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Iridoid and Phenylethanoid Glucosides from *Veronica lavaudiana*

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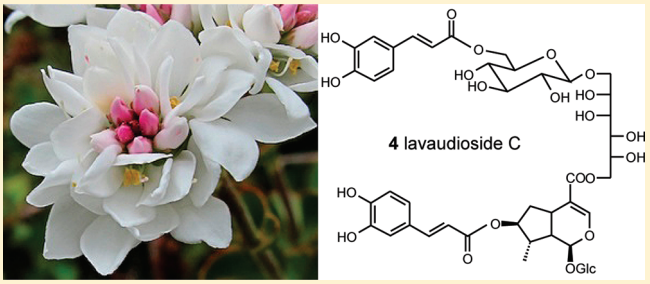
Supporting Information

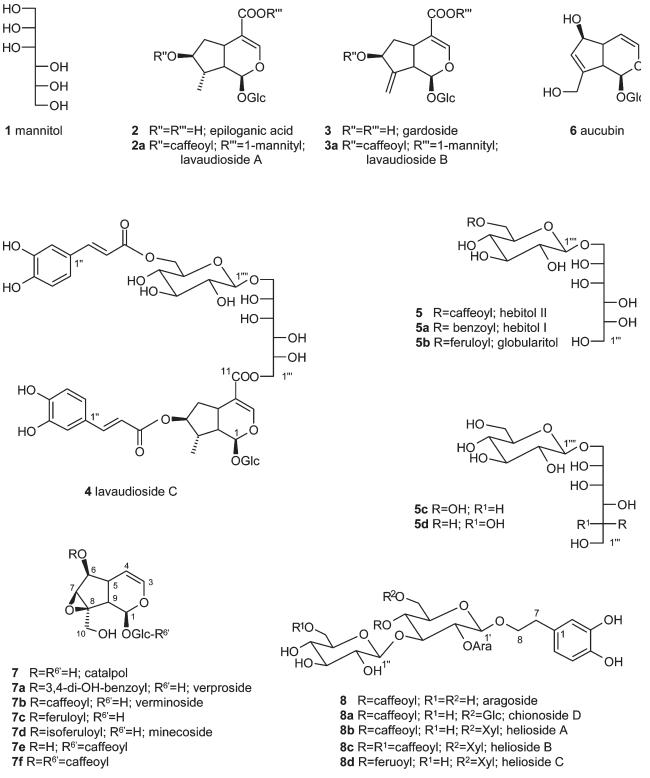
ABSTRACT: From an extract of *Veronica* (sect. *Hebe*) *lavaudiana* we have identified mannitol and isolated 11 iridoid glucosides, the carbohydrate ester hebitol II, and four phenylethanoid glycoside esters. Five of the iridoid glycosides are new; of these, lavaudiosides A, B, and C (**2a**, **3a**, and **4**) are 1-mannityl esters of 8-epiloganic acid, while **7e** and **7f** are 6′-*O*-caffeoyl derivatives of catalpol. The new phenylethanoid glycoside esters, heliosides A, B, and C (**8b**−**d**), are 6′-xylosyl derivatives of aragoside. The structures of the new compounds were elucidated mainly by spectroscopic analysis, but also by chemical degradation. We also demonstrated that the structures of the known glycosides globularitol and hebitols I and II should be revised. These compounds are derivatives of mannitol and not glucitol as previously believed.



Veronica (sect. *Hebe*) *lavaudiana* Raoul (Plantaginaceae) is an endemic subshrub that grows on basalt outcrops and cliffs above 150 m altitude on Banks Peninsula on the east coast of the South Island of New Zealand.[1] The peninsula, reaching an altitude of about 900 m, is formed from two extinct volcanoes and has at times in the past been separated as an island. *V. lavaudiana* and its nearest relatives in the sect. *Hebe* belong in the sun hebe clade.[2] It has been formerly treated as the genus *Heliohebe*[1] or as part of *Hebe*[3] or *Parahebe*,[4] all of which are now included in a broad and monophyletic concept of *Veronica*.[5] Little chemical work has been undertaken on *V. lavaudiana*. Thus, this species was included in an early chemotaxonomic survey of iridoids and flavonoids in *Veronica* and related genera[6] and in a recent LC/MS investigation on the flavonoid chemistry of the five species of *Heliohebe*.[7]

Fresh plant material was blended with cold MeOH, and the water-soluble part of the extract was subjected to a series of chromatographic procedures. Seventeen compounds were isolated and identified including the hexitol mannitol (**1**), the carbohydrate ester hebitol II (**5**), 11 iridoid glucosides (**2a**, **3a**, **4**, **6**, **7**, **7a**−**f**), and four phenylethanoid glycoside esters (**8**, **8b**−**d**). Of these, the five iridoids **2a**, **3a**, **4**, **7e**, and **7f** and the three phenylethanoids **8b**−**d** are new.

The composition of the sugar fraction was deduced by interpretation of the ^{13}C NMR data only. Structural elucidations of the new compounds were carried out by spectroscopic methods (1D and 2D NMR, MS) and chemical degradation. Despite intensive purification, most of the compounds contained minor concentrations of *cis*-caffeoyl or -feruloyl isomers. Compound **8d** contained 8% of the *cis*-form, in accord with our previous experience that feruloyl esters readily isomerize.



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Lavaudioside A (**2a**) was obtained as an amorphous solid, $[\alpha]^{20}_{D}$ −30. The molecular formula was $C_{31}H_{42}O_{18}$, as deduced from the ^{13}C NMR data and the quasimolecular ion obtained by LC-HRESIMS (observed m/z 720.2713 $[M + NH_4]^+$). The ^1H NMR spectrum (Table 1) in methanol-d_4 showed five resonances corresponding to the presence of a caffeoyl group (δ_H 7.51 to 6.24). An additional resonance at δ_H 7.53 (s, H-3), as well as two more at δ_H 5.55 (d, H-1) and 4.68 (d, H-1′), suggested that **2a** was a carboxylated iridoid glucoside. Furthermore, the three-proton doublet at δ_H 1.10 showed the presence of a C-10 methyl group. In the ^{13}C NMR spectrum, 30 resonances were observed, of which one (δ_C 169.0) was of double intensity. Nine of the resonances were consistent with the presence of a caffeoyl group. Of the remaining resonances in the spectrum, 16 were similar to those reported for a 7-*O*-acyl derivative of the iridoid glucoside epiloganic acid (**2**),[8] including those from the 1-*O*-β-glucopyranosyl group. The remaining six resonances were all in the δ_C 65−73 region, which suggested the presence of a hexityl moiety. This was consistent with the ^1H NMR spectrum, where the signals not accounted for were observed in the shift interval δ_H 3.65−4.44. The HMBC spectrum allowed the carbons of attachment between the iridoid moiety and the peripheral parts to be discerned. Thus, the position of the ester group was confirmed by an HMBC correlation between H-7 (δ_H 4.9) and the carbonyl carbon of the caffeoyl group (δ_C 169.0). The 1‴-CH_2 resonances of the hexityl moiety at δ_H 4.44 and 4.21 showed correlations with the carbonyl carbon (δ_C 169.0) of the iridoid core and with a carbinol signal (δ_C 70.5), which could therefore be assigned as C-2‴. The HSQC spectrum designated the remaining resonances at δ_C 65.1, 72.8, 70.9, and 70.8 as one CH_2OH and three CHOH groups, respectively, thereby fulfilling the molecular formula determined by the MS. The first three shift values closely matched those of mannitol (**1**). Confirmation that the hexitol was indeed mannitol was achieved by an in-NMR tube hydrolysis of **2a** with ammonia in D_2O. After 48 h at room temperature, ^{13}C NMR resonances corresponding to mannitol were observed at δ_C 64.5 (C-1 and C-6), 72.1 (C-2 and C-5), and 70.5 (C-3 and C-4).[9] These increased in intensity following the addition of authentic mannitol (see Supporting Information).

Lavaudioside B (**3a**) was obtained as an amorphous solid, $[\alpha]^{22}_{D}$ −24. The molecular formula was $C_{31}H_{40}O_{18}$, as deduced from the ^{13}C NMR data and LC-HRESIMS (observed m/z 718.2558 $[M + NH_4]^+$). The NMR data (Table 1) in methanol-d_4 was assigned as above. The ^1H NMR spectrum was similar to that of **2a**, including a caffeoyl and a mannityl group, as well as the resonance at δ_H 7.62 (1H, s, H-3) and others at δ_H 5.41 and 4.70, assigned to H-1 and H-1′, respectively. The major differences were in the iridoid moiety. Thus, additional resonances (δ_H 5.48 and 5.45) were present, while that from a methyl group was missing. In the ^{13}C NMR spectrum of **3a**, the expected 31 resonances were observed. When compared with the spectrum of **2a**, only the resonances for the iridoid aglucone differed. The two resonances at δ_C 148.9 and 116.5 suggested an iridoid with an 8,10-double bond, in accordance with the ^1H NMR data. A comparison with the data reported[8] for a 7-*O*-acyl derivative of gardoside (**3**) showed a satisfactory coincidence for the chemical shift values.

Lavaudioside C (**4**) was isolated as an amorphous solid, $[\alpha]^{22}_{D}$ −48, and the molecular formula was $C_{46}H_{58}O_{26}$, deduced by the ^{13}C NMR data and LC-HRESIMS (observed m/z 1027.3269 $[M + H]^+$). The ^1H NMR spectrum (Table 1) was similar to that of **2a**, but resonances from two caffeoyl groups were present. Also, two β-hexopyranosyl groups were considered present due to the two doublets at δ_H 4.69 and 4.36. In the ^{13}C NMR spectrum (Table 1) of **4**, 39 resonances were observed, of which five were of double and one (δ_C 71.6) was of triple intensity, as also confirmed by the HSQC data. Eighteen of the resonances could be assigned to the two caffeoyl groups, and comparing with the data for **2a**, a further 16 could be assigned to an epiloganyl moiety including its 1-*O*-glucosyl group. This left 12 ^{13}C NMR resonances to be accounted for. That at δ_C 105.0 was assigned to an anomeric carbon atom, and the remaining 11, resonating at δ_C 64−78, suggested the presence of a hexopyranosyl and a hexityl moiety. 2D NMR data suggested that the hexopyranosyl moiety was an additional β-glucopyranosyl group, but the full structure of **4** could be resolved only in relation to that of hebitol II (**5**).

The sugar ester hebitol II (**5**) was considered to be composed of a 6-*O*-caffeoyl-β-D-glucopyranose moiety with a 1-glucityl group as the aglucone.[10] The structure of **5** was originally elucidated by comparison of the NMR data of an analogue, the feruloyl ester globularitol (**5b**) isolated from *Globularia orientalis*.[11] The structure of **5b** was in turn inferred by deacylation and comparison by TLC of the resulting disaccharide with authentic β-D-glucopyranosyl-(1→6)-glucitol (**5d**), obtained by $NaBH_4$ reduction of gentiobiose. However, because the corresponding ^{13}C NMR shift values of **5** (for C-1‴ to C-3‴ in Table 1) compared better with those of β-D-glucopyranosyl-(1→6)-mannitol (**5c**)[12] than with those of the glucitol derivative, we therefore decided to re-examine the structure of **5**. Thus, treatment of **5** with ammonia in D_2O gave a disaccharide with a ^{13}C NMR spectrum (see Supporting Information) identical to that reported for **5c**. The similar levorotatory activity of **5** and **5a**−**c** pointed to the β-D-form of the glucose moiety in the four compounds (see Supporting Information). Consequently, the structures of the three analogues hebitol II (**5**), hebitol I (**5a**),[10] and globularitol (**5b**)[11] must be revised to the β-D-glucosylmannitol derivatives shown in the formula chart.

Returning to the structure of **4**, the resonances of the caffeoyl and β-glucopyranosyl groups found in **4**, but not in **2a**, were coincident with the corresponding resonances of **5**. In addition, those from C-1‴ through C-3‴ for **4** were almost coincident with the resonances of the corresponding carbon atoms of **2a**, and the resonances of C-4‴ to C-6‴ were coincident with those of **5**. This provided the hypothetical structure for **4**. In the HMBC spectrum, correlations could be seen between the 1‴-CH_2 group (δ_H 4.42 and 4.20) and C-2‴ (δ_C 70.7) as well as C-11 of the iridoid core (δ_C 169.0) since a cross-peak with H-5 (δ_H 3.13) was also found at this shift value. Other correlations were seen between the 6‴-CH_2 group (δ_H 4.14 and 3.76) and C-5‴ (δ_C 71.6) as well as the anomeric carbon atom C-1⁗ of the central β-glucopyranosyl moiety (δ_C 105.0). Also, H-5⁗ (δ_H 3.55) displayed a correlation with C-1⁗, and furthermore, cross-peaks could be found between 6⁗-CH_2 (δ_H 4.52 and 4.28) and C-5⁗ (δ_C 75.5) as well as one of the low-field carbonyl carbon atoms (δ_C 169.1). Therefore, it was concluded that the latter could be assigned to one of the caffeic acid moieties. The structure of lavaudioside C has therefore been established as that shown by **4**.

Compound **7e** was obtained as an amorphous solid, $[\alpha]^{22}_{D}$ −70, and the molecular formula was $C_{24}H_{28}O_{13}$, determined by the ^{13}C NMR data and LC-HRESIMS (observed m/z 523.1465 $[M - H]^-$). The ^1H NMR data (Table 2) were somewhat similar

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Lavaudiosides A−C and Model Compounds in Methanol-d_4 (or D_2O)

	lavaudioside A (**2a**)		lavaudioside B (**3a**)		lavaudioside C (**4**)		hebitol II (**5**)		glu-mannitol (**5c**)
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C (D_2O)[a]
Irid. agluc									
1	95.9	5.55, d (4.3)	96.3	5.41, d (5.6)	96.0	5.54, d (4.5)			
3	152.9	7.53, s	154.2	7.62, s	152.9	7.52, s			
4	113.7		111.2		113.6				
5	31.4	3.14, br q (8)	33.2	3.28, obsc[b]	31.5	3.13, br q (8)			
6	39.0	2.27, ddd (14, 8, 3)	38.6	2.23, dt (13, 6)	39.0	2.26, ddd (14, 8, 3)			
		2.00, dt (14, 7)		2.12, dt (13, 6)		1.99, dt (14, 6.5)			
7	82.6	4.90, obsc	76.4	5.58, br t (5)	82.6	4.90, obsc			
8	43.2	2.44, m	148.9		43.2	2.43, dq (14, 8)			
9	43.1	2.62, dt (4.3, 8)	44.8	3.00, br t (6)	43.1	2.60, dt (8, 4.5)			
10	14.3	1.10, d (7.3)	116.5	5.48, 5.45, br s′s	14.3	1.09, d (7.4)			
11	169.0		169.0[c]		169.0				
Irid. Glc									
1′	99.7	4.68, d (7.9)	99.9	4.70, d (7.9)	99.7	4.69, d (7.9)			
2′	74.7	3.20, dd (9.2, 7.9)	74.8	3.23, dd (9.2, 7.9)	74.8	3.20, dd (9.2, 7.9)			
3′	77.9	3.38, t (9.1)	78.0	3.38, t (9.0)	78.0	3.37, obsc			
4′	71.6	3.25, t (9.1)	71.7	3.26, obsc	71.6	3.24, obsc			
5′	78.3	3.32, obsc	78.5	3.32, obsc	78.4	3.32, obsc			
6′	62.8	3.92, obsc	62.9	3.90, obsc	62.9	3.90, dd (11.9, 1.7)			
		3.66, obsc		3.64, obsc		3.64, dd (11.9, 6.4)			
Caffeoyl									
CO″	169.0		168.9[c]		169.0/169.1		169.1		
α″	115.3	6.24, d (15.9)	115.3	6.26, d (15.9)	115.3/114.8	6.24/6.29, d's (15.9)	114.8	6.29, d (15.9)	
β″	146.9	7.51, d (15.9)	147.1	7.53, d (15.9)	147.3/146.9	7.51/7.57, d's (15.9)	146.8	7.57, d (15.9)	
1″	127.6		127.7		127.7/127.7		127.6		
2″	115.1	7.04, d (1.6)	115.1	7.05, br s	115.2/115.1	2H, 7.04, m	115.1	7.05, d (1.9)	
3″	146.8		146.8		146.8/147.3		147.3		
4″	149.5		149.7		149.6/149.6		149.6		
5″	116.5	6.77, d (8.2)	116.5	6.77, d (8.2)	116.5/116.5	2H, 6.77, d (8.2)	116.5	6.77, d (8.2)	
6″	123.0	6.94, dd (8.2, 1.6)	123.1	6.94, br d (8.2)	123.0/123.1	2H, 6.94, m	123.1	6.95, dd (8.2, 1.9)	
Hexitol									
1‴	67.4	4.44, dd (11.5, 2.1)	67.5	4.45, dd (11.6, 2.4)	67.4	4.42, dd (11.5, 2.3)	65.2	3.80, obsc	65.1
		4.21, dd (11.5, 5.9)		4.21, dd (11.6, 5.9)		4.20, dd (11.5, 5.8)		3.61, dd (11.1, 6.0)	
2‴	70.5	3.91, obsc	70.5	3.91, obsc	70.7	3.87, obsc	72.9	3.69, m	72.8
3‴	70.8[c]	3.81, obsc	70.8[c]	3.81, obsc	70.7[c]	3.80, obsc	70.9[c]	3.80, obsc	71.0
4‴	70.9[c]	3.81, obsc	71.0[c]	3.81, obsc	71.6[c]	3.80, obsc	71.1[c]	3.82, obsc	71.6
5‴	72.8	3.69, obsc	72.9	3.70, obsc	71.6	3.75, obsc	71.6	3.80, obsc	71.6
6‴	65.1	3.82, obsc	65.2	3.82, obsc	73.6	4.14, dd (10.5, 1.3)	73.7	4.15, dd (10.5, 2.3)	73.7
		3.65, obsc		3.64, obsc		3.76, m		3.72, dd (10.5, 6.1)	
Centr. Glc									
1⁗					105.0	4.36, d (7.8)	105.0	4.36, d (7.8)	104.7
2⁗					75.1	3.25, obsc	75.2	3.27, dd (8.9, 7.8)	75.1
3⁗					77.7	3.41, t (9.2)	77.6	3.40, t (8.9)	77.5
4⁗					71.6	3.38, obsc	71.6	3.36, t (8.9)	71.3
5⁗					75.5	3.55, m	75.5	3.55, m	77.8
6⁗					64.6	4.52, dd (11.9, 1.7)	64.6	4.51, dd (11.9, 1.9)	62.6
						4.28, dd (11.9, 5.8)		4.29, dd (11.9, 5.7)	

[a] Data from ref 12. For direct comparison, C-6‴ was aligned with C-6‴ in **5**. [b] Obsc: signal obscured. [c] Interchangeable within column.

to those of verminoside (**7b**),[13] both containing a caffeoyl and a catalpol moiety. However, in **7e** H-6 (δ_H 3.78) resonated 1.2 ppm upfield while the H-6′ signals (δ_H 4.41 and 4.49) were 0.6 and 1.1 ppm downfield compared to those of **7b**. This indicated that in **7e** the caffeoyl moiety was located at the 6′-*O*-position. This conclusion was supported by the relative changes observed

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Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Catalpol Esters (7e and 7f) in Methanol-d_4

	6′-*O*-caffeoylcatalpol (**7e**)		6,6′-di-*O*-caffeoylcatalpol (**7f**)	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
Agluc				
1	96.3	4.86, d (9.9)	95.2	4.96, d (9.7)
3	141.8	6.31, dd (6.0, 1.5)	142.4	6.33, dd (5.9, 1.5)
4	104.1	5.02, dd (6.0, 4.5)	103.1	4.92, dd (5.9, 4.8)
5	39.1	2.22, m	36.8	2.53, m
6	79.7	3.78, br d (8.1)	81.3	4.89, br d (9.0)
7	62.4	3.40, br s	61.6	3.67, br s
8	66.1		66.7	
9	43.3	2.53, dd (9.9, 7.8)	43.0	2.61, dd (9.7, 7.8)
10	61.9	4.15, d (13.0)	60.2	4.19, d (13.1)
		3.64, d (13.0)		3.70, d (13.1)
Glc				
1′	99.7	4.77, d (7.9)	99.8	4.79, d (7.9)
2′	74.7	3.29, obsc[a]	74.8	3.30, obsc
3′	77.4	3.41, m	77.5	3.43, m
4′	71.5	3.41, m	71.7	3.39, m
5′	75.9	3.53, m	76.1	3.55, m
6′	63.9	4.49, dd (11.9, 2.0)	63.9	4.50, dd (12.0, 2.7)
		4.41, dd (11.9, 5.6)		4.47, dd (12.0, 5.9)
Caffeoyl				
CO″	169.0		169.0/168.9	
α″	114.8	6.27, d (15.9)	114.8/114.8	6.30/6.28, d (15.9)
β″	146.8	7.54, d (15.9)	146.8/146.8	2H, 7.58, d (15.9)
1″	127.7		127.7/127.6	
2″	115.1	7.04, d (1.7)	115.2/115.3	2H, 7.06, br s
3″	147.3		147.4/147.7	
4″	149.7		149.7/149.8	
5″	116.5	6.77, d (8.2)	116.5/116.5	6.78/6.73, d (8.2)
6″	123.1	6.94, dd (8.2, 1.7)	123.2/123.3	6.97/6.95, dd (8, 2)

[a] Obsc: signal obscured.

in the ^{13}C NMR data. In the HMBC spectrum, correlations between 6′-CH_2 (δ_H 4.49 and 4.41) and the carbonyl carbon atom of the caffeoyl group (δ_C 169.0) confirmed that the structure of **7e** was 6′-*O*-caffeoylcatalpol.

Compound **7f** was also an amorphous solid, $[\alpha]^{20}_D$ −20, with the molecular formula $C_{33}H_{34}O_{16}$, deduced by the ^{13}C NMR data and LC-HRESIMS (observed *m*/*z* 709.1791 $[M + Na]^+$). The ^{1}H NMR data (Table 2) showed that the molecule contained one catalpol and two caffeoyl units. Comparison with the NMR data of **7b** and **7e** showed that in **7f** the resonances for the catalpol aglucone moiety were very similar to those of the former, while the resonances of the β-D-glucopyranosyl unit were similar to those of the latter. This suggested that the two caffeoyl groups were placed at the 6-*O*- and the 6′-*O*-positions of catalpol. Analysis of the HMBC spectrum confirmed this. Thus, the positions of the ester groups were confirmed by correlations between H-6 (δ_H 4.89) as well as by one of the 6′-CH_2 protons (δ_H 4.47) and the carbonyl carbon atoms of the caffeoyl groups (δ_C 169.0 and 168.9), respectively. The structure of compound **7f** was therefore 6,6′-di-*O*-caffeoylcatalpol.

Helioside A (**8b**), $[\alpha]^{20}_D$ −23, had a molecular formula of $C_{39}H_{52}O_{24}$, as deduced by the ^{13}C NMR data and LC-HRESIMS (observed *m*/*z* 922.3200 $[M + NH_4]^+$). The NMR data (Table 3) were assigned by 2D NMR spectroscopy and by comparison with the model compounds aragoside (**8**)[14] and its 6′-*O*-β-glucopyranosyl derivative chionoside D (**8a**) from *V. thomsonii* (Buchanan) Cheeseman.[15] The ^{13}C NMR spectrum showed 37 resonances, of which two (δ_C 115.3 and 77.8) were of double intensity. This included four signals at δ_C 103−106 arising from anomeric carbon atoms showing correlations with protons in the δ_H 4.2−4.7 region (HSQC). The NMR data (Table 2) were almost superimposable with those of **8a**, a 3,4-dihydroxyphenylethyl β-D-glucopyranoside esterified with a caffeoyl group at C-4′ and substituted with an α-arabinopyranosyl at C-2′ and β-glucopyranosyl groups at C-3′ and C-6′. However, one of the peripheral glucosyl groups of **8a** was replaced by a pentosyl moiety in **8b**, identified as a β-xylopyranosyl group by the NMR data. The correlations in the HMBC spectrum confirmed the sites of attachment of the peripheral units. Thus, a cross-peak between H-1′ (δ_H 4.51) of the central glucosyl moiety and C-8 (δ_C 72.2) proved the position of the aglycone. A correlation between H-4′ (δ_H 4.96) and the carbonyl carbon atom of the caffeoyl ester (δ_C 168.6) showed the position of the acyl moiety. Cross-peaks between H-1‴ (δ_H 4.52) of the arabinosyl group and C-2′ (δ_C 82.5), as well as between H-1″ (δ_H 4.63) of the peripheral glucosyl group and C-3′ (δ_C 81.2), demonstrated the positions of these sugar moieties. Finally, a correlation between H-1⁗ of the xylosyl moiety (δ_H 4.24) and the 6′-CH_2 group (δ_C 69.1) proved the structure as shown by **8b**.

Helioside B (**8c**), $[\alpha]^{20}_D$ −25, had the molecular formula $C_{48}H_{58}O_{27}$ as deduced by the ^{13}C NMR data and LC-HRESIMS (observed *m*/*z* 1084.3451 $[M + NH_4]^+$). The NMR data (Table 3) were assigned as above. When the NMR and MS data were compared with those of **8b**, it became apparent that **8c** was a caffeoyl ester of the former. Besides the additional caffeoyl group, the 6″-CH_2 resonances (δ_H 4.34, 4.27) of the peripheral glucopyranosyl group were found 0.6 and 0.8 ppm, respectively, downfield in the ^{1}H NMR spectrum. Similarly, in the ^{13}C NMR spectrum of **8c**, C-6″ (δ_C 64.7) and C-5″ (75.1) of the peripheral β-glucopyranosyl group were found 1.6 ppm downfield and 2.7 ppm upfield, respectively, when compared to the data for **8b**. Therefore, the additional caffeoyl group was positioned at the C-6″ oxygen atom in helioside B. The correlations in the HMBC spectrum were consistent with this, including the cross-peak between one of the C-6″ protons (δ_H 4.27) and the ester carbonyl carbon atom assigned to the additional caffeoyl group (δ_C 169.1). Helioside B is therefore the 6″-caffeoyl ester of helioside A.

Helioside C (**8d**), $[\alpha]^{20}_D$ −22, had the molecular formula $C_{40}H_{54}O_{24}$, deduced by the ^{13}C NMR data and LC-HRESIMS (observed *m*/*z* 936.3333 $[M + NH_4]^+$). The NMR data (Table 3) were coincident with those of **8b**, except for the resonances of the aroyl moiety and the presence of a methoxy group in **8d**. The chemical shift values (δ_H 3.90; δ_C 56.5), as well as a cross-peak between the *O*-methyl protons (δ_H 3.90) and C-3⁗ (δ_C 149.4), were consistent with the presence of a feruloyl substituent in **8d**. Consequently, helioside C is the feruloyl analogue of helioside A.

Our study confirms the previous report[6] of aucubin (**6**), catalpol (**7**), and the two catalpol esters **7a** and **7b** in *V. lavaudiana*. Epiloganic acid (**2**) and gardoside (**3**) and their methyl esters are often found in plants containing **6** and **7** and have been reported from several *Veronica* species.[10,16−20]

Table 3. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Heliosides A−C (8b−d) in Methanol-d_4

	helioside A (8b)		helioside B (8c)		helioside C (8d)	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
Aglucone						
1	131.9		132.0		131.9	
2	117.5	6.74, d (1.8)	117.5	6.72, d (1.9)	117.5	6.74, d (1.9)
3	145.8		146.0		146.0	
4	144.1		144.7		144.7	
5	116.4	6.67, d (8.0)	116.3	6.67, d (8.1)	116.3	6.68, d (7.9)
6	121.6	6.57, dd (8.0, 1.8)	121.5	6.56, dd (8.0, 1.9)	121.5	6.57, dd (7.9, 1.9)
7	36.6	2H, 2.77, m	36.7	2H, 2.74, m	36.7	2H, 2.77, m
8	72.2	4.06/3.68, m's	72.2	4.01/3.62, m's	72.3	4.06/3.67, m's
Central Glc						
1′	103.2	4.51, d (7.9)	103.3	4.40, d (7.7)	103.3	4.51, d (7.9)
2′	82.5	3.70, obsc[a]	82.8	3.69, dd (9.5, 7.7)	82.8	3.69, obsc
3′	81.2	4.10, t (9.5)	81.6	3.94, t (9.5)	81.4	4.09, t (9.7)
4′	70.2	4.96, t (9.5)	70.0	4.98, t (9.5)	70.3	4.97, t (9.7)
5′	74.1	3.77, obsc	74.5	3.65, obsc	74.4	3.78, obsc
6′	69.1	3.87, dd (11.4, 1.5)	69.1	3.81, dd (11.5, 1.5)	69.4	3.87, dd (11.5, 1.5)
		3.60, dd (11.5, 6.4)		3.60, obsc		3.61, dd (11.5, 6.1)
2′-Ara						
1‴	104.7	4.52, d (7.4)	104.7	4.51, d (7.3)	104.3	4.52, d (7.6)
2‴	73.1	3.56, dd (9.1, 7.4)	73.2	3.57, obsc	73.2	3.56, dd (9.0, 7.4)
3‴	73.9	3.46, obsc	74.0	3.46, obsc	74.0	3.46, obsc
4‴	69.8	3.73, obsc	69.8	3.74, obsc	69.8	3.73, obsc
5‴	67.3	3.76, obsc	67.4	3.74, obsc	67.4	3.78, obsc
		3.16, br d (11.7)		3.09, obsc		3.16, obsc
3′-Glc						
1″	104.1	4.63, d (7.9)	104.5	4.67, d (7.9)	104.9	4.64, d (7.8)
2″	75.2	3.08, dd (9.2, 7.9)	75.1[b]	3.14, obsc	74.8	3.08, dd (9.0, 7.8)
3″	77.8[b]	3.28, obsc.	78.0[b]	3.34, obsc	78.0[b]	3.27, obsc
4″	72.2	3.02, t (9.3)	71.7	3.20, t (9.3)	72.0	3.00, t (9.3)
5″	77.8	3.21, obsc	75.1	3.45, obsc	78.1[b]	3.22, obsc
6″	63.1	3.77, obsc	64.7	4.34, dd (1.9, 11.5)	63.2	3.77, obsc
		3.44, obsc		4.27, dd (6.4, 11.5)		3.41, obsc
4′-Acyl		4′-caffeoyl		4′-caffeoyl		4′-feruloyl
CO⁗	168.6		168.7		168.6	
α⁗	115.3	6.33, d (15.9)	115.0	6.29, d (15.9)	115.7	6.44, d (15.9)
β⁗	147.3	7.56, d (15.9)	147.5	7.52, d (15.9)	147.2	7.62, d (15.9)
1⁗	127.6		127.6		127.7	
2⁗	115.3	7.07, d (1.9)	115.3	7.00, br s	111.6	7.24, d (1.6)
3⁗	146.6		146.7		149.3	
4⁗	149.5		149.6		150.6	
5⁗	116.6	6.77, d (8.2)	116.6	6.77, d (8.2)	116.5	6.81, br d (8.1)
6⁗	123.2	6.98, dd (8.2, 1.9)	123.1	6.90, br d (8.2)	124.3	7.10, dd (8.1, 1.6)
OMe					56.5	3.90, s
6′-Xyl						
1⁗′	105.1	4.24, d (7.4)	105.2	4.20, d (7.4)	105.3	4.24, d (7.4)
2⁗′	74.7	3.19, obsc	75.3[b]	3.20, dd (9.0, 7.4)	75.3	3.19, dd (9.0, 7.4)
3⁗′	77.3[b]	3.39, obsc	77.5[b]	3.30, obsc	77.5[b]	3.30, obsc
4⁗′	70.9	3.45, obsc	71.0	3.46, obsc	71.1	3.45, obsc
5⁗′	66.7	3.82, dd (11.5, 5.3)	66.8	3.81, obsc	66.9	3.82, dd (11.5, 5.4)
		3.14, obsc		3.13, obsc		3.15, obsc

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Table 3. Continued

	helioside A (**8b**)		helioside B (**8c**)		helioside C (**8d**)	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
6′′-caffeoyl						
CO′′′′′′			169.1			
α′′′′′′			115.0	6.18, d (15.9)		
β′′′′′′			147.1	7.50, d (15.9)		
1′′′′′′			127.6			
2′′′′′′			115.5[b]	7.00, br s		
3′′′′′′			146.6[b]			
4′′′′′′			149.5[b]			
5′′′′′′			116.5	6.69, d (8.2)		
6′′′′′′			123.1	6.90, br d (8.2)		

[a] Obsc: signal obscured. [b] Interchangable within column.

However, iridoid 11-carboxylic acids esterified with alditols such as **2a**, **3a**, and **4**, found in the present study, have not been reported before. Hebitol II (**5**), isolated in the present work, adds to the variety of sugar esters found in *Veronica* sect. *Hebe*.[10,15,18−20]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. One-dimensional ^{1}H and ^{13}C NMR and 2D DQF-COSY, gHSQC, gHMBC, and NOESY NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer in methanol-d_4, and the chemical shifts are given as δ values with reference to the solvent peaks (δ_H 3.30 or δ_C 49.0, respectively). LC-HRESIMS was performed on an Agilent HP 1100 HPLC equipped with a BDS-C_{18} reversed-phase column running a H_2O−MeCN (50 ppm TFA in H_2O) gradient. The LC was coupled to a LCT of a TOF MS (Micromass, Manchester, UK) operated in the positive electrospray ion mode using 5-leucineenkephalin as lock mass. UV spectra were recorded on a Shimadzu UV-1601 instrument. Preparative HPLC was performed on an 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 a Waters system (Watford, UK) with a 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 known compounds were identified by NMR and compared with published data: mannitol (**1**);[9] epiloganic acid (**2**), gardoside (**3**), aucubin (**6**), and catalpol (**7**);[21] verproside (**7a**);[22] verminoside (**7b**) and minecoside (**7d**);[13] 6-*O*-feruloylcatalpol (**7c**).[23]

Plant Material. *Veronica lavaudiana* Raoul was collected from plants cultivated in the grounds of Landcare Research, Lincoln, New Zealand. A voucher specimen has been deposited at the Allan Herbarium, Landcare Research (CHR).

Extraction and Isolation. Plant material (40 g, fresh wt) was blended with MeOH and filtered. The concentrated extract (14 g) was partitioned between Et_2O−H_2O, and the aqueous phase was loaded on a 4 × 45 cm Diaion HP-20 (Supelco, Bellefonte, US) column and eluted with H_2O−MeOH mixtures. The fraction eluted with H_2O (5.5 g dry wt) contained mainly mannitol (**1**). The fraction eluted with 10% MeOH (780 mg) contained mainly aucubin (**6**) and catalpol (**7**).

The fraction eluted with 30% MeOH (250 mg) was separated on a silica gel column eluted with $CHCl_3$−MeOH−H_2O (60:15:4, 60:22:4) followed by HPLC (Luna C_{18}, linear gradient from 25% to 40% MeOH over 10 min) to give hebitol II (**5**, 4.2 mg).

The fraction eluted with 50% MeOH (2.2 g) was separated on a Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden; 3 × 30 cm) column eluted with 90% MeOH to give Fr. A (550 mg), which was further separated by HPLC (Luna C_{18}, 3% to 50% MeOH over 10 min) to give helioside A (**8b**, 191 mg) and helioside C (**8d**, 25 mg). The final purification of these was performed by HPLC on Genesis C_{18} with 30% MeOH for **8b** (135 mg) and 35% MeOH for **8d** (4.1 mg). Fr. B (492 mg) was further separated by HPLC (Luna C_{18}, 30% to 60% MeOH over 10 min) to give aragoside (**8**, 63.4 mg), lavaudioside B (**3a**, 12.6 mg), helioside B (**8c**, 24.0 mg), lavaudioside A (**2a**, 41.6 mg), and 6′-*O*-caffeoylcatalpol (**7e**, 2.7 mg), all additionally purified by HPLC (Genesis C_{18}, 35% MeOH). Fr. C (545 mg) was further separated by HPLC (Luna C_{18}, 30% to 50% MeOH over 10 min) to give 6′-*O*-caffeoylcatalpol (**7e**, 3.3 mg) and verminoside (**7b**, 268 mg).

The fraction eluted with 60% MeOH (3.9 g) was separated on a Sephadex LH-20 column (3 × 40 cm) eluted with 90% MeOH to give Fr. D (215 mg), which was further separated by HPLC (Luna C_{18}, 35 to 45% MeOH over 15 min) to give aragoside (**8**, 11.0 mg), lavaudioside B (**3a**, 23.1 mg), verminoside (**7b**, 39.1 mg), and a fraction (35.6 mg), purified by HPLC (Genesis C_{18}, 42% MeOH) to give lavaudioside A (**2a**, 17.1 mg). Fr. E (1.0 g) was further separated on a silica gel column ($CHCl_3$−MeOH−H_2O, 60:22:4) to give verminoside (**7b**, 645 mg).

The fraction eluted with MeOH (1.8 g) was separated on a Sephadex LH-20 column (2.5 × 50 cm) eluted with MeOH: Fr. F (866 mg) was further separated by HPLC (Luna C_{18}, 45% MeOH over 10 min) to give verminoside (**7b**, 202 mg), 6-*O*-feruloylcatalpol (**7c**, 71.5 mg), minecoside (**7d**, 31 mg), and a fraction (50.5 mg), which was separated on silica gel ($CHCl_3$−MeOH−H_2O, 60:22:4) and additionally purified by HPLC (Genesis C_{18} with 45% MeOH) to give lavaudioside C (**4**, 2.0 mg). Fr. G (589 mg) was further separated by HPLC (Luna C_{18}, 40−50% MeOH over 20 min) to give verminoside (**7b**, 311 mg). Fr. H (131 mg) was separated by HPLC on a Luna C_{18} (35−60% MeOH over 20 min) and additionally purified on a Genesis C_{18} (48% MeOH) to give 6,6′-di-*O*-caffeoylcatalpol (**7f**, 1.4 mg).

*Lavaudioside A (**2a**):* colorless, amorphous solid; $[\alpha]^{20}_D$ −30 (*c* 0.4; MeOH); UV (MeOH) λ_{max} (log ε) 330 (4.24), 294 (4.10, sh), 239 (4.30); ^{1}H and ^{13}C NMR, Table 1; LC-HR ESIMS *m*/*z* 720.2713 [M + NH_4]$^+$ (calcd for $C_{31}H_{46}NO_{18}$, 720.2715).

*Lavaudioside B (**3a**):* colorless, amorphous solid; $[\alpha]^{22}_D$ −24 (*c* 0.5; MeOH); UV (MeOH) λ_{max} (log ε) 330 (4.16), 293 (4.02, sh), 236 (4.22); ^{1}H and ^{13}C NMR, Table 1; LC-HR ESIMS *m*/*z* 718.2558 [M + NH_4]$^+$ (calcd for $C_{31}H_{44}NO_{18}$, 718.2558).

*Lavaudioside C (**4**):* colorless, amorphous solid; $[\alpha]^{22}_D$ −48 (*c* 0.1; MeOH); UV (MeOH) λ_{max} (log ε) 329 (4.26), 297 (4.14, sh), 240 (4.25); ^{1}H and ^{13}C NMR, Table 1; LC-HR ESIMS *m*/*z* 1027.3269 [M + H]$^+$ (calcd for $C_{46}H_{59}O_{26}$, 1027.3289).

*6′-O-Caffeoylcatalpol (**7e**):* colorless, amorphous solid; $[\alpha]^{22}_{D}$ −70 (*c* 0.2; MeOH); UV (MeOH) λ_{max} (log ε) 332 (4.12), 300 (3.99, sh), 246 (3.89); ^{1}H and ^{13}C NMR, Table 2; LC-HR ESIMS *m*/*z* 523.1465 $[M - H]^-$ (calcd for $C_{24}H_{27}O_{13}$, 523.1452).

*6,6′-Di-O-caffeoylcatalpol (**7f**):* colorless, amorphous solid; $[\alpha]^{20}_{D}$ −20 (*c* 0.2; MeOH); UV (MeOH) λ_{max} (log ε) 331 (4.43), 289 (4.33, sh), 246 (4.21); ^{1}H and ^{13}C NMR, Table 2; LC-HR ESIMS *m*/*z* 709.1791 $[M + Na]^+$ (calcd for $C_{33}H_{34}NaO_{16}$, 709.1741).

*Helioside A (**8b**):* colorless, amorphous solid; $[\alpha]^{20}_{D}$ −23 (*c* 0.5; MeOH); UV (MeOH) λ_{max} (log ε) 332 (4.21), 292 (4.08), 249 (3.94, sh); ^{1}H and ^{13}C NMR, Table 3; LC-HR ESIMS *m*/*z* 922.3200 $[M + NH_4]^+$ (calcd for $C_{39}H_{56}NO_{24}$, 922.3175).

*Helioside B (**8c**):* colorless, amorphous solid; $[\alpha]^{20}_{D}$ −25 (*c* 0.1; MeOH); UV (MeOH) λ_{max} (log ε) 331 (4.14), 290 (4.00, sh), 247 (3.93, sh); ^{1}H and ^{13}C NMR, Table 3; LC-HR ESIMS *m*/*z* 1084.3451 $[M + NH_4]^+$ (calcd for $C_{48}H_{62}NO_{27}$, 1084.3490).

*Helioside C (**8d**):* colorless, amorphous solid; $[\alpha]^{20}_{D}$ −22 (*c* 0.4; MeOH); UV (MeOH) λ_{max} (log ε) 328 (4.25), 293 (4.08), 233 (4.09, sh); ^{1}H and ^{13}C NMR, Table 3; LC-HR ESIMS *m*/*z* 936.3333 $[M + NH_4]^+$ (calcd for $C_{40}H_{58}NO_{24}$, 936.3331).

*Treatment of **2a** with Ammonium Hydroxide.* Lavaudioside A (**2a**) (17 mg) was dissolved in D_2O (0.7 mL) in an NMR tube (5 mm), and 25% ammonium hydroxide (70 μL) was added. After 48 h at room temperature the ^{13}C NMR spectrum was recorded (D_2O, 75 MHz): δ_C 64.5 (C-1 and 6), 72.1 (C-2 and 5), 70.5 (C-3 and 4); after addition of excess mannitol, the three relevant resonances increased in size (see Supporting Information).

*Treatment of **5** with Ammonium Hydroxide.* Hebitol II (**5**) (13 mg) was dissolved in D_2O (0.7 mL) in an NMR tube (5 mm), and 25% ammonium hydroxide (70 μL) was added. After 72 h at room temperature the ^{13}C NMR spectrum was recorded (D_2O, 75 MHz): δ_C 64.5 (C-1), 72.1 (C-2), 70.3 (C-3), 70.9 (C-4), 70.9 (C-5), 73.1 (C-6), 104.2 (C-1′), 74.5 (C-2′), 76.9 (C-3′), 70.4 (C-4′), 77.3 (C-5′), 62.0 (C-6′) (see Supporting Information), coinciding with that reported for **5c**.[12]

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra (^{1}H, ^{13}C) of lavaudiosides A−C (**2a**, **3a**, and **4**), the catalpol esters (**7d** and **7e**), heliosides A−C (**8b**−**d**), and hydrolysis products of **2a** and **5** are available free of charge via the Internet at http://pubs.acs.org.

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