an uncertain position of some of its members. Comprehensive chemical information is provided for genera such as *Camptoloma*, *Sibthorpia*, *Hebe*, *Veronicastrum* and *Wulfenia*, which have not been studied properly for iridoid and other non-flavonoid glycosides before. The new phytochemical evidence is compared with previously published data and used to evaluate relationships proposed on the basis of morphological and molecular characters.

2. Results and discussion

The plant material was extracted with cold ethanol and the water-soluble part of the extract was subjected to reverse phase column chromatography to give the compounds listed in Table 1. The isolated compounds were identified mainly by NMR spectroscopy and this also included the sugar fraction for which the composition was deduced solely by ^{13}C NMR.

2.1. Camptoloma

No previous work has been reported on this genus which includes about three species. One of these (*C. lyperiiflorum*) was available and a number of compounds were isolated. The sugar fraction consisted of a mixture of glucose and sucrose. The only caffeoyl phenylethanoid glycoside (CPG) found was verbascoside (**20**). Several iridoids were present, namely the acids **1**, **2** and **3** as well as bartsioside (**9**), mainly precursors for aucubin (**10**) and catalpol (**11**). Additionally, we isolated two esters of 6-*O*-rhamnopyranosylcatalpol, namely verbascoside A (**15e**) and scrovalentinoside (**15k**).

2.2. Veronica subg. Pentasepalae

Extensive work has been done on *Veronica;* recently we have reviewed the content of iridoids and other glycosides

Table 1
Plants investigated and compounds isolated in the present work

Species name and voucher	Main carbohydrate(s)	CHO/COOH-iridoids	Decarboxylated iridoids (**9**–**13**)	6-Esters of **11**	10-Esters of **10**,**11**,**13**	6-*O*-Rhamnosyl-catalpol esters	CPGs and other phenolic glycosides
Camptoloma lyperiiflorum (Vatke) Hilliard IOK-32/2002	Sucrose Glucose	**1**,**2**,**3**	**9**, **10**, **11**			**15e**, **15k**	**20**
Veronica austriaca L. IOK-10/2003	**16**		**10**, **11**	**11a**			**20**
Veronica (*Hebe*) *ligustrifolia* A. Cunn IOK-29/2002	**16**	**5**	**10**, **11**	**11b**		**15**, **15f**, **15g**, **15h**	**21**, **22**
Veronica (*Hebe*) × *andersonii* Lindl. et Pax IOK-28/2002	**16**	**1**	**10**, **11**	**11b**		**15**, **15a**, **15b**, **15c**, **15f**, **15g**	**22**
Veronica (*Hebe*) *salicifolia* G. Forst. IOK-27/2002	**16**	**2**, **4**, **5**	**10**, **11**	**11a**, **11b**		**15**, **15g**	**22**, **26**
Veronicastrum sibiricum (L.) Pennell IOK-23/2002	**16**	**1**	**10**, **11**, **12**			**15**, **15a**, **15b**, **15d**	**25**, **28**
Veronicastrum virginicum (L.) Farw. IOK-24/2002	**16**		**10**, **11**, **12**			**15**, **15b**, **15d**, **15i**, **15j**	**27**, **28**
Wulfenia baldaccii Degen IOK-16/2002	**16**	**1**, **14**	**10**, **11**, **13**		**10a**, **11d**, **11e**, **13a**		**23**, **24**
Wulfenia orientalis Boiss. IOK-8/2003	**16**	**2**, **3**, **5**	**10**, **11**		**10a**, **11d**		**24** Shikimic acid
Wulfenia blechicii Lakusic subsp. *rohleanae Lakusic* IOK-30/2002	Mixture	**1**, **2**, **5**, **6**	**10**, **11**		**10a**, **11d**, **11e**		Shikimic acid
Sibthorpia africana L. IOK-22/2002	Glucose Sucrose						**20**, **22** Isoverbascoside
Sibthorpia europea L. IOK-25/2002	Glucose						**20**, **22**
Ellisiophyllum pinnatum (Wall. ex Benth.) MakinoIOK-31/2002	Mixture	**1**					**17**, **18**, **19**, **20**
Globularia vulgaris L. IOK-11/2002	**16**	**2**, **3**, **4**, **7**, **8**	**10**, **11**		**11c**, **11d**		

Table 2
NMR data (CD_3OD) for the new compounds **8**, **13a** and **26**

Atom	Besperuloside (**8**)		Baldaccioside (**13a**)		Hebeoside (**26**)			
	^1H	^{13}C	^1H	^{13}C	^1H		^{13}C	
Agluc								
1	6.05 (*d*, 1.5)	93.5	5.62 (*d*, 3.7)	93.0			131.5	
2					6.70 (*d*, 2)		117.2	
3	7.31 (*d*, 1.8)	150.3	6.22 (*dd*, 6.2; 1.8)	140.8			146.0	
4		106.9	5.10 (*dd*, 6.2; 3.5)	105.8			144.6	
5	3.70 (*dt*, 1.8; 6.7)	37.5	2.72 (*dddd*, 11; 6; 3.5; 1.8)	38.1	6.67 (*d*, 8.1)		116.3	
6	5.59 (*br.d*, 6.5)	86.3	3.92 (*dd*, 8.1; 6.1)	83.5	6.57 (*dd*, 8.1; 2.0)		121.3	
7	5.83 (*m*)	129.1	4.06 (*d*, 8.1)	73.4	2.77 (*t*-like, 7.4)		36.6	
8		144.2		79.5	3.69, 4.05 (*m's*)		72.1	
9	3.39 (*m*)	45.5	2.63 (*dd*; 10.9; 3.6)	49.1				
10	5.03 (*br.d*, 14.2)	62.5	4.56 (*d*, 11.8)	66.3				
	4.92 (*br.d*, 14.2)		4.33 (*d*, 11.8)					
11		172.5						
Glc								
1′	4.67 (*d*, 7.9)	100.1	4.62 (*d*, 7.9)	99.8	4.53 (*d*, 7.8)		103.0	
2′	3.19 (*dd*, 9.0; 8.1)	74.6	3.20 (*dd*, 9.0; 7, 9)	74.7	3.66 (*dd*, 8.7, 7.8)		82.5	
3′	3.37 (*t*, 9.0)	77.9	3.32–3.34 (obsc.)	77.9	3.98 (*t*, 9.0)		81.2	
4′	3.28 (*t*, 9.0)	71.5	3.32–3.34 (obsc.)	71.4	4.94 (*t*, 9.3)		70.8	
5′	3.3 (obsc.)	78.3	3.24 (*m*)	78.1	ca. 3.5		75.8	
6′	3.83 (*dd*, 11.9; 2.0)	62.7	3.82 (*dd*, 11.9; 2.2)	62.7	3.5–3.6 (obsc.)		62.4	
	3.63 (*dd*, 11.9; 5.8)		3.66 (*dd*; 11.9; 5.5)		3.5–3.6 (obsc.)			
Rha/Xyl								
1″ 1‴					5.17 (*d*, 1.5);	4.56 (*d*, 7.3)	103.1	104.5
2″ 2‴					4.05 (*d*, 3.4, 1.8.);	3.21 (*dd*, 9.0, 7.6)	72.1	75.3
3″ 3‴					3.56 (*dd*, 3.4, 9.6)	3.3 (obsc.)	72.1	78.0
4″ 4‴					ca. 3.3	ca. 3.5	73.8	71.1
5″ 5‴					ca. 3.5	3.11 (*dd*, 11.5; 9.9)	70.6	67.0
						3.86 (*dd*, 11.5; 5.2)		
6″					1.09 (*d*, 6.2)		18.4	
Aroyl								
1‴		130.9		135.7			127.6	
2‴	8.04 (*d*-like, 7.5)	130.6	7.61 (*m*)	129.3	7.05 (*d*, 2)		115.2	
3‴	7.49 (*t*-like, 7.5)	129.7	7.40 (*m*)	130.0			146.8	
4‴	7.62 (*t*-like, 7.5)	134.5	7.40 (*m*)	131.6			149.8	
5‴	7.49 (*t*-like, 7.5)		7.40 (*m*)	130.0	6.77 (*d*, 8.2)		116.5	
6‴	8.04 (*d*-like, 7.5)		7.61 (*m*)	129.3	6.94 (*dd*, 8.3; 2.0)		123.2	
α			6.52 (*d*, 16.0)	118.7	6.26 (*d*, 15.9)		114.8	
β			7.73 (*d*, 16.0)	146.6	7.57 (*d*, 15.9)		147.9	
CO		167.3		168.2			168.3	

in this genus (Taskova et al., 2002, 2004; Jensen et al., 2005). In the present work, we have included a single species, *V. austriaca*. As might be expected, the main carbohydrate was found to be mannitol (**16**) and the standard iridoids **10** and **11** were found together with verproside (**11a**), the 6-*O*-protocatechuoyl ester of **11**. The sole CPG present in significant amount was verbascoside (**20**).

2.3. *Veronica subg. Hebe*

Little chemical work has been done on subgenus *Hebe* and their closest relatives, a large taxon with more than 100 species. Mannitol (**16**) has been isolated from leaves of four species (Grady et al., 1967). In his chromatographic investigation of the iridoids in Scrophulariaceae *s. l.*, Kooiman (1970) found that seeds of the three species examined contained both aucubin (**10**) and catalpol (**11**), and the presence of some unidentified iridoid compounds was inferred. In her similar, but more comprehensive studies on many species of *Hebe*, Grayer-Barkmeijer (1973, 1979) identified mainly verproside (**11a**) and verminoside (**11b**) in addition to **10** and **11**. Also a single CPG, namely forsythiaside (an isomer of **20**), has been isolated from *V. stricta* Banks & Sol. ex Benth. var. *atkinsonii* Allan (Kellam et al., 1993).

In the present work, we investigated three species (Table 3) and found mannitol (**16**) and one or more iridoid acids in all samples. Notably, two of the investigated species contained arborescosidic acid (**5**) in trace amounts. All of them contained **10** and **11** together with verminoside (**11b**) and in one species also verproside (**11a**), the latter two both being 6-*O*-esters of catalpol. Thus, subgenus *Hebe* contain the compounds typical for *Veronica*. Surprisingly, we find that all the three species also contain 6-*O*-rhamnopyranosylcatalpol (**15**) together with a number of (substituted) cinnamoyl esters of this compound, the 3″-caffeoyl ester **15g** being common and a main constituent in the three species.

In addition to the iridoid glycosides, CPGs were also present in the three species investigated with poliumoside (**22**) being common and arenarioside (**21**) and the new hebeoside (**26**) found in one species each.

Hebeoside was isolated as a glass with the elemental composition $C_{34}H_{44}O_{19}$ as established by HRESIMS. The ^{13}C NMR spectrum of **26** (Table 2) showed the expected 34 signals of which 29 were almost superimposable with those of ehrenoside (**25**), namely from (i) a 3,4-dihydroxyphenylethyl, (ii) a substituted β-glucopyranosyl, (iii) a caffeoyl and (iv) an α-rhamnopyranosyl moiety, while the remaining five signals indicated the presence of a β-xylopyranosyl moiety instead of the α-arabinopyranosyl moiety found in **25**. The low field positions of the signals for C-2′ and C-3′ (δ 82.5 and 81.2, respectively) and the high field position of C-1′ (δ 103.0) of the central β-glucopyranosyl moiety was consistent with glycosylation at C-2′ and C-3′ (Rønsted et al., 2003a). The assignments of the NMR spectra were partly based on the DQF-COSY, gHSQC and gHMBC spectra, and the expected correlations were seen. Thus, in the gHMBC spectrum, a connectivity from the H-2′ of the of the glucopyranosyl moiety (δ 3.66) to C-1‴′ (δ 104.5) of the of the xylopyranosyl group verified the position of the latter. Similarly, a connectivity from H-3′ (δ 3.98) to C-1″ (δ 103.1) of the rhamnopyronosyl moiety proved that this was placed at the 3′-*O*-position. The structure of compound **26** corresponded therefore to 2′-*O*-β-xylopyranosyl verbascoside, and we have named it hebeoside. Apparently, this is the first CPG reported with a xylopyranosyl group in the 2′-*O*-position.

2.4. Veronicastrum

A few reports exist on the constituents of this genus which has about 20 species. Aucubin (**10**), mannitol (**16**) and arbutin (**28**) were isolated from *V. axillare* (Sieb. et Zucc) T. Yamaz (Liu et al., 1999), while **10** and **16** together with catalpol (**11**), minecoside (6-*O*-isoferuloylcatalpol), 6-*O*-veratroylcatalpol and 6-deoxy-8-isoferuloylharpagide were reported from *V. sibiricum* (Lee et al., 1987, 1988).

Table 3
NMR data (CD_3OD) for **15i** and **15j** and the model compound **15**

Atom	**15i**		**15j**		**15**[a]	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
Agluc						
1	5.11(*d*, 9.7)	95.2	5.10 (*d*, 9.2)	95	5.07 (*d*, 10)	95.2
3	6.40 (*dd*, 6.0; 1.6)	142.4	6.40 (*dd*, 6.0; 1.6)	142	5.35 (*dd*, 6; 2)	142.2
4	5.12 (*dd*, 6.0; 4.5)	103.4	5.13 (*dd*, 6.0; 4.5)	103	5.05 (*dd*, 6; 5)	103.6
5	2.49 (*m*)	37.3	2.52 (*m*)	37	2.38 (*m*)	37.4
6	4.08 (*br.d*, 8.1)	84.4	4.09 (*br.d*, 8.1)	84	3.99 (*dd*, 8; 2)	83.7
7	3.69 (*br.s*)	59.4	3.68 (*br.s*)	59	3.62 (*d*, 2)	59.4
8		66.6		66		66.6
9	2.59 (*dd*, 9.7; 7.6)	43.4	2.59 (*dd*, 9.4; 7.8)	43.3	2.54 (*dd*, 10; 8)	43.4
10	4.16 (*d*, 13.1)	61.5	4.15 (*d*, 12.9)	61.5	4.13 (*d*, 13)	61.5
	3.82 (*d*, 13.1)		3.82 (*d*, 12.9)		3.81 (*d*, 13)	
Glc						
1′	4.78 (*d*, 7.9)	99.7	4.75 (*d*, 9.0)	99	4.77 (*d*, 8)	99.8
2′	3.26 (*dd*, 9; 8)	74.8	ca. 3.3	74	3.25 (*dd*, 9; 8)	74.9
3′	3.40 (*t*, 8.9)	77.7	3.40 (*t*, 8.8)	77	3.38 (*t*, 9)	77.8
4′	3.25 (*t*, 9)	71.8	ca. 3.3	71	3.24 (*dd*, 8; 10)	71.8
5′	3.3 (obsc.)	78.6	ca. 3.3	78	3.3 (*m*)	78.7
6′	3.92 (*dd*, 11.9; 2.0)	63.0	ca. 3.9	63	3.91 (*dd*, 12; 2)	63.0
	3.63 (*dd*, 11.9; 6.6)		3.63 (*dd*, 12.0; 6.5)		3.61 (*dd*, 12; 6)	
Rha						
1″	5.05 (*d*, 1.7)	100.4	5.10 (*d*, 1.9)	98	4.92 (*d*, 2)	100.4
2″	4.17 (*dd*, 2.6; 1.8)	70.3	5.46 (*dd*, 3.5; 1.9)	70	3.84 (*dd*, 3; 2)	72.3
3″	5.39 (*dd*-like)	73.3	5.29 (*dd*, 9.7; 3.5)	?	3.67 (*dd*, 9; 3)	72.4
4″	5.36 (*t*-like, 10.2)	72.7	3.73 (*t*, 9.7)	?	3.38 (*t*, 9)	73.9
5″	4.07 (obsc.)	68.3	ca. 3.9	?	3.65 (*m*)	70.2
6″	1.24 (*d*, 6.3)	17.9	1.37 (*d*, 6.3)	18	1.25 (*d*, 6)	17.9
Aroyl						
1‴		135.6; 135.5		135		
2‴/6‴	7.54 (4H)	129.9	7.62; 7.52 (4H)	130		
3‴/5‴	7.35 (4H)	129.3; 129.2	7.41 (4H)	129; 129		
4‴	7.41, 7.35 (2H)	131.6; 131.5	7.35 (2H)	131		
β	7, 69; 7.68 (*d′s*, 16.0)	147.3; 147.1	7, 72; 7.67 (*d′s*, 16)	147		
α	6.49; 6.48 (*d′s*, 16.0)	118.4; 118.1	6.63; 6.49 (*d′s*, 16)	118; 118		
CO		167.7		167; 167		

[a] Data from Helfrich and Rimpler (1999).

Later, this species was also reported to give scrophularioside (6′-cinnamoylaucubin), 6-dimethoxycinnamoylmyoporoside and 6-*O*-feruloylcatalpol (Lin et al., 1995).

In the present work, we had access to *V. sibiricum* and *V. virginicum* and the two species were very similar in content. Thus both species contained mannitol (**16**) as the main carbohydrate, they contained the simple iridoids **10** and **11** as well as ajugol (**12**) and in addition both had arbutin (**28**) as a constituent. These observations are similar to the previous findings. The CPG ehrenoside (**25**) was isolated from *V. sibiricum*. The other species also contained one or more CPGs, but none of these were isolated in the pure state. However, we could not detect any 6-*O*-esters of catalpol, instead we found that both species contained 6-*O*-rhamnopyranosylcatalpol (**15**) and a number of its esters (Table 1). *V. sibiricum* had thus the 2″-cinnamoyl (**15a**) and 3″-cinnamoyl (**15b**) as well as 3″-coumaroyl (**15d**) esters of **15**, the latter being the major constituent. In *V. virginicum* **15b** was the major component; **15d** was also present and in addition two previously unknown dicinnamoyl esters of **15** were found, namely **15i** and **15j**. However, only the former was isolated in the pure state, the other compound was solely characterized by NMR.

Compound **15i** was found to have the elemental composition $C_{39}H_{44}O_{16}$ by HRESIMS. The ^{13}C NMR spectrum (Table 3) had the expected number of signals representing: (i) a catalpol moiety (15 C), (ii) two cinnamoyl moieties (18 C) and finally a substituted α-rhamnopyranosyl moiety (6 C). The latter is obviously attached to the catalpol moiety at the 6-position as seen from the low field position of the signal from C-6 (δ 84.4) when compared to that of catalpol (δ 79.6) (Chaudhuri et al., 1980). The positions of the two cinnamoyl moieties were deduced from the ^1H NMR spectrum (Table 3) where large downfield shifts were seen for the H-3″ and the H-4″ signals (δ 5.39 and 5.36, respectively) whereas the H-2″ signal was found at δ 4.17, demonstrating that this position was not esterified. The compound **15i** is therefore 3″,4″-dicinnamoyl-6-*O*-rhamnopyranosylcatalpol.

The NMR spectra of **15j** (Table 3) were of poorer quality, yet they were very similar to those of **15i** showing that the two compounds were analogues and that they contained the same functionalities. Again it was evident that the α-rhamnopyranosyl moiety was attached to C-6 (δ 84) of the catalpol moiety, and again the positions of the two cinnamoyl moieties were deduced from the ^1H NMR spectrum (Table 3). In this case, large downfield shifts were seen for the H-2″ and the H-3″ signals (δ 5.46 and 5.29, respectively) whereas the H-4″ signal was found at δ 3.73, showing that compound **15j** is 2″,3″-dicinnamoyl-6-*O*-rhamnopyranosylcatalpol. The reported (Lee et al., 1987; Lin et al., 1995) presence of 6-*O*-catalpol esters in *V. sibiricum* could not be confirmed. In our experience, ^1H NMR spectra in MeOH-d_4 can conveniently be used to distinguish between the two types of catalpol esters, even in complex mixtures. Thus, in the compounds derived from **15** the signals of the 5- and 9-protons are consistently well separated by ca. 0.15 ppm so each of the two signals can be clearly distinguished at 300 MHz and above. Conversely, the signals from these protons in the 6-*O*-catalpol esters are consistently more or less overlapped at ca. δ 2.6. This makes it possible to inspect spectra of inseparable mixtures and decide whether one or the other type of compounds is present. A signal from CPGs are also present in this NMR-region, namely at δ 2.8, but this does not interfere. Therefore, we are convinced that no 6-*O*-catalpol esters were present in either of the two species investigated in the present work. We have no explanation regarding the findings of Lee et al. (1987), but the spectrum given for 6-*O*-feruloylcatalpol by Lin et al. (1995) is not the same as that previously reported for the compound by Stuppner and Wagner (1989). As seen from the shift values given for H-5 (δ 2.48) and H-9 (δ 3.00) it is obviously not a 6-*O*-acylester of catalpol, and we therefore conclude that compounds of this type are not present in *Veronicastrum* species.

2.5. Wulfenia

Only one report on constituents of this genus, which comprise four species, has been published (Arnold et al., 2002). In this study, the underground parts of *W. carinthiaca* Jack. were shown to contain the esters of iridoid glucosides isoscrophularioside (**10a**), globularin (**11d**) and the bis-iridoid wulfenoside (**14**) together with the CPGs plantamajoside (**23**) and its mono- and diacetyl (**24**) esters.

In the present work we have investigated whole plants of the three remaining species. Mannitol (**16**) was again shown to be the major carbohydrate present in two of the species, but not in *W. blechicii* subsp. *rohleanae*. On the other hand, shikimic acid was detected in the carbohydrate fraction from this species and from *W. orientalis*. The three species investigated contained one or more of the iridoid acids epiloganic acid (**1**), gardoside (**2**), mussaenosidic acid (**3**) or arborescosidic acid (**5**). In addition they all had aucubin (**10**) and catalpol (**11**) as well as the esters **10a** and **11d** and also some minor components which were not common to all three species. Notably, 6-*O*-esters of catalpol were not found in any of these species. *W. baldaccii* was the species most similar to *W. carinthiaca* in containing **14**, **23** and **24**, whereas *W. blechicii* subsp. *rohleanae* had neither of these compounds but instead dihydromonotropein (**6**) an iridoid glucoside not previously known from the family. *W. baldaccii* was the only one producing the chlorine containing compound asystasioside E (**13**) and its ester, the new iridoid **13a**.

Compound **13a** was isolated as an amorphous solid with the elemental composition $C_{24}H_{29}O_{11}Cl$ as established by HRESIMS. The ^{13}C NMR spectrum of **13a** (Table 2) showed 22 signals of which six could be assigned to a β-glucopyranosyl moiety, another set of seven (including two of double intensity) was assigned to a cinnamoyl moiety. The remaining nine signals corresponded to an iridoid aglucone very similar to that of asystasioside E (**13**) (Demuth et al., 1989), except that the signal arising from the 10-CH_2 group in **13** resonated at δ 66.3 instead of δ

62.4 (in D_2O), the low field shift indicating that this is the point of acylation. A low field shift for the 10-CH_2 protons is also seen in the ^1H NMR spectrum (δ 4.56/4.33 versus 3.91/3.70 for **13** in D_2O). The assignments of the NMR spectra were partly based on the DQF-COSY and gHSQC spectra and the position of the cinnamoyl group was confirmed by a clear correlation between the 10-CH_2 protons and the carbonyl carbon at δ 168.2 in the gHMBC spectrum. Also, direct comparison with spectra of a series of substituted cinnamoyl esters of **13** isolated from *Premna subscandens* Merr. (Verbenaceae; Sudo et al., 1997) showed a convincing similarity. We have named the new compound baldaccioside. Asystasioside E has so far only been isolated from *Mackaya* (*Asystasia*) *bella* Harv. (Acanthaceae; Demuth et al., 1989). It is notable that all the investigated species of *Wulfenia* contained 8,9-unsaturated iridoids, either arborescosidic acid (**5**) or its derivative wulfenoside (**14**).

2.6. Sibthorpia

The genus *Sibthorpia* (five species) has not been investigated previously for chemical constituents other than flavonoids. The two species available for the present work were unusual in not containing mannitol (**16**) and being devoid of iridoid glucosides. However, the CPGs verbascoside (**20**) and poliumoside (**22**) were found in both species.

2.7. Ellisiophyllum

This small Asian genus of only one species (*E. pinnatum*) has never been chemically investigated before. Again, mannitol (**16**) was absent and 8-epiloganic acid (**1**) was isolated in trace amount as the sole iridoid. The main CGP was verbascoside (**20**) accompanied by calceolarioside C and E (**18** and **19**) together with a trace of calceolarioside A (**17**).

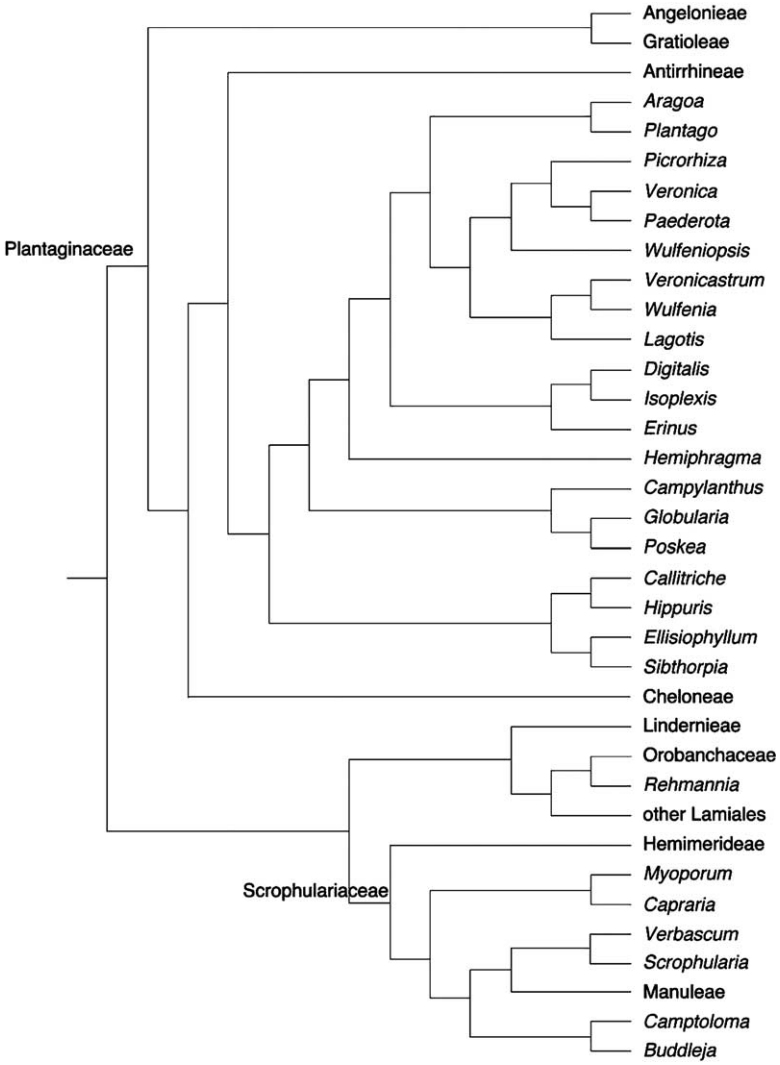
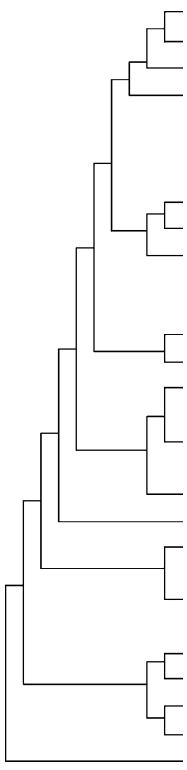


Fig. 1. Phylogenetic relationships of tribes and genera in the "new" Plantaginaceae based on the combined analysis of ITS, plastid *trnL-F, rps16* intron, and *matK-trnK* intron DNA regions (by courtesy of Dirk Albach; modified from Albach et al., 2005).

Table 4
Collected chemical data[a] compared to the phylogenetic tree based on DNA analysis[b]

Cladogram based on DNA sequence data	Genus	Main sugar(s)	Auc/cat (**10/11**)	6-*O*-Esters of **11**	10-*O*-Esters of **10/11**	6-*O*-Rha-esters of **11**	8,9=[c]	CPGs or simple phenylethanoids	References
	*Veronica s. str.*	Mannitol	**10**, **11**	+	–	–	+	**23**-like, + cornoside	Jensen et al. (2005)
	Veronica subg. *Hebe*	Mannitol	**10**, **11**	+	–	+	+	**20**, **21**, **22**, **26**,	This work; Kellam et al. (1993)
	Paederota	Mannitol	**10**, **11**	+	–	–	+	None	Albach et al. (2004b)
	Picrorhiza	Mannitol	**10**, **11**	+	+	n.i.	n.i.	**23** and similar	Kitagawa et al. (1971), Jia et al. (1999), Mandal and Mukhopadhyay (2004), Wang et al. (1993), Li et al. (1998)
	Veronicastrum	Mannitol	**10**, **11**	–	–	+	–	**25**, **27**, **28**	This work
	Wulfenia	Mannitol	**10**, **11**	–	+	–	+	**23**, **24**	This work
	Lagotis	n.i.	**10**, **11**	n.i.	+	n.i.	n.i.	**20**, **23**, **25**, lagotoside	Kooiman (1970), Calis et al. (1991), Zong and Che (1995), Yang et al. (2003, 2004a,b)
	Aragoa	Sorbitol	**10**, **11**	–	+	–	–	**23**-like	Rønsted et al. (2003a)
	Plantago	Sorbitol	**10**, **11**	–	+	–	+	**20**, **23**	Rønsted et al. (2000, 2003b)
	Digitalis	Sorbitol	–	–	–	–	–	**17**, cornoside forsythiaside	Taskova et al. (2005)
	Isoplexis	Sorbitol	–	–	–	–	–	**17**, **27**, cornoside forsythiaside	Taskova et al. (2005)
	Erinus	Mannitol[d]	**10**	–	–	+	+	**22**	Taskova et al. (2005)
	Hemiphragma	Mixture[d]	**10**, **11**[d]	–	+	–	–	**23** +	Ma et al. (1995)
	Campylanthus	Mannitol + sorbitol	**10**	–	–	–	–	**27**	Rønsted and Jensen (2002)
	Globularia	Mannitol	**10**, **11**	–	+	–	+	**20** and more	This work; and see references in the text
	Callitriche	Mixture[d]	**10**, **11**	–	+	–	–	**20**	Damtoft et al. (1994)
	Hippuris	Mixture[d]	**10**, **11**	–	–	–	–	**20**	Damtoft et al. (1994)
	Ellisiophyllum	Sucrose	–	–	–	–	–	**17**, **18**, **19**, **20**	This work
	Sibthorpia	Mixture	–	–	–	–	–	**20**, **22**	This work
	Camptoloma	Mixture	**10**, **11**	–	–	+	–	**20**	This work

[a] "+" present; "–" not detected; n.i. not investigated.
[b] Combined analysis of ITS, plastid *trnL-F, rps 16* intron, and *matK–trnK* intron DNA regions; modified from Albach et al. (2005).
[c] Iridoids with an 8,9-double bond (i.e., **5** and analogues).
[d] Jensen (unpubl.); re *Erinus* (see Note added in proof).

2.8. Globularia

This genus of ca. 20 species has been exceptionally well investigated. Globularin (**11d**) was first described from *G. alypum* by Di Maio and Panizzi (1966). Mannitol (**16**) and **11d** were reported from *G. salicina* by Fikenscher et al. (1969). Later, asperuloside (**7**), aucubin (**10**) and catalpol (**11**) together with a number of compounds related to **11d** were described from *G. cordifolia* and *G. alypum* (Chaudhuri and Sticher, 1980, 1981). More recently, Calis and co-workers (Calis et al., 1999, 2001, 2002a,b; Kirmizibekmez et al., 2003, 2004a,b) have investigated a number of Turkish species and reported many of the previously known iridoids together with new ones of which some were related to arborescosidic acid (**5**). In addition, a number of CPGs were identified from this genus.

In the present work, we had access to *G. vulgaris*, which had not previously been investigated, and this species contained as expected mannitol (**16**) as the main carbohydrate. We isolated the iridoid acids gardoside (**2**), mussaenosidic acid (**3**) and geniposidic acid (**4**), of which **2** and **3** one are new for the genus. Aucubin (**10**), catalpol (**11**) and the known iridoid esters asperuloside (**7**), 10-benzoylcatalpol (**11c**) and globularin (**11d**) were also present in this species and in addition the new benzoyl ester **8**.

Compound **8** was isolated as an amorphous solid with the elemental composition $C_{23}H_{24}O_{11}$ as established by HRESIMS. The ^{13}C NMR spectrum of **8** (Table 2) showed 21 signals of which six could be assigned to a β-glucopyranosyl moiety, another set of five (including two of double intensity) to a benzoyl moiety; the remaining ten signals were in accordance with an iridoid aglucone very similar to that of asperuloside (**7**) except for the signals from the acetyl group in the latter. The ^1H NMR spectrum also demonstrated that the new compound was very similar to asperuloside, but with a benzoyl group at C-10 instead of an acetyl group. The assignments of the NMR spectra were partly based on the DQF-COSY and gHSQC spectra and the position of the benzoyl group was confirmed by a clear correlation between the 10-CH_2 protons (δ 4.92 and 5.03) and the carbonyl carbon at δ 167.3 in the gHMBC spectrum. Due to the similarity of the two compounds we have named the new compound besperuloside.

2.9. Chemotaxonomic implications

The ''new'' Plantaginaceae consist of a central core of tribes and genera more or less closely related to *Veronica* as well as the less related tribes Cheloneae, Antirrhineae, Gratioleae and Angelonieae. The phylogenetic relationships found by the DNA sequence analysis are illustrated in Fig. 1. The chemical characters are summarized in Table 4, including present and previously published results, and plotted against the relevant parts of the phylogram based on DNA sequences.

Two types of iridoid glucosides encountered in the present work are taxonomically distinctive. Firstly, compounds with a 8,9-double bond, e.g., arborescosidic acid (**5**) and its derivative wulfenoside (**14**), have so far solely been encountered in the central core of tribes and genera, namely in *Plantago*, *Veronica*, *Paederota*, *Wulfenia*, *Erinus* and *Globularia* (Taskova et al., 2005, and references therein). Secondly, the 6-*O*-catalpol esters (e.g., **11a** and **11b**) have a very limited distribution. These compounds are almost obligatory in *Veronica*, *Paederota* and *Picrorhiza*, otherwise they have only been reported from some Bignoniaceae (von Poser et al., 2000) and as a minor constituent in *Ballota undulata* (Sieb. ex Fresen.) Bentham (Siciliano et al., 2005).

Regarding the presence of 6-*O*-rhamnopyranosylcatalpol esters, these have previously been reported from Lamiaceae: *Gmelina*, *Premna* and *Holmskioldia* (Helfrich and Rimpler, 1999, and ref. cited therein); in Scrophulariaceae *s. str.*: *Scrophularia*, *Verbascum*, *Buddleja* and *Jamesbrittenia* (see references below – Section 2.9.7); and in Plantaginaceae: *Erinus* (Taskova et al., 2005). We have now found them in *Camptoloma*, *Hebe* and *Veronicastrum*. Although this chemosystematic character seems to have evolved independently at least three times within Lamiales, it may become a useful marker at lower taxonomic levels.

The chemotaxonomic discussion below is concentrated on the central Plantaginaceae taxa and deals with each tribe separately.

2.9.1. Veroniceae

According to the latest circumscription of Albach et al. (2004a), the tribe Veroniceae comprises nine genera and DNA sequence results for seven of these are known (Fig. 1). The tribe consists of two branches, one with *Veronica*, *Paederota* and *Picrorhiza*, the other with *Wulfenia*, *Veronicastrum* and *Lagotis*. *Wulfeniopsis* occupies an intermediate position, but seems closest to the former group. For six of those chemical data are available (Table 4). The observed complex iridoid profiles show interesting affinities and may imply extensive hybridization processes within the tribe.

Mannitol (**16**) was found to be the sugar characteristic for Veroniceae. Most of its representatives contain also CPGs (excluding *Paederota*), aucubin (**10**), catalpol (**11**) and a variety of catalpol esters. The latter are of two main types, namely 6-*O*-esters and 10-*O*-esters. The 6-*O*-catalpol esters are limited to *Veronica* (including subg. *Hebe*) and its closest allies, *Paederota* and *Picrorhiza*. Within this group, the most similar chemical profiles are those of *Veronica* and *Paederota*.

In the first DNA sequence investigation (ITS sequences; Albach and Chase, 2001), *Paederota* was found to have originated from within *Veronica*, emerging as a clade sister to subgenus *Veronica* ($x = 9$), the latter being characterized by mussaenoside and 6-*O*-catalpol esters of cinnamic acid. The iridoid evidence, however, is not in direct support of such a relationship since *Paederota* contains only 6-*O*-catalpol esters of benzoic acid derivatives (Grayer-Barkmeijer, 1979; Albach et al., 2004b). Therefore the chemical evidence corroborates the recent plastid DNA sequence

analyses (Albach and Chase, 2004) where *Paederota* appears as sister to genus *Veronica*.

In the last century, New Zealand and South American *Hebes* were segregated from *Veronica* sensu von Wettstein (1891) and accepted as four separate genera, *Hebe*, *Parahebe*, *Chionohebe*, and *Heliohebe* (Pennell, 1921; Garnock-Jones, 1993). Recently, however, Albach et al. (2004a) placed these taxa back into a large genus *Veronica*. Our finding of the 6-*O*-catalpol esters, verproside (**11a**) and veminoside (**11b**), in the three species of *Hebe* is indeed in support of their close affinity. However, **11a** and **11b** are only found in small amounts and the main iridoids in *Hebe* are the rhamnopyranosylcatalpol esters, which clearly distinguishes it from all the other studied subgenera of *Veronica*. Interestingly, rhamnopyranosylcatalpol esters are the main iridoid constituents in *Veronicastrum* also. This could imply a separate alloploid origin for *Hebe* and *Veronicastrum* from a common ancestor containing rhamnopyranosylcatalpol and its esters. However, the apparent lack of 6-*O*-catalpol esters in *Veronicastrum* is not consistent with this view.

The results from nuclear ribosomal and plastid DNA sequences did not agree on the relationships between *Veronicastrum* and the other members of the wulfenoid grade (Albach and Chase, 2004). In the analyses of the different DNA regions *Veronicastrum* was either a sister to *Lagotis*, a sister to *Wulfenia* and *Lagotis*, or a sister to *Picrorhiza* and *Wulfeniopsis*. Based on the chromosome numbers and molecular data, the authors hypothesized that an ancestor of *Wulfeniopsis* ($x = 8$) had hybridized with an ancestor of *Wulfenia* ($x = 9$) to give the ancestor of *Veronicastrum* ($x = 17$) or with *Veronica*/*Paederota* ($x = 9$) to give the ancestor of *Picrorhiza* ($x = 17$). The iridoid composition of *Veronicastrum* does not support the hypothesis of its alloploid origin involving ancestors of *Wulfenia*. The latter contains 10-*O*- and 6′-*O*-esters of catalpol which have not been found in *Veronicastrum* where the main constituents are rhamnopyranosylcatalpol esters. However, iridoid evidence clearly supports the alloploid origin *of Picrorhiza* with ancestors of *Veronica* as a parental group. *Picrorhiza* contains 6-*O*-catalpol esters of benzoic and cinnamic acid derivatives, characteristic for *Veronica*, and in addition, a number of 6′-*O*-esters of catalpol (picroside I–III) and 10-*O*-catalpol esters found also in *Wulfenia*, a close relative to the suggested other parent, *Wulfeniopsis* (which was segregated from the former by Hong, 1980). Further investigations of the iridoid composition of *Wulfeniopsis* would be of great interest and expected to shed more light on the phylogeny of this group.

The second group of esters, characteristic for Veroniceae, the 10-*O*-catalpol esters are usually accompanied by 10-*O*-esters of aucubin. They are the main iridoid constituents of *Wulfenia* and *Lagotis* and were also found in *Picrorhiza*. Mannitol is the main sugar in *Wulfenia*, which corroborates molecular evidence and shows it to be a member of Veroniceae. However, its relationship with the other tribe members remains unclear. *Wulfenia* was found to be sister to *Veronicastrum* and *Lagotis* in analyses of the *trnL-F* DNA region, sister to *Lagotis* in the *rps16* analysis or sister to *Paederota* and *Veronica* in analysis of ITS sequences (Albach and Chase, 2004). Indeed, *Wulfenia* and *Lagotis* have a similar chemical composition, both containing 10-*O*-esters of catalpol and aucubin. But some iridoid markers, such as 6′-*O*-esters of catalpol (picroside I) and bisiridoids, link *Wulfenia* also to *Picrorhiza* and *Globularia*. The chemical evidence indicate a history of hybridization and possible common ancestors for those three genera.

2.9.2. Plantagineae

Plantago used to be accepted as a separate family, and *Aragoa* was traditionally considered as part of Digitaleae/Veroniceae but the DNA sequence data placed it as a sister to *Plantago*, not to *Veronica* and its closest allies (Bello et al., 2002). The chemical evidence, in agreement with the molecular data (Fig. 1), supports the recognition of *Plantago* and *Aragoa* as the tribe Plantagineae. The chemical features specific to this plant group are: sorbitol, 10-*O*-esters of catalpol, plantamajoside (**23**) and its derivatives (Rønsted et al., 2003a,b). The iridoids found in *Aragoa* are typical for *Plantago* subg. *Albicans* where catalpol and 10-*O*-esters of catalpol are the main iridoid constituents.

2.9.3. Digitaleae

Digitalis and *Isoplexis* contain cardenolides and completely lack iridoids. However, their main sugar compound, sorbitol (a hexitol like mannitol) and the type of CPGs link this group to Veroniceae–Plantagineae clade (Taskova et al., 2005). In *Erinus*, the presence of 6-*O*-rhamnopyranosylcatalpol esters are rather unexpected, but the latter may occasionally replace other esters of catalpol like in *Veronicastrum* (and *Veronica* subg. *Hebe*).

2.9.4. Hemiphragma

This genus contains **10**, **11** and some 10-*O*-esters of these (i.e., globularin (**11d**)) as well as the CPG plantamajoside (**23**) and fits well as a member of Plantaginaceae. Some of the compounds present in the plant are very similar to those found in *Wulfenia* and *Lagotis*, and others are similar to those of *Globularia*.

2.9.5. Globularieae

The systematic position and relationships of *Globularia* have been subject of much discussion. However, based on the DNA sequence results, Albach et al. (2005) placed *Globularia* in tribe Globularieae together with *Poskea* and *Campylanthus* (Fig. 1). Chemical evidence shows *Globularia* as a close relative of Veroniceae. It contains mannitol (**16**), 10-*O*-catalpol esters and CPGs that are characteristic for the members of Veroniceae. Furthermore, interesting similarities of the iridoid composition *of Globularia*, *Wulfenia* and *Lagotis* are evident. Globularin (**11d**) is the main iridoid constituent in all studied representatives of the two genera and wulfenoside (**14**) is very similar to the *Globularia* bisiridoids (Calis et al., 2001; Kirmizibekmez et al., 2003).

Campylanthus has traditionally been considered as a member of Digitaleae/Veroniceae (von Wettstein, 1891). However, in the latest revisions of the genus, Miller (1980) and Hjertson (2003) stated that there are no obvious relatives of *Campylanthus* in Digitaleae and even within the Scrophulariaceae *s.l.* The anomaly of the genus has been confirmed also based on intranuclear inclusions (Bigazzi, 1993) and root systems development (Licht, 1983). The molecular systematic studies have indicated a position of *Campylanthus* near *Globularia* and *Poskea* or as sister to Digitalideae–Veroniceae clade (Albach et al., 2005). Chemical characters again suggest an alloploid origin, but also show the genus as a true member of Plantaginaceae. Thus, the main carbohydrates in *Campylanthus salsoides* (L.f.) Roth are the hexitols: mannitol, characteristic for Veroniceae and *Globularia*, and sorbitol, found in Plantagineae and Digitaleae. Lack of catalpol and presence of aucubin and iridoids of melittoside type are characters similar to those found in *Plantago* subg. *Coronopus* section *Maritima* but also in *Globularia cordifolia* (Kirmizibekmez et al., 2004b). The CPGs isolated from *Campylanthus* are typical for the representatives of *Plantago*, Veroniceae and Digitaleae.

2.9.6. Sibthorpieae and Callitricheae

Sibthorpia was also originally included in Digitaleae (von Wettstein, 1891). Chemically, the genus is peculiar in lacking iridoids (Table 4). However, when comparing it with the other taxa lacking iridoids, namely *Digitalis* and *Isoplexis*, considerable differences are found. Thus, (i) the carbohydrates were mainly glucose and sucrose, not sorbitol, (ii) cardenolides have not been reported for *Sibthorpia*, and (iii) no C_6–C_2 glycosides (**27** or cornoside) were found which are main compounds in both *Digitalis* and *Isoplexis* (Taskova et al., 2005). On the other hand, the chemical evidence supports the recent molecular analysis (Fig. 1), where *Sibthorpia* form a clade with another genus almost devoid of iridoids, namely *Ellisiophyllum*. The main compound in both genera is verbascoside (**20**) and both lack simple phenylethanoids which are otherwise widespread within Plantaginaceae. The carbohydrate composition, the presence of verbascoside (**20**) and the lack of simple phenylethanoids may also relate Sibthorpieae to *Callitriche* and *Hippuris* (Callitricheae), although these genera are capable of producing iridoids (Damtoft et al., 1994). This is in agreement with the molecular data, which place Sibthorpieae and Callitricheae in a single clade, sister to the central core of Veroniceae and its closest allies (Albach et al., 2005).

2.9.7. Camptoloma

Camptoloma was initially placed in Digitaleae by Bentham (1876), although with reservation, since he had not seen the aestivation of the corolla lobes. Subsequently, floral morphology linked this group with the representatives of the tribe Manuleeae and many authors included *Camptoloma* in *Sutera*/*Lyperia*. Hilliard (1994) did not find *Camptoloma* to be closely allied to *Sutera* and accepted it as a small genus (of 3 species) in Manuleeae. Most recently, the DNA sequence results (Oxelman et al., 2005) have demonstrated that *Scrophularia* and *Verbascum* form a group sister to an extended Manuleeae, and to *Camptoloma* and *Buddleja* in Scrophulariaceae (Oxelman et al., 2005).

The chemical data is in line with this: neither sorbitol nor mannitol (**16**) were detected in *Camptoloma lyperiiflorum* and the main compound was aucubin (**10**), accompagnied by small amounts of catalpol (**11**) and bartsioside (**9**). Two rhamnopyranosylcatalpol esters (**15e** and **15k**) in low concentrations were isolated, but not the parent rhamnopyranosylcatalpol (**15**). The compound **15e** is known from *Buddleja* (Miyase et al., 1991), *Jamesbrittenia* (Cogne et al., 2003), *Scrophularia* (Miyase and Mimatsu, 1999) and *Verbascum* (Agababyan et al., 1982; Warashina et al., 1991; Akdemir et al., 2004), while **15k** is only known from *Scrophularia* (Giner et al., 1998; Miyase and Mimatsu, 1999). Mannitol (**16**) was not found in the investigated species of *Scrophularia* and *Verbascum* (Hegnauer, 1973). Thus, the chemical profile of *Camptoloma* do not show particular affinity to the studied representatives of Plantaginaceae, but rather to members of the new Scrophulariaceae *s. str.* (sensu APG, 2003), and this is consistent with the molecular data analysis.

3. Experimental

3.1. General

Fresh or frozen plant material was homogenized with EtOH (4 × weight) and filtered. The dry plant sample was ground, blended with EtOH and left for 7 days. The concentrated extracts were partitioned in Et_2O–H_2O. The aq. phase was taken to dryness and separated by prep. chromatography using reverse phase Merck Lobar RP-18 SiGel columns (size B and C) or Merck HiBar column (250–25) packed with LiChrosorb RP-18 (7μm) and H_2O–MeOH mixtures as the eluents. Compounds are listed in order of elution; the proportion of mannitol (**1**) was estimated from the ^{13}C NMR spectrum of the crude (polar) sugar fraction. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova-500 or Mercury-300 instruments in D_2O or MeOH-d_4 using the solvent peak (δ 4.75, 3.31 or 49.0) as the internal standard. In the cases where ^{13}C NMR spectra were recorded in D_2O the C-6′ shift was set to 61.5 ppm (Damtoft et al., 1981). LC-HR ESIMS was performed on an Agilent HP 1100 Liquid Chromatograph equipped with a BDS-C18 reversed phase column running a water–acetonitrile (50 ppm TFA in water) gradient. The LC was coupled to a LCT of a TOF MS (Micromass, Manchester, UK) operated in the negative or positive electrospray ion mode using 5-leucineenkephalin as lock mass. The known compounds isolated were identified by their NMR data: iridoids (**1**–**5**, **7**, **9**–**11**, **11c**, **11d**), verbascoside (**20**) and plantamajoside (**23**) were compared with authentic samples (Rønsted et al., 2000, 2003b). Spectra of the remaining compounds were compared with published data: dihydromonotropein (**6**)

(Jensen et al., 2002); isoscrophularioside (**10a**) (Junior, 1981); verproside (**11a**) (Afifi-Yazar and Sticher, 1980); verminoside (**11b**) (Sticher and Afifi-Yazar, 1979); picroside I (**11e**) (Chaudhuri et al., 1980); ajugol (**12**) (Damtoft et al., 1982); asystasioside E (**13**) (Demuth et al., 1989); wulfenoside (**14**) (Arnold et al., 2002); 6-α-*O*-rhamnopyranolsylcatalpol (**15**) and cinnamoyl esters (**15a**–**15c**) (Helfrich and Rimpler, 1999); **15d**, (Tatli et al., 2003); verbascoside A (**15e**) (Akdemir et al., 2004); caffeoyl esters **15f** and **15g** (Otsuka et al., 1989); 2″-feruloyl ester (**15h**) (Otsuka et al., 1991); scrovalentinoside (**15k**) (Giner et al., 1998; and by direct comparison to a spectrum recorded by Miyase and Mimatsu, 1999); mannitol (**16**) (Bock and Pedersen, 1983); calceolarioside A (**17**) (Nicoletti et al., 1986); calceolarioside C (**18**) (Nicoletti et al., 1988b); calceolarioside E (**19**) (Nicoletti et al., 1988c); arenarioside (**21**) (Andary et al., 1985); poliumoside (**22**) (Zhou et al., 1998); diacetyl-plantamajoside (**24**) (Arnold et al., 2002).

3.2. Plant material

Globularia vulgaris was collected on Öland, Sweden (Alvaren near Viddeby, June, 2002) and frozen at −23 °C. *Camptoloma lyperiiflorum* was grown from seeds originating from a specimen collected in S. W. Soqotra, Yemen (7.3.1996, A. Millar 14118 (E)). *Wulfenia baldaccii, W. orientalis* and *W. blechicii* subsp. *rohleanae* were grown from seeds at the experimental field of The Botanical Garden of Copenhagen in Tåstrup, Sealand, Denmark; the seeds were obtained from German botanical gardens and are all of unspecified origin. Cuttings of *Ellisiophyllum pinnatum* was donated from Crûg Farm Plants, UK, and grown in Tåstrup. Fresh plant material from *Veronica austriaca*, *V.* (*Hebe*) *salicifolia*, *V.* (*Hebe*) × *andersonii*, *V.* (*Hebe*) *ligustrifolia*, *Sibthorpia africana*, *Veronicastrum virginicum*, *V. sibiricum* were obtained from The Botanical Garden of Copenhagen, Denmark. Dry material from *Sibthorpia europaea* L. (IOK-25/2002) from the Botanical Garden of Bonn, Germany. Vouchers (see Table 1) were authenticated by Dr. Dirk Albach and deposited at the Herbarium of Vienna.

3.2.1. Camptoloma lyperiiflorum

Fresh plant material (117 g) gave 2.9 g of crude extract. Chromatography (C-column; 1:0 to 1:1.5) gave a sugar fraction (0.7 g) consisting of mixed sugars, a 3:1 mixture (60 mg) of salt of mussaenosidic acid (**3**) and catalpol (**11**), a fraction with mainly gardoside (**2**, 125 mg), aucubin (**10**, 560 mg), a fraction (120 mg) with a 3:3:1 mixture of epiloganic acid (**1**), mussaenosidic acid (**3**) and bartsioside (**9**), verbascoside (**20**, 330 mg), followed by fraction B (170 mg) containing a mixture of 6-*O*-rhamnopyranosylcatalpol esters. Part of fraction B (90 mg) was additionally separated (HiBar column, 1:1.5 to 1:2) to give 6-*O*-(4″-*O*-methoxycinnamoyl) rhamnopyranosylcatalpol (verbascoside A; **15e**, 14 mg) and 6-*O*-(2″,4″-diacetyl-3″-*O*-*p*-methoxycinnamoyl) rhamnopyranosylcatalpol (scrovalentinoside; **15k**, 3 mg).

3.2.2. Ellisiophyllum pinnatum

Fresh plant material (124 g) gave 2.8 g of crude extract. Chromatography (C-colum, 1:0 to 1:1) gave a sugar fraction (1.3 g) consisting of mixed sugars, impure epiloganic acid (**1**, 10 mg), calceolarioside A (**17**, 20 mg), a 1:1 mixture (315 mg) of calceolarioside C (**18**) and E (**19**) and impure verbascoside (**20**, 400 mg).

3.2.3. Globularia vulgaris

Frozen plant material (67 g) gave 3.5 g of crude extract. Chromatography (C-column, 1:0 to 1:1) gave a sugar fraction (1.3 g) with mainly mannitol (**16**, 85%), catalpol (**11**, 80 mg), aucubin (**10**, 10 mg), a 1:1 mixture (20 mg) of gardoside (**2**) and geniposidic acid (**4**), pure geniposidic acid (20 mg), mussaenosidic acid (**3**, 75 mg), asperuloside (**7**, 55 mg), 10-benzoylcatalpol (**11c**, 40 mg), globularin (**11d**, 670 mg), and a mixture (190 mg), 100 mg of which was rechromatographed (HiBar column, 1:1) to give globularin (**11d**, 25 mg) and besperuloside (**8**,15 mg).

3.2.4. Veronica (Hebe) ligustrifolia

Fresh plant material (66 g) gave 3.5 g of crude extract. Chromatography (C-column, 1:0 to 1:1) gave a sugar fraction (1.3 g) with mainly mannitol (**16**, >90%), catalpol (**11**, 225 mg), a 1:2:4 mixture (180 mg) of arborescosidic acid (**5**), aucubin (**10**), and 6-*O*-rhamnopyranosylcatalpol (**15**), 6-*O*-(3″-*O*-caffeoyl) rhamnopyranolsylcatalpol (**15g**, 200 mg), 6-*O*-(2″-*O*-caffeoyl) rhamnopyranolsylcatalpol (**15f**, 225 mg), followed by fractions A and B (85 and 100 mg, respectively). Fraction A (85 mg) was rechromatographed (HiBar column, 1.7:1 and 1.5:1) to give arenarioside (21, 10 mg) and 6-*O*-(2″-*O*-*trans*-feruloyl) rhamnopyranolsylcatalpol (**15h**, 10 mg). Fraction B gave similarly (HiBar column, 1.5:1) poliumoside (**22**, 32 mg), and verminoside (**11b**, 10 mg).

3.2.5. Veronica (Hebe) salicifolia

Fresh plant material (67 g) gave 3.5 g of crude extract. Chromatography (Lobar C-column; 1:0 to 1:1.5) provided a sugar fraction (1.3 g) with mainly mannitol (**16**, >90%), catalpol (**11**, 45 mg), a 10:1 mixture (70 mg) of aucubin (**10**) and rhamnopyranosylcatalpol (**15**), a 1:2:1 mixture (20 mg) of gardoside (**2**), geniposidic acid (**4**) and arborescosidic acid (**5**), followed by three fractions A–C containing mixtures of catalpol esters and CPGs. Fraction A (50 mg) gave by rechromatography (B-column; 3.5:1) 6-*O*-(3″-*O*-caffeoyl)-rhamnopyranosylcatalpol (**15g**; 10 mg). Fraction B (875 mg) gave similarly (B-column; 2.5:1) an addnl amount of **15g** (65 mg), verproside (**11a**, 65 mg) and a fraction with CPGs (450 mg) which was not further explored. Fraction C (540 mg) gave (HiBar column, 2.5:1; several injections) the new hebeoside (2′-β-xylopyranosyl-verbascoside; **26**, 23 mg), verminoside (**11b**, 33 mg), and poliumoside (**22**, 78 mg).

3.2.6. Veronica (Hebe) × andersonii

Fresh plant material (75 g) gave 3.5 g of crude extract. Chromatography (C-column, 1:0 to 1:1) gave a sugar fraction

(1.1 g) with mainly mannitol (**16**, >85%), catalpol (**11**, 45 mg), a 2:1 mixture (180 mg) of aucubin (**10**) and rhamnopyranosylcatalpol (**15**), a fraction (10 mg) with mainly 8-epi-loganic acid (**1**), 6-*O*-(3″-*O*-caffeoyl)-rhamnopyranosylcatalpol (**15g**, 170 mg), 6-*O*-(2″-*O*-caffeoyl)-rhamnopyranosylcatalpol (**15f**, 275 mg), fractions A and B (410 and 190 mg), 6-*O*-(2″-*O*-cinnamoyl)-rhamnopyranosylcatalpol (**15a**, 185 mg) and fraction C (65 mg). Fraction A contained both 6-*O*-rhamnopyranosylcatalpol esters and CPGs, but was not further explored. Part of fraction B (120 mg) gave (HiBar column, 1.5:1) poliumoside (**22**, 35 mg), verminoside (**11b**, 20 mg) and 6-*O*-(3″-*O*-cinnamoyl)-rhamnopyranosylcatalpol (**15b**, 15 mg). Fraction C gave (HiBar column, 1:1.5) 6-*O*-(4″-*O*-cinnamoyl)-rhamnopyranosylcatalpol (**15c**, 5 mg).

3.2.7. *Sibthorpia africana*

Fresh plant material (97 g) gave a crude extract (3.0 g). Chromatography (C-column; 1:0 to 1:1.5) gave first a sugar fraction (1.7 g) with mainly glucose and sucrose, descaffeoylverbascoside (50 mg), verbascoside (**20**, 540 mg), poliumoside (**22**, 180 mg), and isoverbascoside (80 mg).

3.2.8. *Sibthorpia europaea*

Dry plant material (15 g) was brought to boiling with EtOH (100 ml), homogenized and left to stand for 1 week. Work-up gave crude extract (0.1 g), and chromatography (B-column; 1:0 to 1.5:1) gave a sugar fraction (85 mg; mainly glucose), verbascoside (**20**, 6 mg) and poliumoside (**22**, 4 mg).

3.2.9. *Veronica austriaca*

Fresh plant material (40 g) gave a crude extract (2.0 g). Chromatography (B-column, 1:0 to 1:1) gave a sugar fraction (0.7 g) with mainly mannitol (**16**, >90%), catalpol (**11**, 25 mg), aucubin (**10**, 60 mg), a 3:1 mixture (50 mg) of veproside (**11a**) and verbascoside (**20**), unseparated mixture of verbascoside-like compounds (160 mg), and isoscutellarein 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl(1‴→2″)-β-glucopyranoside (100 mg).

3.2.10. *Veronicastrum sibiricum*

Fresh plant material (52 g) gave a crude extract (3.5 g). Chromatography (C-column; 1:0 to 1:1.5) gave a sugar fraction (1.4 g) with mainly mannitol (**16**, 90%), a 2:1 mixture (85 mg) of arbutin (**28**) and catalpol (**11**), aucubin (**10**; 55 mg), 6-rhamnopyranosyl-catalpol (**15**; 55 mg), a 4:1 mixture (45 mg) of ajugol (**12**) and epiloganic acid (**1**), 6-*O*-(3″-*O*-*p*-coumaroyl)-rhamnopyranosylcatalpol (**15d**, 140 mg), followed by five fractions, A–E (total 750 mg) containing mixtures of esters. By chromatography (HiBar column, 2:1 to 1:2) of these fractions, A gave ehrenoside (**25**, 180 mg), B gave 6-*O*-(3″-*O*-cinnamoyl)-rhamnopyranosylcatalpol (**15b**, 50 mg), and D gave 6-*O*-(2″-*O*-cinnamoyl)-rhamnopyranosylcatalpol (**15a**, 10 mg).

3.2.11. *Veronicastrum virginicum*

Frozen plant material (38 g) gave 3.5 g of crude extract. Chromatography (C-column, 1:0 to 0:1) gave a sugar fraction (1.7 g) with mainly mannitol (**16**, 85%), a fraction with mainly arbutin (**28**; 60 mg), a 10:1 mixture (190 mg) of **28** and catalpol (**11**), a 4:1 mixture (55 mg) of aucubin (**10**) and rhamnopyranosylcatalpol (**15**), ajugol (**12**; 30 mg), salidroside (**27**, 10 mg), followed by three fractions A–C (total 1.05 g), containing mixtures of CPGs and iridoid esters. By chromatography (HiBar column, 2:1 to 1:2) of these fractions, A gave 6-*O*-(3″-*O*-*p*-coumaroyl)-rhamnopyranosylcatalpol (**15d**, 60 mg), B gave 6-*O*-(3″-*O*-cinnamoyl)-rhamnopyranosylcatalpol (**15b**, 135 mg). Finally, C gave the new compounds 6-*O*-(3″,4″-*O*-di-cinnamoyl)-rhamnopyranosylcatalpol (**15i**, 10 mg) and 6-*O*-(2″,3″-*O*-di-cinnamoyl)-rhamnopyranosylcatalpol (**15j**, 2 mg).

3.2.12. *Wulfenia baldaccii*

Fresh plant material (61 g) gave 3.5 g of crude extract. Chromatography (C-column, 1:0 to 1:1) gave a fraction with sugars (1.6 g) with mainly mannitol (**16**, 90%), a 3:1 mixture (180 mg) of catalpol (**11**) and asystasioside E (**13**) with a trace of epiloganic acid (**1**), aucubin (**10**,180 mg), plantamajoside (**23**, 305 mg), globularin (**11d**, 425 mg), followed by three fractions A–C (480, 115 and 80 mg), containing mixtures of CPGs and iridoids. An aliquot (150 mg) of fraction A was rechromatographed (HiBar column, 1.5:1) to give 2′,6″-diacetyl plantamajoside (**24**, 120 mg) and picroside I (**11e**, 10 mg). Fraction B (115 mg) gave (HiBar column, 1:1) 2′,6″-diacetyl plantamajoside (**24**,10 mg), wulfenoside (**14**, 15 mg) and the new baldaccioside (**13a**, 25 mg). Fraction C (80 mg) gave (HiBar column, 1:1) isoscrophularioside (**10a**, 10 mg).

3.2.13. *Wulfenia orientalis*

Fresh plant (56 g) gave a crude extract (2.1 g), which was chromatographed (B-column, 1:0 to 1:1) to give a sugar fraction (740 mg) with mainly (40% each) of mannitol (**16**) and shikimic acid, a 5:1 mixture (140 mg) of catalpol (**11**) and gardoside (2), aucubin (**10**, 230 mg), mussaenosidic acid (**3**, 10 mg), arborescosidic acid (**5**, 50 mg), globularin (**11d**, 575 mg), 2′,6″-di-*O*-acetyl-plantamajoside (**24**, 10 mg) and isoscrophularioside (**10a**, 80 mg).

3.2.14. *Wulfenia blechicii subsp. rohleanae*

Frozen plant material (170 g) gave a crude extract (3.1 g), which was chromatographed (C-column, 1:0 to 1:1) to give a fraction with mixed sugars and shikimic acid (2.1 g), a fraction with mainly catalpol (**11**, 10 mg), 6,7-dihydromonotropein (**6**, 30 mg), aucubin (**10**, 30 mg), gardoside (**2**, 30 mg), a 1:1 mixture (60 mg) of epiloganic acid (**1**) and arborescosidic acid (**5**), a mixture of *cis*-globularin and globularin (**11d**, 140 mg), pure **11d** (380 mg), picroside I (**11e**, 60 mg), and isoscrophularioside (**10a**, 90 mg).

3.3. Besperuloside (*8*)

Amorphous solid: $[\alpha]_D^{20} = -115^\circ$ (MeOH; *c* 0.2); LC-HR ESIMS *m/z*: 521.1266 $[M + HCOO]^-$; ($C_{24}H_{25}O_{13}$ requires

521.1295); ^{1}H and ^{13}C NMR (500 and 125 MHz, CD_3OD) in Table 2.

3.4. Baldaccioside (*13a*)

Amorphous solid: $[\alpha]_D^{20} = -119°$ (MeOH; *c* 0.8); LC-HR ESIMS *m/z*: 527.1323 $[M - H]^-$; ($C_{24}H_{28}O_{11}^{35}Cl$ requires 527.1320); ^{1}H and ^{13}C NMR (500 and 125 MHz, CD_3OD) in Table 2.

3.5. 6-O-(3″,4″-O-Di-cinnamoyl)-rhamnopyranosylcatalpol (*15i*)

Amorphous solid: $[\alpha]_D^{20} = -60°$ (MeOH; *c* 0.5); LC-HR ESIMS *m/z*: 786.2910 $[M + NH_4]^+$; ($C_{39}H_{48}NO_{16}$ requires 786.2973); ^{1}H and ^{13}C NMR (500 and 125 MHz, CD_3OD) in Table 3.

3.6. 6-O-(2″,3″-O-Di-cinnamoyl)-rhamnopyranosylcatalpol (*15j*)

Impure sample solely characterized by NMR in Table 3.

3.7. Hebeoside (*26*)

Amorphous solid: $[\alpha]_D^{20} = -59°$ (MeOH; *c* 0.2); LC-HR ESIMS *m/z*: 774.2784 $[M + NH_4]^+$; ($C_{34}H_{48}NO_{19}$ requires 774.2820); ^{1}H and ^{13}C NMR (500 and 125 MHz, CD_3OD) in Table 2.

Note added in proof

A reinvestigation of fresh whole *Erinus alpinus* plants extracted with boiling ethanol showed that the sugar fraction contained almost exclusively mannitol.

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