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Phenylethanoid and iridoid glycosides in the New Zealand snow hebes (*Veronica*, Plantaginaceae)

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Snow hebes are the alpine cushion-forming plants of New Zealand *Veronica,* formerly classified as *Chionohebe.* The chemical composition of *Veronica pulvinaris* and *Veronica thomsonii* was studied and 33 water-soluble compounds were isolated. The structure of 14 previously unknown esters of phenylethanoid glycosides were elucidated by spectroscopic analyses. Further, eight known phenylethanoids, nine iridoids, 6'-feruloyl-sucrose and mannitol are also reported. It was found that the iridoid profile of the snow hebes was different from the other species of *Veronica* in New Zealand but similar to the alpine Northern Hemisphere representatives of the genus.

Key words *Veronica*; *Chionohebe*; Phenylethanoid glycoside; Iridoid glucoside; Chemotaxonomy

As currently circumscribed, *Veronica* is a genus of 450 species found in temperate regions of both hemispheres. It now includes the segregate genus *Hebe* and its relatives from New Zealand, New Guinea and South America, *Derwentia* from Australia, *Besseya* and *Synthyris* from North America, and *Pseudolysimachion* from Europe and Asia.[1—3] The New Zealand clade *Veronica* sect. *Hebe* comprises plants formerly treated as *Chionohebe*, *Detzneria*, *Hebe*, *Hebejeebie*, *Heliohebe*, *Parahebe*, and *Leonohebe*. This monophyletic complex is the largest plant group in New Zealand and has been the object of many biological investigations.[4]

The chemistry of sect. *Hebe*, however, has received relatively limited attention. The early studies of the New Zealand clade were performed as part of a chemotaxonomic survey of iridoids and flavonoids in *Veronica* and related genera.[5—7] Later investigations focused mainly on flavonoid chemistry,[8—10] including a paper-chromatography study of *Hebe* and *Leonohebe*[11,12] and on a LC/MS survey of *Heliohebe*.[13] In a series of recent studies, Jensen and co-authors reported the isolation of water-soluble compounds (mainly iridoids and phenylethanoids) of 12 species of *Veronica* from the Southern Hemisphere.[14—17]

The cushion-forming plants of New Zealand *Veronica,* formerly classified as *Chionohebe,* are found in high-elevation habitats of the South Island. Molecular studies[18,19] show that they form a well-defined clade of four species: *Veronica chionohebe* Garn.-Jones, *Veronica ciliolata* (Hook. f.) Cheeseman with two subspecies (var. according to Meudt and Bayly[19]), *Veronica pulvinaris* (Hook. f.) Cheeseman, and *Veronica thomsonii* (Buchanan) Cheeseman. Another species, *Veronica densifolia* (F. Muell.) F. Muell., formerly included in *Chionohebe*, was shown to be distinct from these four species.

In this paper the isolation and structural elucidation of the water-soluble compounds of *V. pulvinaris* and *V. thomsonii* are reported. The evolutionary trends and phylogenetic implications of these new data are discussed. We will report elsewhere on our wider chemotaxonomic investigations in the group.

Results and discussion

Plant material was extracted with cold ethanol and the water-soluble part of the extract was subjected to a series of chromatographic procedures. The isolated compounds were identified by NMR spectroscopy including those of the sugar fraction, for which the composition was deduced by interpretation of the ^{13}C NMR data.

A total of 33 compounds were isolated and identified in the present work. These comprised one sugar alcohol, mannitol (**1**), nine iridoid glucosides (**2-4** and **4a-4f**), 6'-feruloyl-sucrose (**27**) and 22 esters of phenylethanoid glycoside (**5-26**) (Table 1). For the latter we use the generic term CPG (caffeoyl phenylethanoid glycoside) despite the fact that in some cases caffeic acid was replaced with ferulic acid. Fourteen of the isolated CPGs had not been previously reported, while eight were known. Aragoside (**5**) has been isolated from *Aragoa cundinamarcensis* Fern. Alonso,[20] persicoside (**12**) from *Veronica persica* Poir.,[21] two unnamed CPGs (**14** and **25**) and isopersicoside (**23**) from another collection of *V. persica*,[22] one more unnamed CPG (**22**) from *V. undulata* Wall.,[23] ehrenoside (**16**) from *V. bellidioides* L.,[24] and lagotoside (**17**) from *Lagotis stolonifera* Maxim.[25] Since the above three unnamed CPGs are analogues of the series of compounds isolated in the present work, we have termed them chionosides or isochionosides for systematic reasons.

Chionoside A (**6**) was obtained as a colourless glass with the molecular formula $C_{35}H_{46}O_{20}$, determined by HRESI-MS, which corroborated with the 35 signals observed in the ^{13}C NMR spectrum. The NMR spectral data (Table 2) were assigned by comparison with data of analogous structure as well as interpretation of 1D and 2D (gCOSY, HSQC and gHMBC) spectra. The 1D ^{1}H- and ^{13}C NMR spectral data of **6** (Table 2) were very similar to those of aragoside (**5**) [20] which is a 3,4-dihydroxy-phenylethyl glucoside esterified with a caffeoyl group at C-4' as well as α-arabinopyranosyl and β-glucopyranosyl groups at C-2' and C-3', respectively. A comparison of the ^{13}C NMR spectra of **5** and **6** pointed to the downfield shift of C-3'''' (δ_C 149.4) by 2.7 ppm, besides the presence of an additional methoxy carbon at δ_C 56.5 ppm. The remaining carbons were assigned to one substituted cinnamoyl (C_9) and one 3,4-dihydroxyphenylethyl (C_8), and three glycosyl moieties as indicated by the three signals at δ_C 103-105 arising from anomeric carbon atoms showing correlations with protons in the δ_H 4.5-4.7 region (HSQC). Correlations in the gHMBC spectrum confirmed the sites of attachment among the structural units. Thus, a cross-peak between H-1' (δ_H 4.50) of the central glucosyl moiety and C-8 (δ_C 72.2), demonstrated the position of the aglycone. Another cross-peak between H-1''' (δ_H 4.53) of the arabinosyl group and C-2' (δ_C 82.8), as well as between H-1'' (δ_H 4.64) of the peripheral glucosyl group and C-3' (δ_C 81.4), demonstrated the positions of the two sugar moieties. The protons of the methoxy group (δ_H 3.89) correlated with C-3'''' (δ_C 149.4). Finally, a weak correlation could be seen between H-4' (δ_H 4.92) and the carboxyl group CO'''' of the feruloyl substituent (δ_C 168.5), which showed the position of the acyl moiety. Therefore, **6** was the feruloyl analogue of aragoside

5.

Compound **7** was crystalline with mp 155-158 °C and with the molecular formula $C_{36}H_{48}O_{20}$ determined by HRESI-MS. The NMR spectral data (Table 2) were assigned as above. The ^{13}C NMR spectrum showed the expected 36 signals including two methoxyl signals (δ_C 56.4 and 56.5) and comparison with that of **6** showed that the spectra were superimposable within 0.1 ppm, except for the signals arising from the aglucone of **7**, which was evidently substituted with the additional methyl group. The HMBC spectrum showed the same connectivities as above, while the additional methoxy group (δ_H 3.80) was correlated with C-4 (δ_C 147.6). Therefore, **7** was the 4-*O*-methyl analogue of **6**, named chionoside B.

Compound **8** had the molecular formula $C_{44}H_{52}O_{23}$ (HRESI-MS) and the NMR spectral data (Table 2) were assigned as above. The ^{13}C NMR spectrum showed 42 signals including two of double intensity (δ_C 127.6 and 116.5). Two signals arising from carboxyl carbon atoms indicated the presence of two ester groups. After sorting out the signals from a 3,4-dihydroxy-phenylethyl moiety (eight C), and a caffeoyl group (nine C), eight signals in the aromatic region remained to be assigned. These, together with a carbonyl and a methoxyl signal (δ_C 169.0 and 56.5) matched well with those of an additional feruloyl substituent. The remaining 17 signals were consistent with the presence of three carbohydrate moieties as in the compounds above. All the ^{13}C NMR signals from **5** and **8** (and partly also **6**, Table 2) were almost superimposable, except for three (δ_C 71.6, 75.2 and 64.7, of which the latter was a methylene signal). Comparison of these with the corresponding signals from C-4'', C-5'' and C-6'' of **6** (δ_C 72.0, 78.2 and 63.3) established that compound **8** was esterified with the additional feruloyl group at the C-6'' oxygen atom. Consistent with this, the two ^{1}H NMR signals of this methylene group were found downfield by 0.9 and 0.5 ppm compared to those of **6**. The gHMBC spectrum showed the expected connectivities including the cross-peaks between H-6'' (δ_H 4.33) and CO''''' of the feruloyl group (δ_C 169.0) and between the methoxyl group (δ_H 3.88) and C-3''''' (δ_C 149.3). Thus, compound **8** was 6''-*O*-feruloyl-aragoside, named chionoside C.

Compound **9** was obtained as crystals with mp 166-168 °C; it had the molecular formula $C_{40}H_{54}O_{25}$ (HRESI-MS) and the ^{13}C NMR spectrum showed 39 signals (Table 2) including one of double intensity (δ_C 78.0). The six carbon atoms more than in aragoside (**5**) corresponded to an additional hexosyl moiety, as also indicated by the four peaks in the region for anomeric signals (δ_C 103-105). Again, most of the signals could be assigned by comparison with the spectrum of **5**; however, two signals deviated significantly, namely those of C-5' and C-6' (δ_C 74.2 and 69.3) which were seen upfield and downfield, respectively, from those of **5** (δ_C 75.4 and 62.3) showing that the C-6' oxygen atom was the site of attachment for the additional hexosyl group. The remaining six signals matched well with a β-glucopyranosyl group situated in the 6'-*O*-position of similar compounds. Thus, comparison with the signals arising from the 6'-*O*-β-glucopyranosyl group of cuproside from *Veronica cupressoides* Hook. f.[16)] showed coincidence within 0.2 ppm for these six signals. Consistent with this, the HMBC spectrum H-1''''' (δ_H 4.29; *d*, J = 7.7 Hz) showed a cross-peak with C-6' (δ_C 69.3), and the latter had another with H-4' (δ_H 5.01), proving the assignment. Compound **9** was therefore 6'-*O*-β-glucopyranosyl-aragoside, named chionoside D.

Compound **10** was also crystalline, mp 166-168 °C, with the molecular formula $C_{41}H_{56}O_{25}$ (HRESI-MS) and the ^{13}C NMR spectrum showed the expected 41 signals (Table 2) and was almost superimposable with that of **9**, except for an additional peak from a methoxyl group (δ_C 56.5) and some of the aromatic signals. These differences indicated that the caffeoyl moiety in **9** was replaced with a feruloyl group in **10**. This was confirmed by the HMBC spectrum where all the expected correlations could be seen, including one between the methoxyl protons (δ_H 3.89) and C-3'''' (δ_C 149.4). Compound **10** was therefore the 3''''-*O*-methyl analogue of chionoside D named chionoside E.

Compound **11** had the molecular formula $C_{40}H_{54}O_{24}$ (HRESI-MS). The compound could not be obtained completely pure. The ^{13}C NMR spectrum presented 37 signals (Table 2) including three of double intensity (δ_C 78.1, 72.3 and 72.0) and was almost superimposable with that of **9**, except for a change in some of the sugar signals. This included the presence of a methyl group (δ_C 18.0) combined with the loss of one hydroxymethyl group (δ_C 62.6). The differences could be explained by an exchange of the 6'-*O*-β-glucopyranosyl moiety in **9** for an α-rhamnopyranosyl group in **11**. In the HMBC spectrum, a cross-peak was found between the anomeric proton H-1''''' of the rhamnosyl group (δ_H 4.62; br. *s*) and C-6' (δ_C 67.5). The 1.8 ppm upfield shift seen in the $\delta_{C\text{-}6'}$ of **11** when compared to that of **9** is in line with what was found for the corresponding methyl glycosides, namely from β-glucopyranoside (δ_C 58.1) to α-rhamnopyranoside (δ_C 55.8).[26)] Thus, compound **11** was 6'-*O*-α-rhamnopyranosyl aragoside, named chionoside F.

Chionoside G (**13**) was also only obtained in an impure state; it had the molecular formula $C_{36}H_{48}O_{21}$ (HRESI-MS). Nevertheless, the NMR data (Table 3), was sufficient to elucidate the structure of the compound. The ^{13}C NMR spectrum presented 34 signals of which two (δ_C 75.8 and 72.0) had double intensity and a third showed the presence of a methoxyl group (δ_C 56.5). A comparison with the spectrum of persicoside (**12**) from *V. persica*[21)] showed near identity (within 0.2 ppm) for the signals of the ester part and for the three sugar residues. The ^1H NMR spectrum (Table 3) could be partly assigned by the gCOSY spectrum; it was also very similar to that of **12**, and consequently, we could determine the structure of **13** to be 4-*O*-methyl persicoside.

Chionoside I (**15**) had the molecular formula $C_{37}H_{50}O_{21}$ (HRESI-MS). The ^{13}C NMR spectrum showed 34 signals (Table 3) of which two (δ_C 72.0 and 56.5) had double intensity, the latter being indicative of two aromatic methoxyl groups. Comparison with the spectra of **7**, **12** and **13** showed that the compound was 3'''',4-di-*O*-methylpersicoside, and this was consistent with the ^1H NMR spectrum (Table 3). The HMBC spectrum presented all the expected cross-peaks including those involving the methoxyl groups, namely between (δ_H 3.80) and C-4 (δ_C 147.5) and between (δ_H 3.88) and C-3'''' (δ_C 149.3).

Compound **18**, with the molecular formula $C_{35}H_{46}O_{21}$ (HRESI-MS), was isomeric with persicoside (**12**). The ^{13}C NMR spectrum had 34 signals (Table 3) of which one (δ_C 77.6) had double intensity. The spectrum was very similar to those of the previous compounds with three carbohydrate entities, except for the unusually low field positions of two signals (δ_C 89.9 and 85.1) in **18** arising from the carbon atoms linking the sugar moieties; these were otherwise consistently seen between δ_C 81 and 83 ppm (Tables 2 and 3) in all the compounds (**6-15**) discussed above. The ^1H NMR spectral data of **18** (Table 3) were very similar to those of **12** in the aromatic region, but between δ 4 and 5 ppm important differences were noted. The three signals arising from anomeric protons were present, but the usual signal from the site of esterification (δ_H ca. 4.9) was missing and was replaced by two signals (δ_H 4.33 and 4.50), which proved to be the AB part of an ABX system, i. e. an oxymethylene group. This indicated that **18** was an *iso*-form with the ester group attached to O-6' instead of at O-4'; it could, however, also be bonded to either of the other carbohydrate moieties. Due to the similarity in shift values between many of the signals in the ^1H NMR spectrum, it was difficult to assign all carbohydrate signals with confidence even using 2D NMR techniques, but all signals were consistent with the presence of three β-glucopyranosyl moieties in **18**. Searching the literature for compounds with similar characteristics, we found the 3'''',4-di-*O*-methyl-substituted analogue scroside A, reported from *Picrorhiza scrophulariiflora* (Pennell)

D.Y. Hong,[27)] which exhibits such a pair of unusually low field signals for C-3' and C-2". Comparison of the NMR spectral data showed satisfactory similarity, allowing for the different solvent (pyridine-d_5) used by Li et al.[27)] The HMBC spectrum was consistent with the structure given, including a correlation between H-6' (δ_H 4.33) and CO'''' of the caffeoyl residue (δ_C 169.1). The structure of **18** was therefore the *iso*-form: 2"-*O*-glucosyl-substituted plantainoside D, a compound reported from *Plantago asiatica* L. [28)] We have named the compound isochionoside J.

Compound **19** had the molecular formula $C_{34}H_{44}O_{20}$ (HRESI-MS), isomeric with aragoside (**5**). The ^{13}C NMR spectrum had 33 signals (Table 4) of which one (δ_C 78.2) had double intensity. The spectral data were almost coincident with those of aragoside except for the downfield shift changes seen for C-3' and C-6' (5 and 2 ppm, respectively) of the central glucosyl moiety. These changes indicated that the caffeoyl substituent was positioned at the O-6' in **19,** which therefore was the *iso*-form of **5**. The ^{1}H NMR spectral data (Table 4) were consistent with this, since H-4' was found 1.5 ppm upfield and the C-6' protons ca 0.8 ppm downfield compared to the values of **5**. Finally, the HMBC spectrum showed correlations between the C-6' protons (δ_H 4.31 and 4.51) and CO'''' of the caffeoyl residue (δ_C 169.0). Compound **19** was therefore named isoaragoside.

Isochionosides K (**20**) and A (**21**) both had the same molecular formula $C_{35}H_{46}O_{20}$ (HRESI-MS) as well as an additional methoxy group compared to **19** in their ^{13}C NMR spectra. On the bases of the chemical shift differences and gHMBC correlations the methyl groups were placed on the O-4 and O-3'''' in compounds **20** and **21**, respectively. Similar approaches were taken to establish the structures of isochionosides G (**24**; $C_{36}H_{48}O_{21}$, HRESI-MS) and I (**26**; $C_{37}H_{50}O_{21}$, HRESI-MS), which were iso-forms of **13** and **15**, respectively.

The absolute configuration of the sugar units in the new compounds have not been elucidated directly in this work. However, using the ^{13}C NMR data we propose that the sugars involved are in all cases D-glucose, L-arabinose and L-rhamnose. In their comprehensive paper on glucosylation shifts of alcohols, Seo et al.[29)] have demonstrated that the ^{13}C NMR spectra of diastereomeric glucosides of chiral secondary alcohols are so different that they can be used to determine the absolute configuration of the aglucones. Consequently, it is possible to determine the absolute configuration of one sugar moiety in an oligosaccharide if that of the other sugars are known, simply by comparing the 1D ^{13}C NMR spectra with those of known analogues. An example of this is seen when comparing the spectra of the two iridoid glucosides 8-epiloganic acid and 1,5,9-epideoxy loganic acid.[30] These two are β-D-glucopyranosides with a pair of enantiomeric aglucones and the shift differences between the two carbons forming the linkage are significant (ca. 4 ppm). In the original report[20] on aragoside (**5**), the absolute stereochemistry of the sugars was not directly determined; however, the ^{13}C NMR data were compared to those of persicoside[21] (**12**) and those of ehrenoside[24] (**16**) and the relevant parts showed excellent agreement. Of these, the absolute configuration of the sugars of **16** have been determined to be D-glucose, L-arabinose and L-rhamnose. Compounds **12** and **16** are reported to be levorotary and so are all compounds reported here. Additionally, the 3'-*O*-glucoside plantamajoside (= desarabinosyl-aragoside) has been synthesised[31] using a D-glucosyl derivative, and the chemical shift data for this compound also compare well with the data for the 3'-*O*-glucopyranosyl units in the Tables 2-4. We are unable to offer an argument for the 6'-*O*-terminal sugars in compounds **9-11** for they are bonded to a primary alcohol; however, they are most likely derived from D-glucose and L-rhamnose.

Based on their morphology alone, cushion-forming species of *Veronica* are difficult to distinguish from each other. The widely used classification of snow hebes in Flora of New Zealand (as *Pygmea*)[32] was based on leaf trichomes, which show significant interspecific and intraspecific variations.[18—19] The DNA sequence data and AFLP analyses show well-defined lineages within the group.[19] The chemical profile of *V. thomsonii* is distinct from *V. pulvinaris* (Table 1). The latter contains mussaenoside (**2**) and veronicoside (**4a**), which are not found in *V. thomsonii*. The main CPGs in *V. thomsonii* are aragoside and isoaragoside derivatives (**5-11**, **20-22**) where the central glucose bears an arabinosyl group at its 2'-*O*-position. In contrast, *V. pulvinaris* accumulates persicoside- and isopersicoside-based metabolites, where the 2'-*O*-position is glucosylated (**12-15**, **23-26**). Within *V. pulvinaris*, the iridoid minecoside (**4f**) was found only in the collection from Mt Arthur, whereas the CPGs lagotoside (**17**), persicoside (**12**) and its derivatives (**13-15**), were characteristic only for the collection from Black Birch Range.

The iridoid profile of cushion-forming species differs from those found in other representatives of New Zealand *Veronica*. The snow hebes contain only 6-*O*-esters of catalpol (**4a-f**) but not 6-*O*-rhamnopyranosylcatalpol or its derivatives, which are characteristic of some of the shrubby species of *Veronica* sect. *Hebe*.[14—17] The iridoid composition of snow hebes is very similar to those reported from the Northern Hemisphere montane to alpine species of *Veronica* subg. *Veronica*. In particular, mussaenoside (**2**) has not been found outside subg. *Veronica* and the snow hebes of sect. *Hebe*, and 6-*O*-catalpol esters with cinnamic acid derivatives, feruloylcatalpol and minecoside (**4e** and **4f**) are also common in these groups. The snow hebe clade diverges from a relatively basal node within *V*. sect. *Hebe*[33)] and thus similarities with subg. *Veronica* could be interpreted as ancestral for genus *Veronica*, or it might have been derived independently in the two lineages in response to alpine environments. When comparing with other southern lineages that are attached to nodes more basal than the snow hebes,[33)] we find a different chemical composition in *V. cupressoides*,[16)] and this may support convergent or parallel evolution of this specific chemical profile in these alpine groups.

In conclusion, our results show that *Veronica* sect. *Hebe* has a considerable chemical diversity that might parallel its morphological diversity. Chemical profiles, especially from iridoid and phenylethanoid glycosides, may provide valuable data in the search for informative monophyletic groupings that could be given taxonomic recognition at subsection rank.

Experimental

General Procedures Two HPLC systems were used, Agilent 1100 Series LC System (Agilent, Santa Clara, CA, USA) with a guarded Luna C_{18} column (10 × 250 mm, 5 µm, Phenomenex) kept at 40 °C and Waters system comprising a 600 pump, a 717 autosampler and a 2996 PDA detector (Manchester, UK) with Genesis C_{18} column (10 × 250 mm, 5 µm, Jones Chromatography, Mid Glamorgan, UK) at 30 °C, with MeOH-H_2O mixtures as eluents at a flow rate of 4 ml/min. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova-500 in D_2O or CD_3OD using the solvent peak (δ_H 4.75, 3.30 or δ_C 49.0) as an internal reference. 2D gCOSY, HSQC and gHMBC spectra were acquired using standard pulse sequences. HRESI-MS was performed on a TOF MS Micromass (DTU, Denmark; for compounds **13** and **15**) and on MAT900 (Mariner TOF, EPSRC National Mass Spectrometry Service Centre Swansea). UV and IR spectra were recorded on a Shimadzu UV-1601 and a Bruker Alpha FT-IR instrument, respectively. Fresh plant material was homogenised with EtOH and filtered. The concentrated extracts were partitioned between Et_2O–H_2O. The aqueous phase was loaded on a 3.5 (i.d.) × 8.5 cm cellulose column (microcrystalline cellulose, Merck, Germany) and eluted with water. The eluate was then loaded on a 4 × 45 cm Diaion HP-20 (Supelco, Bellefonte, US) column and subsequently eluted with water,

water-methanol mixtures and methanol. The fractions were further separated on Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) columns and/or with the aid of HPLC coupled to a photodiode array (PDA) detector.

Plant material This was collected from the species natural habitats in the South Island, New Zealand. *V. pulvinaris* was collected in Black Birch Range, 1669 m above sea level, in March 2006 and on Mount Arthur Ridge, 1549 m above sea level, in January 2007. *V. thomsonii,* was collected at Shotover Saddle, Matukituki Valley, 1600 m above sea level, in February 2007. The voucher specimens have been deposited at the Herbarium of Victoria University of Wellington (WELTU).

Isolation The fresh plant material of *V. pulvinaris* (Black Birch Range) (300 g) gave 9.2 g of crude extract. Chromatography on Diaion HP-20 gave a fraction containing mainly mannitol (**1**; eluted with H_2O, 2.5g), a fraction containing mainly aucubin (**3**) and catalpol (**4**; eluted with 30% MeOH, 430 mg), a fraction containing mainly mussaenoside (**2**; eluted with 40% MeOH, 180 mg) and three next fractions were subjected to further fractionation as described below. The fraction eluted with 50% MeOH (1.6 g) was loaded on a Sephadex LH-20 column (3 × 30 cm) and eluted with 90% MeOH to give: **fr. A** (345 mg), containing mainly **2**; **fr. B** (687 mg) separated by HPLC (Luna C18, linear gradient from 25 to 42% MeOH over 15 min) to obtain pure 6'-feruloylsucrose (**27**;10.2 mg), persicoside (**12**; 245 mg), **2** (8.6 mg), chionoside H (**14**; 18 mg) and isopersicoside (**23**; 6.3 mg); and **fr. C** (365 mg) separated by HPLC (40% MeOH) to obtain persicoside (**12**; 124 mg), verproside (**4b**; 52 mg) and isopersicoside (**23**; 51 mg).A fraction eluted with 60% MeOH (1.2 g) was loaded on a Sephadex LH-20 column (2.5 × 55 cm) and eluted with 90% MeOH to give: **fr. D** (260 mg), further separated by HPLC (40% MeOH) to give **14** (29.5 mg), chionosides G (**13**; 15.8 mg) and I (**15**; 72.4 mg); **fr. E** (480 mg), separated by HPLC (40% MeOH) to give **12** (99 mg), **4b** (28.4 mg), **14** (49.4 mg), **23** (75.2 mg), amphicoside (**4c**; 59.3 mg), **15** (9.9 mg), and isochionoside (**25**; 12 mg); **fr. F** (160 mg), separated by HPLC (30 to 40% MeOH over 15 min) to give **4b** (28.4 mg) and **23** (59.4 mg); and **fr. G** (110 mg), separated by HPLC (30 to 40% MeOH over 15 min) to give pure **4b** (15.5 mg), verminoside (**4d**; 17.5 mg) and **23** (20 mg). A fraction eluted with MeOH (1.2 g) was loaded on a Sephadex LH-20 column (2.5 × 45 cm) and eluted with MeOH to give: **fr. H** (361 mg), further separated by HPLC (Luna C18, linear gradient from 35 to 60% MeOH over 15 min) to give pure **4c** (21.5 mg), **15** (62.3 mg), **25** (7.3 mg), lagotoside (**17**; 30.0 mg), isochionoside I (**26**; 41.4 mg), and veronicoside (**4a**; 11.1 mg); **fr. I** (423 mg), separated by HPLC (Luna C18, 40 to 60% MeOH over 10 min) to give **23** (10 mg), **4c** (92.8 mg), **15** (7.7 mg), **25** (27.5 mg), feruloylcatalpol (**4e**; 103.4 mg) and **26** (27.9 mg); and **fr. J** (160 mg), separated by HPLC (Luna C18, 35% MeOH) to give **23** (9.0 mg), **4d** (21.6mg), **25** (8.0 mg) and **4e** (77.7 mg).

The fresh plant material of *V. pulvinaris* (Mt Arthur) (137 g) gave 2.9 g of crude extract. Chromatography on Diaion HP-20 gave a sugar fraction (eluted with H_2O, 800 mg), a fraction containing mainly aucubin (**3**) and catalpol (**4**; eluted with 30% MeOH, 140 mg) and three other fractions subjected to further purification. A fraction eluted with 50% MeOH (320 mg) was separated by HPLC (Genesis C_{18}, 38% MeOH) to give pure verproside (**4b**; 18.9 mg), mussaenoside (**2**; 11.7 mg) and isopersicoside (**23**; 30.1 mg). A fraction eluted with 70% MeOH (800 mg) was loaded on a Sephadex LH-20 column (2.5 × 45 cm) and eluted with 80% MeOH to give: **fr. A** (262 mg) separated further by HPLC (Genesis C_{18}, 45% MeOH for 10 min then 48%, 10 min) to give **2** (17.6 mg) and isochionoside I (**26**; 40.5 mg); **fr. B** (149 mg) was separated by HPLC (Genesis C_{18}, 47% MeOH) to amphicoside (**4c**; 36.9 mg), isochionosides G (**24**; 4.9 mg) and I (**26**; 12.8 mg); **fr. C** (305 mg) separated by HPLC (Genesis C_{18}, 35% MeOH) to give pure **4b** (158 mg); and **fr. D** (14.7 mg) containing mainly verminoside (**4d**). A fraction eluted with MeOH (320 mg) was separated by HPLC (Genesis C_{18}, 48% MeOH) to give pure **4c** (22.8 mg), feruloylcatalpol (**4e**; 24.7 mg), minecoside (**4f**; 5.3 mg), **26** (32.9 mg) and veronicoside (**4a**; 1.5 mg).

The fresh plant material of *V. thomsonii* (145 g) gave 5.6 g of crude extract. Chromatography on Diaion HP-20 gave a sugar fraction (eluted with H_2O, 1.2 g), a fraction containing mainly aucubin (**3**) and catalpol (**4**; eluted with 30% MeOH, 180 mg) and three other fractions: A fraction eluted with 50% MeOH (800 mg) was loaded on a Sephadex column (2.5 × 45 cm) and eluted with 80% MeOH to give: **fr. A** (381 mg) separated by HPLC (Genesis C_{18}, step gradient at 30% MeOH for 12 min followed by 36% MeOH for 10 min) to give pure chionosides D (**9**; 70.2 mg), C (**8**; 27.9 mg) and F (**11**; 10.0 mg); and **fr. B** (191 mg) separated by HPLC (Genesis C_{18}, 38% MeOH) to give verproside (**4b**; 17.1 mg), isochionoside J (**18**; 6.3 mg), isopersicoside (**23**; 8.0 mg) and isoaragoside (**19**; 40.2 mg). A fraction eluted with 70% MeOH (1.6 g) was loaded on a Sephadex LH-20 column (2.5 × 55 cm) and eluted with 90% MeOH to give: **fr. C** (456 mg) separated by HPLC (Genesis C_{18}, step gradient of 42% MeOH for 10 min and 50% MeOH for 13 min) to give pure chionoside A (**6**; 19.8 mg), amphicoside (**4c**, containing **6**; 14.4 mg), chionosides I (**15**; 11.8 mg) and B (**7**; 39.7 mg), lagotoside (**17**; 12.6 mg), isochionosides I (**26**; 4.3 mg) and B (**22**; 5.4 mg); **fr. D** (961 mg) separated by HPLC (Genesis C_{18}, step gradient 38% and 46% MeOH for 8 and 9 min, resp.) to give **4b** (60.4 mg) aragoside (**5**; 88.0 mg), ehrenoside (**16**; 6.7 mg), **4c** (127.3 mg), **19** (145.0 mg), **8** (18.1 mg), isochionosides A (**21**; 9.5 mg) and K (**20**; 3.8 mg); and **fr. E** (75.7 mg) containing mainly verminoside (**4d**). A fraction eluted with MeOH (470 mg) was separated by HPLC (Genesis C_{18}, 48% MeOH) to give pure **4c** (22.1 mg), feruloylcatalpol (**4e**; 89.4 mg), **26** (16.2 mg) and **22** (30.9 mg).

The known compounds were identified by NMR and compared with published data: mannitol and iridoids **1-4** with authentic samples; veronicoside (**4a**);[34] verproside (**4b**);[35] amphicoside (**4c**);[36] verminoside and minecoside (**4d** and **4f**);[37] feruloylcatalpol (**4e**); [38] 6'-*O*-feruloylsucrose (**27**).[39]

Chionoside A (**6**): colourless syrup: $[\alpha]_D^{22}$ – 4 (c=0.1, MeOH). IR (neat) cm^{-1}: 3373, 1746, 1586, 1047. UV λ_{max} (MeOH) nm (log ε): 327 (3.38), 204 (3.68). HRESI-MS *m/z*: 785.2502 $[M-H]^-$ (Calcd for $C_{35}H_{45}O_{20}$: 785.2510); NMR data in Table 2.

Chionoside B (**7**): colourless crystals: mp 155-158 °C. $[\alpha]_D^{22}$ – 35 (c=0.7, MeOH). IR (neat) cm^{-1}: 3359, 1697, 1630, 1593, 1513, 1017. UV λ_{max} (MeOH) nm (log ε): 328 (4.32), 219 (4.29), 206 (4.43). HRESI-MS *m/z*: 799.2659 $[M-H]^-$ (Calcd for $C_{36}H_{47}O_{20}$: 799.2666); NMR data in Table 2.

Chionoside C (**8**): colourless syrup: $[\alpha]_D^{23}$ – 27 (c=0.4, MeOH). IR (neat) cm^{-1}: 3389, 1698, 1630, 1601, 1515, 1078. UV λ_{max} (MeOH) nm (log ε): 329 (4.36), 218 (4.33), 207 (4.40). HRESI-MS *m/z*: 947.2811 $[M-H]^-$ (Calcd for $C_{44}H_{51}O_{23}$: 947.2827); NMR data in Table 2.

Chionoside D (**9**): colourless crystals: mp 166-168 °C. $[\alpha]_D^{22}$ – 31 (c=0.9, MeOH). IR (neat) cm^{-1}: 3325, 1694, 1625, 1600, 1516, 1012. UV λ_{max} (MeOH) nm (log ε): 331 (4.43), 221 (4.46), 206 (4.56). HRESI-MS *m/z*: 933.2868 $[M-H]^-$ (Calcd for $C_{40}H_{53}O_{25}$: 933.2881); NMR data in Table 2.

Chionoside E (**10**): colourless crystals: mp 162-164 °C. $[\alpha]_D^{22}$ – 29 (c=1.2, MeOH). IR (neat) cm^{-1}: 3386, 1701, 1630, 1600, 1516, 1038. UV λ_{max} (MeOH) nm (log ε): 328 (4.30), 220 (4.19), 205 (4.46). HRESI-MS *m/z*: 947.3026 $[M-H]^-$ (Calcd for $C_{41}H_{55}O_{25}$: 947.3038); NMR data in Table 2.

Chionoside F (**11**): impure. HRESI-MS *m/z*: 917.2926 $[M-H]^-$ (Calcd for $C_{40}H_{53}O_{24}$: 917.2932); NMR data in Table 2.

Chionoside G (**13**): impure. HRESI-MS *m/z*: 815.2620 $[M-H]^-$ (Calcd for $C_{36}H_{47}O_{21}$: 815.2615); NMR data in Table 3.

Chionoside I (**15**): colourless syrup: $[\alpha]_D^{22}$ – 10 (c=0.3, MeOH). IR (neat) cm^{-1}: 3391, 1752, 1640, 1610, 1520, 1080. UV λ_{max} (MeOH) nm (log ε): 327 (4.20), 289 (4.03), 232 (4.06), 219 (4.11), 206 (4.29). HRESI-MS *m/z*: 848.3228 $[M+NH_4]^+$ (Calcd for $C_{37}H_{54}NO_{21}$: 848.3188); NMR data in Table 3.

Isochionoside J (**18**): colourless syrup: $[\alpha]_D^{20}$ – 7 (c=0.2, MeOH). IR (neat) cm^{-1}: 3373, 1736, 1597, 1044. UV λ_{max} (MeOH) nm (log ε): 331 (4.00), 292 (3.92), 218 (4.06), 209 (4.07). HRESI-MS *m/z*: 801.2436 $[M-H]^-$ (Calcd for $C_{35}H_{45}O_{21}$: 801.2459); NMR data in Table 3.

Isoaragoside (**19**): colourless syrup: $[\alpha]_D^{22}$ – 9 (c=0.1, MeOH). IR (neat) cm^{-1}: 3373, 1739, 1597, 1080. UV λ_{max} (MeOH) nm (log ε): 330 (3.74), 293 (3.69), 206 (3.94). HRESI-MS *m/z*: 795.2319 $[M+Na]^+$ (Calcd for $C_{34}H_{44}O_{20}Na$: 795.2319); NMR data in Table 4.

Isochionoside K (**20**): colourless syrup: $[\alpha]_D^{23}$ – 7 (c=0.05, MeOH). IR (neat) cm^{-1}: 3352, 1740, 1711, 1593, 1514, 1055. UV λ_{max} (MeOH) nm (log ε): 330 (4.23), 301 (4.13), 289 (4.13), 208 (4.36). HRESI-MS *m/z*: 785.2499 $[M-H]^-$ (Calcd for $C_{35}H_{45}O_{20}$: 785.2510); NMR data in Table 4.

Isochionoside A (**21**): colourless syrup: $[\alpha]_D^{24}$ – 8 (c=0.4, MeOH). IR (neat) cm^{-1}: 3387, 1699, 1630, 1601, 1516, 1078. UV λ_{max} (MeOH) nm (log ε): 327 (4.13), 291 (4.02), 206 (4.30). HRESI-MS *m/z*: 785.2505 $[M-H]^-$ (Calcd for $C_{35}H_{45}O_{20}$: 785.2510); NMR data in Table 4.

Isochionoside B (**22**): colourless crystals: mp 134-136 °C. $[\alpha]_D^{22}$ – 1 (c=0.2, MeOH). IR (neat) cm^{-1}: 3356, 1695, 1590, 1513, 1012. UV λ_{max} (MeOH) nm (log ε): 327 (4.24), 289 (4.07), 232 (4.10), 219 (4.15) 206 (4.33). HRESI-MS *m/z*: 799.2653 $[M-H]^-$ (Calcd for $C_{36}H_{47}O_{20}$: 799.2666); NMR data identical to those published.[23)]

Isochionoside G (**24**) impure. HRESI-MS *m/z*: 815.2607 $[M-H]^-$ (Calcd for $C_{36}H_{47}O_{21}$: 815.2615); NMR data in Table 4.

Isochionoside I (**26**): colourless syrup: $[\alpha]_D^{23}$ – 8 (c=0.1, MeOH). IR (neat) cm^{-1}: 3384, 1708, 1631, 1592, 1515,1077. UV λ_{max} (MeOH) nm (log ε): 327 (4.04), 289 (3.88), 232 (3.91), 219 (3.98), 206 (4.19). HRESI-MS *m/z*: 829.2769 $[M-H]^-$ (Calcd for $C_{37}H_{49}O_{21}$: 829.2772); NMR data in Table 4.

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Table 1. Compounds isolated from *V. thomsonii* and *V. pulvinaris*.

	V. thomsonii	*V. pulvinaris* (Black Birch Range)	*V. pulvinaris* (Mt Arthur)
Iridoids			
Mussaenoside (**2**)		+	+
Aucubin (**3**)	+	+	+
Catalpol (**4**)	+	+	+
Veronicoside (**4a**)		+	+
Verproside (**4b**)	+	+	+
Amphicoside (**4c**)	+	+	+
Verminoside (**4d**)	+	+	+
Feruloylcatalpol (**4e**)	+	+	+
Minecoside (**4f**)			+
CPGs			
Aragoside (**5**)	+		
Chionoside A (**6**)	+		
Chionoside B (**7**)	+		
Chionoside C (**8**)	+		
Chionoside D (**9**)	+		
Chionoside E (**10**)	+		
Chionoside F (**11**)	+		
Isoaragoside (**19**)	+		
Isochionoside K (**20**)	+		
Isochionoside A (**21**)	+		
Isochionoside B (**22**)	+		
Ehrenoside (**16**)	+		
Lagotoside (**17**)	+	+	
Isochionoside J (**18**)	+		
Persicoside (**12**)		+	
Chionoside G (**13**)		+	
Chionoside H (**14**)		+	
Chionoside I (**15**)	+	+	
Isopersicoside (**23**)	+	+	+
Isochionoside G (**24**)			+
Isochionoside H (**25**)		+	+
Isochionoside I (**26**)	+	+	+
Acylsugars			
6'-Feruloyl-sucrose (**27**)		+	

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of Chionosides A-F (**6-11**) in CD_3OD.

Atom	Chionoside A (**6**)		Chionoside B (**7**)		Chionoside C (**8**)		Chionoside D (**9**)		Chionoside E (**10**)		Chionoside F (**11**)	
	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C
Agluc												
1		131.9		133.2		132.0		131.9		131.9		131.8
2	6.74 (d, 1.9)	117.5	6.76 (br.s)	117.4	6.73 (br.s)	117.5	6.75 (d, 1.5)	117.5	6.75 (d,.1.7)	117.5	6.73 (d, 1.5)	117.5
3		146.1		147.3		146.1		146.0		146.0		146.1
4		144.7		147.6		144.7		144.7		144.7		144.8
5	6.67 (d, 8.0)	116.3	6.82 (d, 8.1)	112.9	6.68 obsc.	116.3	6.67 (d, 8.0)	116.3	6.69 (d, 8.0)	116.3	6.68 (d, 8.0)	116.3
6	5.56 (dd, 1.9, 8.0)	121.5	6.68 (br. d, 8.1)	121.4	6.55 (br.d, 8.1)	121.5	6.57 (dd, 1.5, 8.0)	121.5	6.57 (dd, 1.7, 8.0)	121.5	6.57 (dd, 1.5, 8.0)	121.5
7	2.76 2H (m)	36.8	2.80 2H (m)	36.7	2.74 (m)	36.7	2.76 2H (m)	36.7	2.77 2H (m)	36.7		36.8
8	3.73 (m), 4.07 (m)	72.2	3.69 (m),4.07 (m)	72.0	3.60 (m),4.02 (m)	72.1	3.68 (m),4.05 (m)	72.3	3.67 (m), 4.06 (m)	72.3	3.7 (m), 4.01 (m)	72.3
4–OMε			3.80 (s)	56.4								
Centr β-Glc	c-Glc		c-Glc		c-Glc		c-Glc		c-Glc		c-Glc	
1′	4.50 (d, 7.7)	103.4	4.50 (d, 7.7)	103.4	4.40 (d, 7.7)	103.3	4.50 (d, 8.0)	103.3	4.52 (d, 7.7)	103.4	4.49 (d, 7.7)	103.5
2′	3.70 (dd, 7.7/9.4)	82.8	3.69 obsc.	82.7	3.70 (dd, 7.7, 9.3)	82.8	3.70 (dd, 8.0, 9.2)	82.7	3.71 (dd, 7.7, 9.2)	82.8	3.67 obsc.	82.6
3′	4.10 (t, 9.4)	81.4	4.10 (t, 9.3)	81.5	3.96 (t, 9.3)	81.6	4.09 (t, 9.2)	81.2	4.10 (t, 9.2)	81.4	4.09 (t, 9.2)	81.3
4′	4.92 (t, 9.4)	70.4	4.93 (t, 9.3)	70.4	4.93 (t, 9.3)	70.1	5.01 (t, 9.2)	70.4	5.01 (t, 9.2)	70.4	4.98 (t, 9.6)	70.3
5′	3.56 obsc.	75.3	3.56 obsc.	75.3	3.43 obsc.	75.3	3.79 obsc.	74.2	3.79 obsc.	74.4	3.67 obsc.	74.3
6′	3.54, 3.65 obsc.	62.3	3.54/3.65 obsc.	62.3	3.48, 3.57 obsc.	62.1	3.63 obsc.	69.3	3.66 obsc.	69.4	3.49 (dd, 11.7, 6.5)	67.5
							3.95 (br. d, 10.4)		3.96 (br. d, 10.3)		3.76 obsc.	
2'-α-Ara	2'-Ara		2'-Ara		2'-Ara		2'-Ara		2'-Ara		2'-Ara	
1′′′	4.53 (d, 7.3)	104.9	4.53 (d, 7.3)	104.9	4.52 (d, 7.3)	104.7	4.52 (d, 7.3)	104.8	4.52 (d, 7.3)	104.9	4.53(d, 7.3)	104.9
2′′′	3.57 obsc.	73.2	3.57 obsc.	73.2	3.56 obsc.	73.3	3.57 (dd, 7.3, 9.3)	73.2	3.57 (dd, 7.3, 9.1)	73.2	3.56 (dd, 7.3, 9.1)	73.2
3′′′	3.47 (dd, 3.4, 9.3)	74.0	3.48 (dd, 3.3, 9.2)	74.0	3.46 obsc.	74.0	3.48 (dd, 3.0, 9.3)	74.0	3.48 (dd, 3.0, 9.1)	74.0	3.47 obsc.	74.1
4′′′	3.73 (m)	69.8	3.73 obsc.	69.8	3.73 obsc.	69.8	3.73 (m)	69.8	3.73 (m)	69.8	3.73 obsc.	69.8
5′′′	3.14 (br. d, 12.3)	67.4	3.17 (br. d, 12.3)	67.3	3.09 (d, 11.8)	67.4	3.14 (br. d, 11.9)	67.4	3.14 (br. d, 11.9)	67.4	3.16 (br. d, 12.9)	67.4
	3.79 (dd, 2, 11.5)		3.78 obsc.		3.73 obsc.		3.76 obsc.		3.75 obsc.		3.77 obsc.	

Table 2. contd

3'-β-Glc	3'-Glc		3'-Glc		3'-Glc		3'-Glc		3'-Glc		3'-Glc	
1′′	4.64 (d, 7.8)	104.3	4.64 (d, 7.8)	104.3	4.69 (d, 7.9)	104.5	4.63 (d, 7.8)	104.2	4.64 (d, 7.8)	104.3	4.63 obsc.	104.3
2′′	3.09 (dd, 7.8, 9.3)	75.6	3.10 (dd, 7.8, 9.3)	75.6	3.16 (dd, 7.9, 9.2)	75.6	3.10 (dd, 7.8, 9.3)	75.3	3.10 (dd, 7.8, 9.3)	75.3	3.09 (dd, 7.8, 9.3)	75.4
3′′	3.29 (t, 9.3)	78.0	3.31 obsc.	78.0	3.34 (t, 9.2)	78.0	3.30 obsc.	77.7*	3.30 obsc.	77.8*	3.28 obsc.	78.1
4′′	3.00 (t, 9.3)	72.0	3.02 (t, 9.3)	72.0	3.22 (t, 9.2)	71.6	3.03 (t, 9.3)	72.0	3.01 (t, 9.3)	72.0	3.01 (t, 9.3)	72.3
5′′	3.22 (m)	78.2	3.24 (m)	78.1	3.44 obsc.	75.2	3.24 obsc.	78.0	3.24 obsc.	78.1*	3.21 (m)	78.1
6′′	3.43 (dd, 6.9, 11.7)	63.3	3.42 obsc.	63.2	4.33 (2H, m)	64.7	3.44 (dd, 7.1, 11.4)	63.2	3.43 (dd, 7.1, 11.7)	63.3	3.43 (dd, 7.1, 11.7)	63.2
	3.8 (dd 2/12)		3.79 obsc.				3.78 obsc.		3.78 obsc.		3.77 obsc.	
4'-Acyl	4'-Fer.		4'-Fer.		4'-Caff.		4'-Caff.		4'-Fer.		4'-Caff.	
1′′′′		127.8		127.8		127.6		127.7		127.8		127.7
2′′′′	7.23 (d, 1.6)	111.6	7.23 (br. s)	111.6	6.99 (br. s)	115.1	7.09 (d, 1.4)	115.4	7.24 (d, 1.4)	111.7	7.07 (d, 1.5)	115.4
3′′′′		149.4		149.4		146.7		146.7		149.4		146.8
4′′′′		150.6		150.6		149.5		149.6		150.6		149.6
5′′′′	6.81 (d, 8.2)	116.5	6.81 (d, 8.2)	116.5	6.88 (br. d, 8.2)	116.5	6.79 (d, 8.2)	116.5	6.81 (d, 8.2)	116.5	6.78 (d, 8.1)	116.5
6′′′′	7.09 (dd, 1.6, 8.2)	124.3	7.10 (br. d, 8.2)	124.3	6.68 obsc.	123.0	6.99 (dd, 1.4, 8.2)	123.1	7.10 (dd, 1.4, 8.2)	124.3	6.98(dd, 1.5, 8.1)	123.1
β′′′′	7.62 (d 15.9)	147.0	7.62 (d, 15.9)	147.0	7.52 (d, 15.8)	147.3	7.56 (d, 15.9)	147.4	7.63 (d, 15.9)	147.2	7.56 (d, 15.9)	147.2
α′′′′	6.45 (d 15.9)	115.9	6.45 (d, 15.9)	115.8	6.27 (d, 15.8)	115.5	6.35 (d, 15.9)	115.3	6.46 (d, 15.9)	115.8	6.34 (d, 15.9)	115.3
CO′′′′		168.5		168.5		168.5		168.7		168.7		168.3
3′′′′-OMe	3.89 (s)	56.5	3.86 (s)	56.5					3.89 (s)	56.5		
6'/6"-subst					6'''-Fer.		6'-β-Glc		6'-β-Glc		6'-α-Rha	
1′′′′′						127.6	4.29 (d, 7.7)	104.7	4.30 (d, 7.7)	104.7	4.62 (br. s)	102.3
2′′′′′					7.08 (br. s)	111.7	3.21 (dd, 7.7, 9.0)	75.0	3.22 (dd, 7.7, 9.3)	75.1	3.83 (br. d, 3.2)	72.0
3′′′′′						149.3	3.35 (t, 9.0)	77.8*	3.35 (t, 9.3)	77.9*	3.66 obsc.	72.0
4′′′′′						150.5	3.28 (t, 9.0)	71.4	3.29 obsc.	71.4	3.34 (t, 9.4)	73.9
5′′′′′					6.80 (d, 8.1)	116.5	3.23 obsc.	78.0	3.24 obsc.	78.0*	3.6 (dq, 9.4, 6.2)	69.9
6′′′′′					7.00 obsc.	124.1	3.65 obsc.	62.6	3.65 obsc.	62.6	1.19(d, 6.2)	18.0
							3.83 obsc.		3.83 (dd, 1.8, 12.2)			
β′′′′′					7.50 (d, 15.9)	146.9						
α′′′′′					6.23 (d, 15.9)	115.3						
CO′′′′′						169.0						
3′′′′′-OMe					3.88 (s)	56.5						

* Not assigned with certaincy. "obsc.": The signal is obscured by overlapping peaks.

Table 3. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of (**13, 15** and **18**) in CD_3OD.

Atom	Chionoside G (**13**)		Chionoside I (**15**)		Isochionoside J (**18**)	
	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C
Agluc						
1		133.1		133.0		131.4
2	6.76 (d, 1.8)	117.3	6.76 (br.s)	117.2	6.68 (d, 2.0)	117.2
3		147.3		147.2		146.1
4		147.6		147.5		144.6
5	6.81 (d, 8.2)	112.8	6.80 (d, 8.2)	112.8	6.62 (d, 8.0)	116.3
6	6.70 (dd, 1.8, 8.2)	121.3	6.68 (br.d, 8.2)	121.3	6.53 (dd, 2.0, 8.0)	121.3
7	2.80 (2H, m)	36.6	2.80 (2H, m)	36.5	2.77 (2H, m)	36.7
8	3.77, 4.09 (m's)	72.0	3.76, 4.08 (m's)	72.0	3.72, 3.95 (m's)	72.3
4-OMe	3.80 (s)	56.5	3.80 (s)	56.5		
Centr β-Glc						
1′	4.54 (d, 7.7)	103.3	4.53 (d, 7.7)	103.2	4.43 (d, 7.0)	103.5
2′	3.74 obsc.	82.0	3.75 (dd, 7.7, 9.3)	82.0	3.50 obsc.	73.8
3′	4.12 (t, 9.3)	81.4	4.12 (t, 9.3)	81.5	3.50 obsc.	89.9
4′	4.92 (t, 9.3)	70.5	4.93 (t, 9.3)	70.5	3.50 obsc.	70.1
5′	3.58 obsc.	75.8	3.55 obsc.	75.6*	3.59 obsc.	75.0
6′	3.66 obsc.	62.8	3.66 obsc.	62.3	4.33 (dd, 5.9, 11.8)	64.5
	3.55 obsc.		3.55 obsc.		4.50 (dd, 1.5, 11.8)	
2′/2″-β-Glc	2'-Glc		2'-Glc		2"-Glc (outer)	
1‴	4.71 (d, 7.8)	104.0	4.72 (d, 7.8)	103.9	4.61 (d, 7.7)	106.4
2‴	3.20 obsc.	75.8*	3.23 obsc.	75.7*	3.27 (dd 7.7, 9.2)	76.2
3‴	3.3 obsc.	77.8*	3.34 obsc.	77.7*	3.38 obsc.	77.6
4‴	3.3 obsc.	71.5	3.33 obsc.	71.4	3.35 obsc.	71.2
5‴	3.20 obsc.	78.0*	3.24 obsc.	78.1	3.36 obsc.	78.8
6‴	3.43 (dd, 6.8, 11.7)	63.2	3.43 (dd, 6.9, 11.7)	63.2	3.73 (dd, 4.6, 12.0)	62.3
	3.76 obsc.		3.79 obsc.		3.91(dd, 1.8, 12.0)	
3′-β-Glc	3'-Glc		3'-Glc		3'-Glc (inner)	
1″	4.65 (d, 7.8)	104.2	4.66 (d, 7.8)	104.2	4.62 (d, 7.8)	104.0
2″	3.09 (dd, 9.3, 7.8)	75.4	3.11 (dd, 9.3, 7.8)	75.3	3.50 obsc.	85.1
3″	3.27 (t, 9.3)	77.9*	3.30 obsc.	77.7*	3.39 (t, 9.3)	77.6
4″	3.02 (t, 9.3)	72.0	3.03 (t, 9.3)	72.0	3.35 obsc.	71.0
5″	3.07 (m)	78.1*	3.07 (m)	77.9*	3.35 obsc.	78.0
6″	3.64 (br. d, 11.7)	62.8	3.66 (br. d, 11.8)	62.7	3.63 (dd, 5.7, 12.0)	62.4
	3.78 obsc.		3.80 obsc.		3.87 (br. d, 11.5)	
Acyl	4'-Caff.		4'-Fer.		6'-Caff.	
1⁗		127.8		127.7		127.6
2⁗	7.06 (d, 1.8)	115.2	7.23 (br.s)	111.6	7.03 (d, 2.0)	114.8
3⁗		146.8		149.3		146.8
4⁗		149.7		150.5		149.6
5⁗	6.77 (d, 8.2)	116.5	6.81 (d, 8.2)	116.5	6.76 (d, 8.2)	116.5
6⁗	6.98 (dd, 1.8, 8.2)	123.0	7.09 (br. d, 8.2)	124.2	6.89 (dd, 2.0, 8.2)	123.2
β⁗	7.56 (d, 15.9)	147.2	7.62 (d, 15.9)	147.0	7.55 (d, 15.9)	147.2
α⁗	6.34 (d, 15.9)	115.4	6.46 (d, 15.9)	115.8	6.28 (d, 15.9)	115.0
CO⁗		168.5		168.5		169.1
3⁗-OMe			3.88 (s)	56.5		

* Not assigned with certaincy. "obsc.": The signal is obscured by overlapping peaks.

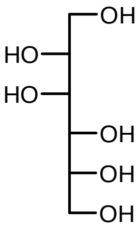
Table 4. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of iso-CPGs **19-25** in CD_3OD and **26** in D_2O.

Atom	Isoaragoside (**19**)		Isochionoside K (**20**)		Isochionoside A (**21**)		Isochionoside G (**24**)		Isochionoside I (**26**)	
	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C	^{1}H - δ (mult. Hz)	^{13}C
Agluc										
1		131.7		132.9		131.7		132.7		132.3
2	6.71 (d, 2.0)	117.4	6.71 (br.s)	117.2	6.70 (d, 1.7)	117.4	6.71 (d, 1.7)	117.2	6.71 (br.s)	117.0
3		146.1		147.3		146.1		147.2		147.2
4		144.7		147.5		144.7		147.5		147.4
5	6.63 (d, 8.0)	116.3	6.68 (d, 8.2)	112.8	6.62 (d, 8.0)	116.3	6.67 (d, 8.2)	112.7	6.65 (d, 8.2)	112.8
6	6.53 (dd, 8.0, 2.0)	121.4	6.61 (br. d, 8.2)	121.3	6.52 (dd, 8.0, 1.7)	121.4	6.62 (dd, 8.2, 1.7)	121.2	6.61 (br. d, 8.2)	121.5
7	2.75 (2H, m)	36.8	2.77(2H, m)	36.8	2.75 (2H, m)	36.8	2.78 (2H, m)	36.6	2.78 (2H, m)	36.3
8	3.67, 3.97 (m's)	72.4	3.69, 3.95 (m's)	72.2	3.67, 3.97 (m's)	72.5	3.75, 3.97 (m's)	72.3	3.77, 3.97 (m's)	72.4
4-OMe			3.75 (s)	56.4			3.75 (s)	56.4	3.74 (s)	56.5
Centr β-Glc	c-Glc		c-Glc		c-Glc		c-Glc		c-Glc	
1′	4.46 (d, 7.7)	103.5	4.47 (d, 7.6)	103.5	4.47 (d, 7.6)	103.6	4.50 (d, 7.7)	103.3	4.51 (d, 7.7)	102.9
2′	3.60 (dd, 7.7, 9.0)	82.0	3.60 obsc.	82.0	3.60 obsc.	82.1	3.65 obsc.	81.3	3.65 obsc.	81.0
3′	3.70 (t, 9.0)	86.6	3.69 obsc.	86.7	3.71 (t, 9.1)	86.7	3.71 (t, 9.2)	86.8	3.75 obsc.	86.2
4′	3.46 (t, 9.0)	70.3	3.45 (t, 9.1)	70.4	3.46 (t, 9.1)	70.3	3.46 (t, 9.2)	70.4	3.47 (t, 9.2)	70.3
5′	3.56 obsc.	74.7	3.57 obsc.	74.7	3.57 obsc.	74.7	3.58 (m)	74.9	3.60 obsc.	74.8
6′	4.51 obsc.	64.6	4.51 obsc.	64.6	4.53 obsc.	64.6	4.52 (br. d, 11.9)	64.5	4.54 (br. d, 11.7)	64.6
	4.31 (dd, 6.0, 11.9)		4.34 (dd, 6.5, 11.8)		4.32 (dd, 6.2, 11.8)		4.34 (dd, 6.3, 11.9)		4.35 (dd, 6.3, 11.9)	
2'-Gly	2'-α-Ara		2'-α-Ara		2'-α-Ara		2'-β-Glc		2'-β-Glc	
1‴	4.51 (d, 7.5)	105.2	4.53 obsc.	105.2	4.51 obsc.	105.2	4.71 (d, 7.9)	104.1	4.70 (d, 7.9)	103.7
2‴	3.55 obsc.	73.3	3.55 (dd, 7.5, 9.0)	73.2	3.55 (dd, 7.1, 9.1)	73.3	3.18 (t-like, 8.5)	75.9	3.18 (t-like, 8.3)	75.3
3‴	3.48 obsc.	74.1	3.47 (dd, 3.3, 9.0)	74.2	3.48 obsc.	74.1	3.33 obsc.	78.2	3.33 obsc.	77.1
4‴	3.74 (m)	69.9	3.74 obsc.	69.9	3.74 (m)	69.9	3.29 obsc.	71.5	3.30 obsc.	70.9
5‴	3.21 (br.d, 12.5)	67.4	3.27 obsc.	67.4	3.23 (br. d, 12.4)	67.4	3.12 (m)	78.0	3.12 (m)	77.5
	3.78 (dd, 2.6, 12.5)		3.80 (br.d 12.3)		3.78 (dd, 2.4, 12.4)					
6‴							3.65 obsc.	62.8	3.65 obsc.	62.1
							3.79 (dd, 2.1, 11.8)		3.77 obsc.	

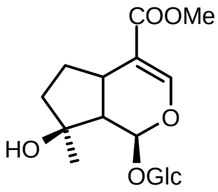
Table 4. contd

3'-β-Glc	3'-Glc		3'-Glc		3'-Glc		3'-Glc		3'-Glc	
1′′	4.62 (d, 7.9)	104.7	4.62 (d, 7.9)	104.8	4.62 (d, 8.0)	104.8	4.63 (d, 7.9)	104.7	4.63 (d, 7.9)	104.1
2′′	3.26 (dd, 7.9, 9.1)	75.2	3.25 obsc.	75.2	3.26 obsc.	75.2	3.26 obsc.	75.3	3.27 obsc.	74.8
3′′	3.37 (t, 9.1)	78.2	3.37 (t, 9)	78.2	3.38 (t, 9)	78.2	3.36 (t, 9.3)	77.8	3.37 obsc.	77.6
4′′	3.29 obsc.	71.5	3.27 obsc.	71.5	3.29 obsc.	71.5	3.32 obsc.	71.5	3.35 obsc.	71.0
5′′	3.33 obsc.	78.2	3.32 (m)	78.2	3.35 (m)	78.2	3.57 (m)	78.2	3.58 (m)	77.6
6′′	3.63 (dd, 6.2, 11.9)	62.5	3.63(dd, 6.1, 11.8)	62.5	3.63 obsc.	62.5	3.63 obsc.	62.5	3.65 obsc.	62.1
	3.88 (dd, 2.1, 11.9)		3.88 (dd, 2, 11.8)		3.88 (dd, 2.4, 11.8)		3.88 (br. d, 11.7)		3.88 (br. d, 11.6)	
6'-Acyl	6'-Caff.		6'-Caff.		6'-Fer.		6'-Caff.		6'-Fer.	
1′′′′		127.6		127.7		127.6		127.6		127.5
2′′′′	7.03 (d, 2.0)	115.1	7.03 (br.s)	115.1	7.14 (d, 1.7)	111.6	7.02 (d, 1.8)	115.1	7.13 (d, 1.7)	111.7
3′′′′		146.8		147.0		149.3		149.7		149.0
4′′′′		149.7		149.7		150.6		150.7		149.8
5′′′′	6.76 (d, 8.2)	116.5	6.76 (d, 8.2)	116.5	6.79 (d, 8.2)	116.5	6.76 (d, 8.2)	116.5	6.78 (d, 8.2)	116.4
6′′′′	6.89 (dd, 8.2, 2.0)	123.2	6.89 (br. d, 8.2)	123.2	7.00 (dd, 8.2, 1.7)	124.5	6.89 (dd, 8.2, 1.8)	123.2	7.00 (dd, 1.7, 8.2)	124.2
β′′′′	7.55 (d, 15.9)	147.2	7.55 (d, 15.9)	147.2	7.60 (d, 15.9)	147.1	7.55 (d, 15.9)	146.8	7.60 (d 15.9)	146.4
α′′′′	6.28 (d, 15.9)	114.8	6.28 (d, 15.9)	114.8	6.37 (d, 15.9)	115.2	6.27 (d, 15.9)	114.8	6.37 (d 15.9)	114.9
CO′′′′		169.0		169.0		169.0		169.0		169.5
3′′′′-OMe					3.86 (s)	56.4			3.85 (s)	56.5

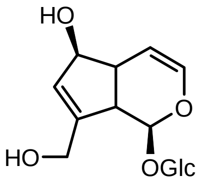
"obsc.": The signal is obscured by overlapping peaks.



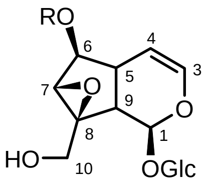
1; Mannitol



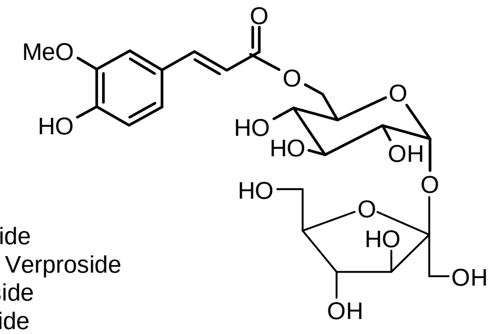
2; Mussaenoside



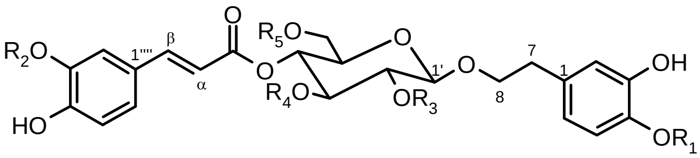
3; Aucubin



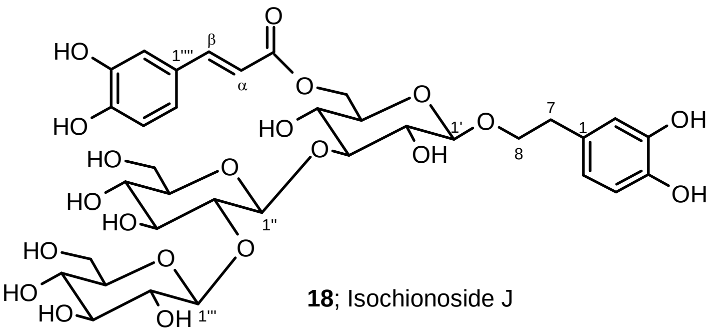
4; R=H; Catalpol
4a; R=Benzoyl; Veronicoside
4b; R=3,4-di-OH-Benzoyl; Verproside
4c; R=Vanilloyl; Amphicoside
4d; R=Caffeoyl; Verminoside
4e; R=Feruloyl; Feruloylcatalpol
4f; R=Isoferuloyl; Minecoside



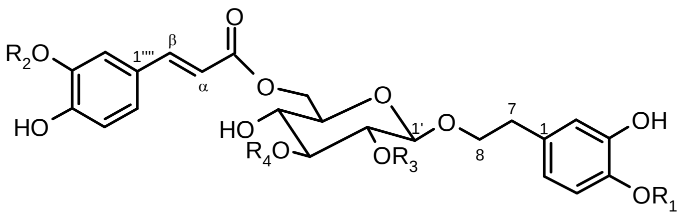
27; 6' Feruloyl-sucrose



5	R_1=H;	R_2=H;	R_3=Ara;	R_4=Glc;	R_5=H;	Aragoside
6	R_1=H;	R_2=Me;	R_3=Ara;	R_4=Glc;	R_5=H;	Chionoside A
7	R_1=Me;	R_2=Me;	R_3=Ara;	R_4=Glc;	R_5=H;	Chionoside B
8	R_1=H;	R_2=H;	R_3=Ara;	R_4=6-Fer-Glc;	R_5=H;	Chionoside C
9	R_1=H;	R_2=H;	R_3=Ara;	R_4=Glc;	R_5=Glc;	Chionoside D
10	R_1=H;	R_2=Me;	R_3=Ara;	R_4=Glc;	R_5=Glc;	Chionoside E
11	R_1=H;	R_2=H;	R_3=Ara;	R_4=Glc;	R_5=Rha;	Chionoside F
12	R_1=H;	R_2=H;	R_3=Glc;	R_4=Glc;	R_5=H;	Persicoside
13	R_1=Me;	R_2=H;	R_3=Glc;	R_4=Glc;	R_5=H;	Chionoside G
14	R_1=H;	R_2=Me;	R_3=Glc;	R_4=Glc;	R_5=H;	Chionoside H
15	R_1=Me;	R_2=Me;	R_3=Glc;	R_4=Glc;	R_5=H;	Chionoside I
16	R_1=H;	R_2=H;	R_3=Ara;	R_4=Rha;	R_5=H;	Ehrenoside
17	R_1=Me;	R_2=Me;	R_3=Ara;	R_4=Rha;	R_5=H;	Lagotoside



18; Isochionoside J



19	R_1=H;	R_2=H;	R_3=Ara;	R_4=Glc;	Isoaragoside
20	R_1=Me;	R_2=H;	R_3=Ara;	R_4=Glc;	Isochionoside K
21	R_1=H;	R_2=Me;	R_3=Ara;	R_4=Glc;	Isochionoside A
22	R_1=Me;	R_2=Me;	R_3=Ara;	R_4=Glc;	Isochionoside B
23	R_1=H;	R_2=H;	R_3=Glc;	R_4=Glc;	Isopersicoside
24	R_1=Me;	R_2=H;	R_3=Glc;	R_4=Glc;	Isochionoside G
25	R_1=H;	R_2=Me;	R_3=Glc;	R_4=Glc;	Isochionoside H
26	R_1=Me;	R_2=Me;	R_3=Glc;	R_4=Glc;	Isochionoside I