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Iridoid glucosides in the genus *Veronica* (Plantaginaceae) from New Zealand

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A B S T R A C T

Four simple iridoid glucosides, three known esters of catalpol, seven esters of aucubin, and two phenylethanoids were isolated from *Veronica hookeri* (syn. *Hebe ciliolata*; Plantaginaceae). Of these, none of four aromatic (*p*-methoxybenzoyl, isovanilloyl, veratroyl, caffeoyl) 6-*O*-esters of aucubin and 6''-*O*-benzoyl mussaenosidic acid, had been reported from nature before. Similarly, three simple iridoid glucosides, two esters of 6-*O*-rhamnopyranosylcatalpol, and two phenylethanoid glucosides, as well as 1-*O*-benzoyl-3- α -glucuronosylglycerol, and 1-*O*- β -benzoyl rutinose were isolated from *Veronica pinguifolia* (syn. *Hebe pinguifolia*). The compound 3''-*O*-benzoyl-2''-*O*-caffeoyl 6-*O*-rhamnopyranosylcatalpol had not been reported previously. The pattern of the structural features of the iridoid glucosides is overlaid onto the latest molecular phylogenetic framework of *Veronica* sects. *Hebe* and *Labiatooides*, and discussed in the context of evolutionary trends.

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1. Introduction

During the last decade we have investigated the chemical constituents of the genus *Veronica*. Historically, the early work of Renée Grayer-Barkmeijer (1973; 1979) indicated that the genus was characterized by the iridoid glucosides aucubin (**5**) and catalpol (**4**) together with a number of aromatic esters of the latter. Those observations from extensive investigations based on paper chromatography were confirmed more recently by actual isolation and de-novo structure elucidation of the compounds from various species (Taskova et al., 2002; 2004). However, most of the above more extensive investigations were based on thin-layer chromatography, although the actual compounds had in fact been isolated from plants belonging to the genus. In a review on isolated compounds (Jensen et al., 2005), we could demonstrate that the subgenus *Chamaedrys* deviated from the common theme of other *Veronica* spp., being characterized mainly by cornoside (an oxidation product of salidroside (**2**)) while only one out of the nine species investigated contained aucubin and none of them catalpol. Later, we undertook an investigation of Bentham's (1846) tribe *Veroniceae* (Taskova et al., 2005; 2006) including three species of *Veronica* sect. *Hebe*, which at that point had never been investigated for iridoid glucosides other than by Grayer-Barkmeijer (1973). It appeared that all of these contained aucubin (**5**) and catalpol (**4**) together with at least one catalpol ester, namely verminoside (**4b**), but in addition they all contained several esters of 6-*O*-rhamnopyranosylcatalpol (e.g. **6a**), compounds which had not previously been detected in *Veronica*. Following this lead, we continued work on *V.* subg. *Pseudoveronica* using cultivated specimens of sect. *Hebe* (Johansen et al., 2007; Pedersen et al., 2007) and sect. *Labiatooides* (Jensen

et al., 2008). This was followed by material collected in the wild in New Zealand (Maggi et al., 2009; Taskova et al, 2010; 2011; 2013). We have now investigated *Veronica hookeri* (Buchanan) Garn.-Jones (syn. *Hebe ciliolata* (Hook.f.) Cockayne & Allan) and *V. pinguifolia* Hook. f. (syn. *Hebe pinguifolia* (Hook.f.) Cockayne & Allan), collected also from wild populations in New Zealand.

2. Results and discussion

Fresh material of *V. hookeri* was extracted with methanol and chromatographed on reverse phase silica gel to obtain the iridoid glucosides mussaenosidic acid (**1**), 6-epiaucubin (**3**), aucubin (**5**), mussaenoside (**1a**), three catalpol esters: verminoside (**4b**), 6-*O*-isovanilloylcatalpol (**4a**), minecoside (**4c**), seven aucubin esters: derwentioside A (**5a**), derwentioside B (**5b**), 6-*O*-*p*-methoxybenzoyl aucubin (**5c**), topiarioside (**5d**), 6-*O*-isovanilloyl aucubin (**5f**), 6-*O*-veratroyl aucubin (**5g**), 6-*O*-caffeoyl aucubin (**5h**), as well as 6'-*O*-benzoyl mussaenosidic acid (**1b**) and the phenylethanoids salidroside (**2**) and ehrenoside (**7b**). Of these, **5c**, **5f**, **5g**, **5h** and **1b** appeared to be previously undescribed.

Compound **5c** was slightly impure but had the elemental composition C₂₃H₂₈O₁₁ as established by HR-ESIMS. The NMR spectra of **5c** in methanol-*d*₄ (Table 1) were assigned by the usual 1 and 2D NMR data. The ¹³C NMR spectrum showed 21 signals, of which two were of double intensity. A set of six peaks could be assigned to a β-glucopyranosyl moiety. Another set of five, including those of double intensity, together with an aromatic methoxyl peak at δ_C 56.4, represented a *p*-methoxy-benzoyl group. The remaining nine signals represented an iridoid aglucone very similar to that of aucubin (**5**), except that the signals assigned to C-6 and C-7 were seen 3-5 ppm downfield and those from C-5 and C-8 were moved upfield when compared to those of **5**. This indicated that the C-6 oxygen atom was esterified with the *p*-methoxy-benzoyl group. Inspection of the ¹H NMR spectrum confirmed that this was indeed the case since the H-6 signal at δ_H 5.53 was more than 1 ppm downfield from that present in the spectrum of **5**. Further proof could not be obtained from the HMBC spectrum since no correlation could be seen between H-6 (δ_H 5.53) and the carbonyl carbon atom of the benzoyl group (δ_C 167.8). However, when comparing the spectra with those of the known derwentioside B (**5b**, 6-*O*-*p*-hydroxy-benzoyl aucubin; Jensen et al., 2008), the signals of the aucubin moiety were almost identical in the two cases. Therefore, compound **5c** was 6-*O*-*p*-methoxy-benzoyl aucubin. According to SciFinder, this structure has been reported before from

Crescentia alata (Bignoniaceae) (Ramírez-Cisneros et al., 2015 as “*Crescentia alata*”). However, the reported ^{13}C NMR data for their compound, which they named 6-*epi-O-p*-methoxybenzoyl aucubin, clearly exhibit the presence of an epoxide ring between C-7 and C-8 rather than a double bond. It follows that their compound **4** in the report must be the catalpol analogue.

Compound **5f** had the elemental composition $\text{C}_{23}\text{H}_{28}\text{O}_{12}$ as established by HR-ESIMS. The NMR data of **5f** in methanol- d_4 (Table 1) were assigned as above and by comparison with the spectra of **5c** it could be shown to contain a 6-*O*-substituted aucubin moiety, accounting for 15 ^{13}C NMR signals. Of the remaining eight signals in the ^{13}C NMR spectrum, seven could be assigned to a benzoyl group substituted in the 3- and 4-positions with oxygen atoms. The last signal (δ_{C} 56.4) proved to be a methoxyl group. This indicated the presence of either a vanilloyl or an isovanilloyl moiety in **5f**; however, comparison with the spectra of derwentioside C (**5e**), the known vanilloyl ester (Jensen et al., 2008), showed that the two compounds were not identical. The presence of an isovanilloyl moiety was confirmed by the NOESY spectrum, where the *O*-methyl protons (δ_{H} 3.90) showed conclusively one correlation, namely to the H-5" signal (δ_{H} 6.97; d , 8.5 Hz). Compound **5f** was therefore determined as 6-*O*-isovanilloyl aucubin.

Compound **5g** was also slightly impure but shown by HR-ESIMS to have the elemental composition $\text{C}_{24}\text{H}_{30}\text{O}_{12}$. The NMR spectra of **5g** in methanol- d_4 (Table 1) were assigned as above and were rather similar to the spectra of **5f** except that the methoxyl signal (δ_{C} 56.5) in the ^{13}C NMR spectrum was of double intensity and that the pattern of the aromatic signals had changed, while those from the aucubin moiety were identical in the two compounds. In the ^1H NMR spectrum, two methoxyl groups appeared at δ_{H} 3.85 and 3.88 in the HMBC spectrum; these could be seen to correlate with C3" (δ_{C} 150.2) and C4" (δ_{C} 154.8), respectively. Therefore compound **5g** was shown to be 6-*O*-veratroyl aucubin.

Compound **5h** had the elemental composition $\text{C}_{24}\text{H}_{28}\text{O}_{12}$ as established by HR-ESIMS. The NMR spectra of **5h** in methanol- d_4 (Table 1) were assigned as above and by comparison with the spectra of **5c** it was again evident that it contained a 6-*O*-substituted aucubin moiety. The ^{13}C NMR spectrum showed only 23 signals, however, one of these (δ_{C} 115.1) was of double intensity. In the ^1H NMR spectrum, signals from a typical 3,4-dioxygenated benzene as well as from a *trans*- α,β -conjugated carbonyl system. Taken together with the elemental composition as well as the ^{13}C

NMR data, this showed that the acid moiety in this case was a caffeoyl unit. In conclusion, compound was deduced to be 6-*O*-caffeoyl aucubin.

The last compound obtained from this plant (**1b**) was shown by HR-ESIMS to have the elemental composition $C_{23}H_{28}O_{11}$. The NMR data (Table 1) were assigned by comparison with those of mussaenosidic acid (**1**). The ^{13}C NMR spectrum contained the expected 21 signals, of which two were of double intensity. Of these signals, 16 could be assigned to a mussaenosidic acid moiety, although the signals for C-5' (δ_C 75.6) and C-6' (δ_C 64.7) had moved upfield and downfield, respectively, relative to those of **1**. The remaining five signals were consistent with the presence of a benzoyl group in **1b**. In the 1H NMR spectrum, signals from a benzoyl group were present as well as a set of signals from a mussaenosidic acid moiety. However, the two H-6' protons resonated about 0.9 ppm downfield at δ_H 4.68 and 4.50, compared to those seen in the spectrum of **1**, showing that this was the point of attachment for the benzoyl group. This was further confirmed by the HMBC spectrum where two protons at C-6' could be seen to correlate with CO" (δ_C 167.8), and this proved that **1b** was 6'-*O*-benzoyl mussaenosidic acid. An analogous compound, namely the *p*-hydroxybenzoyl ester was initially reported from *Vitex negundo* (Sehgal et al., 1983).

Fresh material of *V. pinguifolia* gave similarly the following compounds: mussaenosidic acid (**1**), catalpol (**4**), aucubin (**5**), 1-*O*-benzoyl-3- α -glucuronosylglycerol (**8**), 1-*O*- β -benzoyl rutinose, verbascoside (**7a**), hebeoside (**7c**), 3''-*O*-caffeoyl 6-*O*-rhamnopyranosylcatalpol (**6a**) and 3''-benzoyl-2''-*O*-caffeoyl 6-*O*-rhamnopyranosylcatalpol (**6b**). Of these, compound **6b** had not been reported before.

The elemental composition $C_{37}H_{42}O_{18}$ of compound **6b** was determined by HR-ESIMS. The ^{13}C NMR spectrum in methanol- d_4 (Table 2) showed only 35 signals but two of these (δ_C 129.6 and 130.6) were of double intensity. Comparison with the known compound **6a** also found in the plant showed the presence of a catalpol moiety (15 C) with a substitution at 6-OH, a caffeoyl moiety (9 C) as well as six signals from a rhamnosyl unit somewhat different from the pattern seen in the spectrum of **6a**. The remaining seven signals were coincident with a benzoyl group. This was consistent with the 1H NMR spectrum which was partly similar to that of **6a**. The main differences were seen at low field exhibiting a set of signals from the benzoyl group, but also it was found that the signals from H-2'' and H-3'' (δ_H 5.49 and 5.40) were considerably downfield from those of **6a** (Table 2). In the HMBC spectrum, H-1'' (δ_H 5.10) showed a correlation with C6 (δ_C 84.4), proving

the position of the rhamnosyl moiety. Also, H-2" (δ_{H} 5.49) correlated with CO'" (δ_{C} 168.0), while H3" (δ_{H} 5.40) correlated with CO"" (δ_{C} 167.4), demonstrating that the caffeoyl and the benzoyl groups were esterified to the 2"- and 3"- oxygen atoms, respectively. Therefore, the structure of **6b** was 3"-*O*-benzoyl-2"-*O*-caffeoyl 6-*O*-rhamnopyranosylcatalpol.

A number of different aromatic 6-*O*-esters of catalpol (e.g. **4a-c**) have been found in most members of the genus *Veronica*, including those of Eurasian origin (Table 3; Jensen et al. 2005) and many of these are also present in several other genera and families. Similar esters of aucubin (**5a-h**) are much rarer, in fact they have so far only been reported from *Veronica* subg. *Pseudoveronica*. The esters of 6-*O*-rhamnopyranosylcatalpol (e.g. **6a/b**) were found in this work and are also present in other species of sect. *Hebe* but not among the other subgenera of *Veronica*. Elsewhere in the Plantaginaceae such compounds have been reported from *Erinus* (Taskova et al.; 2005) and *Veronicastrum* (Taskova et al.; 2006). They are otherwise common, particularly in the Scrophulariaceae (De Santos Galindez et al.; 2001) but also in other families. In Table 3, we have listed the occurrence of the above-mentioned esters reported from the investigated species of subg. *Pseudoveronica*, together with the 3-*O*-glucosyl-phenyl-ethanoids (**7**) which also seem to be limited to this subgenus.

The topology of our phylogenetic analysis (Fig. 1) resembles that of previous analyses including more taxa (e.g. Wagstaff et al. 2002; Meudt et al. 2015) with the exception of the basal unsupported branches. However, chemically similar species are also supported by the phylogenetic analysis of DNA sequences. Thus, our results suggest that the production of 6-*O*-rhamnopyranosylcatalpol esters (**6**) was gained once (possibly originating from the basic compound **6** so far only found in *V. catarractae*) in the evolution of the group emerging at the origin of the clade comprising *V. odora* and its sister group, and being lost once in the ancestor of *V. brachysiphon*, *V. stenophylla* and *V. topiaria*.

3. Experimental

3.1. General

One-dimensional ^1H , ^{13}C NMR and 2D DQF-COSY, gHSQC, gHMBC and NOESY NMR spectra were recorded on a 400 MHz Bruker Avance III equipped with a BBO Prodigy probe in CD_3OD and the chemical shifts are given as δ values with reference to the solvent peaks (δ_{H} 3.30 or δ_{C} 49.0). HRMS data were obtained on a Thermo Scientific LTQ-Orbitrap XL mass spectrometer. The

samples were introduced through a Thermo Accela 1250 HPLC system on a 150 mm Phenomenex Luna C₁₈ column with 10–100% MeOH gradient in water (containing 0.1% formic acid throughout), via an electrospray ion source in positive mode. Chromatography was performed on a Merck Lobar RP-18 column (size C) eluting with H₂O-MeOH mixtures (1:0 to 1:1); compounds are listed in order of elution. Semi-preparative HPLC was conducted on a Waters system consisting of 600 pump, 717-plus autosampler, and 996 photodiode array detector (Milford, MA, USA), using a Genesis C₁₈ column (10 mm diam. × 250 mm; Jones Chromatography, Mid Glamorgan, UK) at 30 °C and with MeCN-H₂O mixtures at a flow rate of 4.0 ml/min. The solvent compositions were as detailed below. UV absorption maxima were taken directly from on-line LC-UV detector. The isolated known compounds were identified by comparison of the NMR data with those of authentic compounds (**1-5**, **4a-c**, **6a**, **7a-b**) or the published NMR data: 1-*O*-benzoyl-3- α -glucuronosylglycerol (**8**) (Cai et al., 2011).

3.2 Plant material

Veronica hookeri (Buchanan) Garn.-Jones was collected from the natural habitat, Mount Arthur Ridge, the South Island, 1540 m above sea level, 9th January 2007 (41.212° S, 172.699° E).

Veronica pinguifolia Hook. f. was collected from its natural habitats in Black Birch Range, Marlborough, 1552 m above sea level, the South Island, New Zealand, 23rd March 2006 (41.750° S, 173.772° E). The population of *V. pinguifolia* at Black Birch Range is characterised by smaller leaves and lower growth than is typical of the species elsewhere, and although plants there have sometimes been identified as *V. buechananii* we agree with Bayly and Kellow 2006 who assigned them to *V. pinguifolia* (as *Hebe pinguifolia*). The voucher specimens have been deposited at the Museum of New Zealand Te Papa Tongarewa (WELT).

3.3 Compound isolation

3.3.1 *V. hookeri*

Plant material (209 g, fresh wt) was blended with MeOH and filtered. The concentrated extract (22.3 g) was partitioned between Et₂O-H₂O, and the aqueous phase was taken to dryness. Part of this (1.2 g) crude extract was loaded on a Merck Lobar C-column and eluted with H₂O-MeOH mixtures (25:1 to 1:1) to give mussaenosidic acid (**1**, 50 mg), semipure 6-epiaucubin (**3**, 10 mg),

aucubin (**5**, 20 mg), salidroside (**2**, 5 mg), mussaenoside (**1a**, 20 mg), topiarioside (**5d**, 120 mg), impure ehrenoside (**7b**; 30 mg), derwentioside B (**5b**; 20 mg), a fraction A (40 mg), 6-*O*-isovanilloyl aucubin (**5f**; 30 mg), fractions B (15 mg), C (30 mg) and D (30 mg) followed by semimpure 6-*O*-*p*-methoxybenzoyl aucubin (**5c**; 9 mg).

Fractions A to D were separately rechromatographed with C₁₈ semi-preparative HPLC, yielding from fraction A (MeCN–H₂O, 17:83, v/v) verminoside (**4b**; 5 mg), **5b** (4 mg), 6-*O*-isovanilloylcatalpol (**4a**; 2 mg) and **5f** (11 mg). Fraction B (MeCN–H₂O, 17:83, v/v) 6-*O*-caffeoyl aucubin (**5h**; 4 mg) and a further amount of **5f** (6 mg). Fraction C (MeCN–H₂O, 20:80, v/v) gave 6'-*O*-benzoylmussaenosidic acid (**1b**; 5 mg) and 6-*O*-veratroylaucubin (**5g**; 8 mg). From fraction D were obtained (MeCN–H₂O, 26:74, v/v): **4c** (5 mg) and derwentioside A (**5a**; 4 mg).

3.3.2 *V. pinguifolia*

Plant material (130 g, fresh wt) was blended with MeOH and filtered and treated as above to give the concentrated aqueous extract (25 g). Part of this (1.2 g) was chromatographed as above to give: a mixt. of catalpol and mussaenosidic acid (**4** and **1**; 2:1, 20 mg), 1-*O*-benzoyl-3- α -glucuronosylglycerol (**8**; 25 mg), aucubin (**5**; 5 mg), 1-*O*- β -benzoyl rutinoside (55 mg), fraction E (10 mg), impure verbascoside (**7a**; 45 mg), hebeoside (**7c**; 25 mg), and fraction F (50 mg).

Rechromatography of fraction E on HPLC with MeCN–H₂O (15:85, v/v) gave: 3''-*O*-caffeoyl 6-*O*-rhamnosylcatalpol (**6a**; 2 mg). Working-up of fraction F required a greater proportion of the organic modifier (MeCN–H₂O, 27:73, v/v) in isocratic elution, to obtain 3''-*O*-benzoyl-2''-*O*-caffeoyl 6-*O*-rhamnosylcatalpol (**6b**; 3 mg).

3.4 6'-*O*-Benzoylmussaenosidic acid (**1b**)

UV $\lambda_{\max}$: 231 nm; ¹H and ¹³C NMR data: see Table 1; HR ESIMS *m/z*: 498.1962 [M+NH₄]⁺ (calcd for [C₂₃H₂₈O₁₁+NH₄]⁺, 498.1970).

3.5 6-*O*-*p*-Methoxybenzoylaucubin (**5c**)

¹H and ¹³C NMR data: see Table 1; HR ESIMS *m/z*: 498.1963 [M+NH₄]⁺ (calcd for [C₂₃H₂₈O₁₁+NH₄]⁺, 498.1970).

3.6 6-O-Isovanilloylaucubin (**5f**)

UV λ_{max} : 264, 297 nm; ^1H and ^{13}C NMR data: see Table 1; HR ESIMS m/z : 514.1911 $[\text{M}+\text{NH}_4]^+$ (calcd for $[\text{C}_{23}\text{H}_{28}\text{O}_{12}+\text{NH}_4]^+$, 514.1919).

3.7 6-O-Veratroylaucubin (**5g**)

UV λ_{max} : 264, 295 nm; ^1H and ^{13}C NMR data: see Table 1; HR ESIMS m/z : 528.2076 $[\text{M}+\text{NH}_4]^+$ (calcd for $[\text{C}_{24}\text{H}_{30}\text{O}_{12}+\text{NH}_4]^+$, 528.2068).

3.8 6-O-(E)-Caffeoylaucubin (**5h**)

UV λ_{max} : 329 nm; ^1H and ^{13}C NMR data: see Table 1; HR ESIMS m/z : 526.1917 $[\text{M}+\text{NH}_4]^+$ (calcd for $[\text{C}_{24}\text{H}_{28}\text{O}_{12}+\text{NH}_4]^+$, 526.1919).

3.9 3''-O-Benzoyl-2''-O-(E)-caffeoyl-6-O-rhamnopyranosylcatalpol (**6b**)

UV λ_{max} : 329 nm; ^1H and ^{13}C NMR data: see Table 2; HR ESIMS m/z : 792.2703 $[\text{M}+\text{NH}_4]^+$ (calcd for $[\text{C}_{37}\text{H}_{42}\text{O}_{18}+\text{NH}_4]^+$, 792.2709).

3.10 Phylogenetic analysis

DNA sequences of the internal transcribed spacer region of nuclear ribosomal DNA (ITS) were assembled from previous analyses for a data set of the 23 corresponding species (Table SI1). *Veronica* $\times$ *andersonii* Lindl. & Paxton (*V. speciosa* $\times$ *V. stricta* Benth.) was represented by a sequence of *V. speciosa*, and the hybrid of *V. odora* and whipcord spec. 2 by a sequence of *V. odora*. Six indels were coded manually as separate characters at the end of the data set. The data set was analyzed using the maximum likelihood criterion in PAUP 4.0a144 (Swofford 2002) using at GTR+ Γ -model in a heuristic search with 10 replicates of random taxon addition, gaps treated as missing data and TBR branch swapping. The search was repeated three times. One thousand bootstrap replicates were analyzed under the parsimony criterion starting from a random tree with a single replicate of random taxon addition per replicate and 50 trees maximum per replicate. *Veronica derwentiana* and *V. perfoliata* were coded as outgroup based on previous analyses (e.g. Albach & Meudt 2010).

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Figure legend:

Figure 1. Maximum likelihood tree of ITS analysis in both sections of *Veronica* subg.

Pseudoveronica. *V. derwentiana* and *V. perfoliata* represent sect. *Labiatooides* and the other species represent sect. *Hebe*. The ITS data set of 645 aligned resulted in a single optimal tree. Numbers above the branches are bootstrap percentages. Black and white boxes after species names indicate presence/absence of compounds following Table 3.

1; R=R'=H

1a; R=Me; R'=H

1b; R=H; R'=6'-O-Benzoyl

2

3

4; R=H; Catalpol

4a; R=Vanilloyl

4b; R=Caffeoyl

4c; R=isoFeruloyl

5; R=H; Aucubin

5a; R =Benzoyl

5b; R=p-OH-Benzoyl

5c; R=p-OMe-Benzoyl

5d; R=3,4-diOH-Benzoyl

5e; R=Vanilloyl

5f; R=isoVanilloyl

5g; R=3,4-diOMe-Benzoyl

5h; R=Caffeoyl

6; R'=R''=H

6a; R'=Caffeoyl; R''=H

6b; R'=Benzoyl; R''=Caffeoyl

7a; R'=H; R''=Rha; R'''=Caffeoyl

7b; R'=Ara; R''=Rha; R'''=Caffeoyl

7c; R'=Xyl; R''=Rha; R'''=Caffeoyl

8; 1-O-Benzoyl-3- α -glucuronosyl-glycerol

Table 1. ¹H (400 MHz) and ¹³C NMR (100 MHz) spectra of new esters of aucubin and mussaenosidic acid in methanol-*d*₄.

Atom	6- <i>O</i> -pMeOBz-aucubin (5c)		6- <i>O</i> -isovan-aucubin (5f)		6- <i>O</i> -Di-MeOBz-aucubin (5g)		6- <i>O</i> -Caff-aucubin (5h)		6'- <i>O</i> -Bz-mussaenosidic acid (1b)	
	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C
Agluc										
1	5.16 (<i>d</i> , 6.0)	96.6	5.16 (<i>d</i> , 5.8)	96.6	5.16 (<i>d</i> , 5.9)	96.6	5.14 (<i>d</i> , 5.9)	96.6	5.16 (<i>d</i> , 6.3)	95.6
3	6.31 (<i>dd</i> , 6.1, 1.9)	141.9	6.31 (<i>dd</i> , 6.0, 2.1)	141.9	6.31 (<i>dd</i> , 6.1, 1.9)	141.9	6.30 (<i>dd</i> , 6.2, 1.9)	141.8	7.38 (<i>br. s</i>)	152.0
4	5.09 (<i>dd</i> , 6.1, 3.6)	105.1	5.08 (<i>dd</i> , 6.0, 3.6)	105.1	5.10 (<i>dd</i> , 6.1, 3.6)	105.1	5.06 (<i>dd</i> , 6.2, 3.6)	105.1		113.5
5	3.03 (<i>m</i>)	42.4	3.02 (<i>m</i>)	42.4	3.03 (<i>m</i>)	42.5	2.95 (<i>m</i>)	42.4	3.14 (<i>q</i> -like, 6.2)	33.4
6	5.53 (<i>m</i>)	85.5	5.50 (<i>m</i>)	85.5	5.54 (<i>m</i>)	85.6	5.42 (<i>m</i>)	85.1	1.26, 2.25 (<i>m</i> 's)	30.9
7	5.90 (<i>m</i>)	125.9	5.89 (<i>m</i>)	125.9	5.90 (<i>m</i>)	125.8	5.85 (<i>m</i>)	126.0	1.57 (<i>m</i>)	39.8
8		151.9		151.9		152.0		151.7		81.1
9	3.11 (<i>br. t</i> , 6.7)	48.2	3.10 (<i>br. t</i> , 6.7)	48.2	3.11 (<i>br. t</i> , 6.8)	48.3	3.07 (<i>br. t</i> , 7.5)	48.2	2.06 (<i>dd</i> , 6.3, 8.2)	52.1
10	4.40 (<i>br. d</i> , 15.6)	61.2	4.39 (<i>br. d</i> , 15.6)	61.1	4.40 (<i>br. d</i> , 15.7)	61.2	4.38 (<i>br. d</i> , 15.5)	61.1	1.23 (<i>s</i>)	25.1
	4.22 (<i>br. d</i> , 15.6)		4.22 (<i>br. d</i> , 15.6)		4.22 (<i>br. d</i> , 15.7)		4.21 (<i>br. d</i> , 15.5)			
11										171.0
Glc										
1'	4.67 (<i>d</i> , 7.9)	99.8	4.67 (<i>d</i> , 7.9)	99.8	4.68 (<i>d</i> , 7.9)	99.8	4.67 (<i>d</i> 7.9)	99.8	4.74 (<i>d</i> , 7.9)	99.8
2'	3.22 (<i>dd</i> , 9.1, 7.9)	74.9	3.22 (<i>dd</i> , 9.1, 7.9)	74.9	3.22 (<i>dd</i> , 9.0, 7.9)	74.9	3.21 (<i>dd</i> , 9.2, 7.9)	74.9	3.24 (<i>m</i>)	74.8
3'	3.38 (<i>t</i> -like, 9.1)	77.9	3.38 (<i>t</i> -like, 9.1)	77.9	3.38 (<i>t</i> -like, 9.0)	77.9	3.37 (<i>m</i>)	77.9	3.41 (<i>m</i>)	77.1
4'	3.29 <i>obsc.</i>	71.6	3.29 <i>obsc.</i>	71.6	3.28 <i>obsc.</i>	71.6	3.27 <i>obsc.</i>	71.6	3.43 (<i>m</i>)	71.8
5'	3.29 <i>obsc.</i>	78.3	3.29 <i>obsc.</i>	78.3	3.28 <i>obsc.</i>	78.3	3.27 <i>obsc.</i>	78.3	3.62 (<i>m</i>)	75.6
6'	3.87 <i>obsc.</i>	62.7	3.87 (<i>br. d</i> , 12.1)	62.7	3.87 <i>obsc.</i>	62.7	3.87 (<i>br. d</i> , 11.5)	62.7	4.68 (<i>dd</i> , 11.8, 2.4)	64.7
	3.65 (<i>m</i>)		3.65 (<i>m</i>)		3.65 (<i>m</i>)		3.65 <i>m</i>		4.50 (<i>dd</i> , 11.8, 6.1)	
Aroyl										
CO		167.8		168.0		167.8		169.1		167.8
α''							6.24 (<i>d</i> , 15.9)	115.1		
β''							7.53 (<i>d</i> , 15.9)	146.9		
1''		123.7		124.0		123.8		127.7		131.4
2''	7.94 (<i>d</i> , 8.6)	132.6	7.40 (<i>d</i> , 2.0)	117.1	7.52 (<i>d</i> , 2.0)	113.3	7.02 (<i>d</i> , 2.0)	115.1	8.01 (<i>m</i>)	130.6
3''	6.98 (<i>d</i> , 8.6)	114.8		147.4		150.2		147.4	7.46 (<i>m</i>)	129.6
4''		165.2		153.5		154.8		149.7	7.59 (<i>m</i>)	134.4
5''	6.98 (<i>d</i> , 8.6)	114.8	6.97 (<i>d</i> , 8.5)	111.8	7.01 (<i>d</i> , 8.4)	111.9	6.77 (<i>d</i> , 8.2)	116.5	7.46 (<i>m</i>)	129.6
6''	7.94 (<i>d</i> , 8.6)	132.6	7.52 (<i>dd</i> , 8.5, 2.0)	123.4	7.64 (<i>dd</i> , 8.4, 2.0)	125.9	6.93 (<i>dd</i> , 8.2, 2.0)	123.0	8.01 (<i>m</i>)	130.6
OMe	3.85 (<i>s</i>)	56.0	3.90 (<i>s</i>)	56.4	3.88 (<i>s</i>), 3.85 (<i>s</i>)	2x 56.5				

Table 2. ^1H (400 MHz) and ^{13}C NMR (100 MHz) of 6-*O*-rhamnopyranosylcatalpol esters in methanol- d_4 .

Position	(6a)		(6b)	
	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}
Agluc				
1	5.09, <i>d</i> (9.6)	95.0	5.11, <i>d</i> (9.6)	95.2
3	6.38, <i>dd</i> (6.0, 1.6)	142.0	6.40, <i>dd</i> (6.0, 1.6)	142.4
4	5.10, <i>obsc</i>	103.4	5.14, <i>dd</i> (6.0, 4.5)	103.4
5	2.45, <i>m</i>	37.0	2.52, <i>m</i>	37.3
6	4.04 <i>br. d</i> (8.2)	83.7	4.09 <i>br. d</i> (8.1)	84.4
7	3.64, <i>br. s</i>	58.7	3.69, <i>br. s</i>	59.4
8		66.0		66.6
9	2.56, <i>dd</i> (9.6, 7.7)	43.1	2.59, <i>dd</i> (9.6, 7.7)	43.3
10	4.14, <i>d</i> (13.0)	61.3	4.16, <i>d</i> (13.1)	61.5
	3.80, <i>d</i> (13.0)		3.81, <i>d</i> (13.1)	
Glc				
1'	4.77, <i>d</i> (7.9)	99.5	4.78, <i>d</i> (7.9)	99.7
2'	3.24, <i>obsc</i>	74.7	3.25, <i>obsc</i>	74.9
3'	3.39, <i>t</i> -like (9.0)	77.6	3.39, <i>t</i> -like (9.0)	77.7
4'	3.24, <i>obsc</i>	71.6	3.24, <i>obsc</i>	71.8
5'	3.30, <i>obsc</i>	78.5	3.30, <i>obsc</i>	78.7
6'''	3.91, <i>d</i> (11.8, 1.9)	62.8	3.91, <i>obsc</i>	63.0
	3.61, <i>obsc</i>		3.62, <i>d</i> (11.8, 6.6)	
Rha				
1''	4.96, <i>d</i> (1.6)	100.0	5.10, <i>obsc</i>	97.9
2''	4.07, <i>dd</i> (1.6, 3.2)	70.1	5.49, <i>dd</i> (1.7, 3.5)	71.3
3''	5.08, <i>obsc</i>	75.2	5.40, <i>dd</i> (9.7, 3.5)	73.7
4''	3.66, <i>obsc</i>	71.2	3.78, <i>t</i> (9.7)	71.7
5''	3.80, <i>obsc</i>	70.2	3.95, <i>obsc</i>	70.3
6''	1.30, <i>d</i> (6.2)	17.8	1.38, <i>d</i> (6.2)	18.0
Caff				
1'''		127.7		127.5
2'''	7.05, <i>d</i> (1.9)	114.9	7.05, <i>d</i> (1.9)	115.2
3'''		146.2		147.0
4'''		149.2		150.1
5'''	6.77, <i>d</i> (8.2)	116.3	6.78, <i>d</i> (8.2)	116.6
6'''	6.95, <i>dd</i> (8.2, 1.9)	122.8	6.95, <i>dd</i> (8.2, 1.9)	123.3
α	6.34, <i>d</i> (16.0)	115.1	6.33, <i>d</i> (15.9)	114.1
β	7.63, <i>d</i> (16.0)	147.2	7.52, <i>d</i> (15.9)	148.2
CO'''		168.4		168.0
Benz				
1''''				131.3
2''''			7.97 (<i>m</i>)	130.7
3''''			7.41 (<i>t</i> -like, 7.6)	129.5
4''''			7.56 (<i>t</i> -like, 7.6)	134.1
5''''			7.41 (<i>t</i> -like, 7.6)	129.5
6''''			7.97 (<i>d</i> -like, 7.6)	130.7
CO''''				167.4

Table 3. Species of *Veronica* investigated.

Species name	6-esters of catalpol(4)	esters of 6- <i>O</i> - Rha-catalpol (6)	6-esters of aucubin (5)	3- <i>O</i> -glc-phenyl- ethanoids (7)	Ref.
<i>V. perfoliata</i> Raf.	x			x	Jensen et al., 2008
<i>V. derwentiana</i> Andrews			x		Jensen et al., 2008
<i>V. cheesemanii</i> Benth.	x		x		Maggi et al., 2009
<i>V. raoulii</i> Hook. f.	x			x	Taskova et al., 2012
<i>V. pentasepala</i> (L. B. Moore) Garn.-Jones	x			x	Taskova et al., 2012
<i>V. lavaudiana</i> Raoul	x			x	Taskova et al., 2011
<i>V. hulkeana</i> F. Muell.	x			x	Taskova et al., 2012
<i>V. hookeriana</i> Walp.	x			x	Maggi et al., 2009
<i>V. catarractae</i> G. Forst.		(6) ^a			Jensen et al., 2008
<i>V. pinguifolia</i> Hook. f.		x			This work
<i>V. odora</i> Hook. f. × "whipcord spec."		x			Johansen et al., 2007
<i>V. elliptica</i> G. Forst.		x			Johansen et al., 2007
<i>V. × andersonii</i> Lindl. et Paxton	x	x			Taskova et al., 2006
<i>V. salicifolia</i> G. Forst	x	x			Taskova et al., 2006
<i>V. ligustrifolia</i> A. Cunn	x	x			Taskova et al., 2006
<i>V. topiaria</i> (L.B. Moore) Garn.-Jones	x		x		Pedersen et al., 2007
<i>V. stenophylla</i> Steudel	x				Pedersen et al., 2007
<i>V. brachysiphon</i> (Summerh.) Bean	x				Johansen et al., 2007
<i>V. thomsonii</i> (Buchanan) Cheeseman	x			x	Taskova et al., 2010
<i>V. pulvinaris</i> (Hook. f.) Cheeseman	x			x	Taskova et al., 2010
<i>V. cupressoides</i> Hook. f.	x				Pedersen et al., 2007
<i>V. hookeri</i> (Buchanan) Garn.-Jones			x		This work
Eurasian <i>Veronica</i>	most sp.	none	none	none	Jensen et al., 2005

^a In this plant only the simple unesterified 6 was found.

Figure 1.