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Revised structures of Arillatanosides A–C from *Polygala arillata*

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Arillatanosides A–C are three triterpenoid saponins from *Polygala arillata* Buch–Ham that have been reported previously, but with partially incorrect structures. Further investigation of their NMR data led to the conclusion that the terminal α-L-arabinopyranosyl unit originally proposed for Arillatanosides A–C (I–III) is actually a β-D-xylopyranosyl unit. Thus, the correct structures of Arillatanosides A–C are represented by 1–3. Complete NMR assignments of Arillatanosides A–C (1–3) and the related polygalasaponin XXXV (4) were achieved using modern 2D NMR techniques, such as DQF H–H COSY, HMQC, HMBC, TOCSY, 2D HMQC–TOCSY.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; *Polygala arillata* Buch–Ham; Arillatanosides A–C; triterpenoid saponins; structure revision

INTRODUCTION

Arillatanosides A–C are oleanane triterpenoid saponins that were first obtained from the stem bark of *Polygala arillata* Buch–Ham.[1] The structures of Arillatanosides A–C were proposed as 28-*O*-α-L-arabinopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl presenegenin-3-*O*-β-D-glucopyranoside (**I**), 28-*O*-β-D-galactopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 3)]-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[4-*O*-acetyl]-β-D-fucopyranosyl presenegenin-3-*O*-β-D-glucopyranoside (**II**), 28-*O*-β-D-galactopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 3)]-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl presenegenin-3-*O*-β-D-glucopyranoside (**III**) respectively (Figure 1). In the earlier paper,[1] the structure elucidation of these compounds was mainly based on the glycosylation shift effect and ^{13}C NMR data comparison with similar known compounds, but no 2D NMR spectra were provided to confirm their structures. Furthermore, the resolution of the previous NMR spectra was poor due to the presence of a carboxylic group located at C-23 of the aglycone.

New NMR experiments were carried out on the samples after being treated with ion exchange resin. The resolution of their ^{1}H and ^{13}C NMR spectra was significantly enhanced, and complete NMR assignments of the three saponins by modern 2D NMR techniques, such as DQF H–H COSY, HMQC, HMBC, TOCSY, HMQC–TOCSY, have resulted in their structure revision.

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RESULTS AND DISCUSSION

The identification of the aglycones of Arillatanosides A–C was done by direct comparison with the ^{13}C NMR data of presenegenin in the literature.[2,3] Complete assignments of proton signals belonging to the aglycone were achieved using 2D NMR spectra, such as H–H COSY, HMQC, HMBC, HMQC–TOCSY. The results are shown in Tables 1 and 2.

Arillatanoside A (**1**) was obtained as an amorphous powder. $[\alpha]_D^{19}$ −4.71° (*c* 0.43, MeOH). The high-resolution fast atom bombardment mass spectrometry (HR-FAB-MS) spectrum corresponds to a molecular formula of $C_{58}H_{92}O_{28}$ [*m/z* 1236.5775 $[M]^-$ (requires 1236.5726)]. The FAB-MS spectrum also showed following peaks *at m/z* (rel. int.): 1236$[M]^-$ (100), 1103 $[M - H - 132]^-$ (10), 1073 $[M - H - 162]^-$ (8), 971 $[M - H - 132 - 132]^-$ (2), 679 $[M - H - 132 - 132 - 146 \times 2]^-$(4), 518$[M - 132 - 132 - 146 \times 2 - 162]^-$(2) (the aglycone). The ^{1}H NMR spectrum of **1** showed five anomeric proton signals at δ5.02 (1H, d, $J = 7.3$ Hz), 5.04 (1H, d, $J = 7.2$-Hz), 5.12 (1H, d, $J = 7.4$ Hz), 6.03 (1H, d, $J = 7.9$ Hz), 6.47 (1H, br.s), whereas the ^{13}C NMR spectrum exhibited five anomeric carbon signals at δ94.95, 101.20, 105.34, 105.90, 106.83. Therefore, **1** was assumed to contain five sugar units. On the basis of the ^{13}C NMR data of polygalasaponin XXVIII,[21] **1** has the same four sugars as polygalasaponin XXVIII, except for one additional sugar unit. The HMQC–TOCSY spectrum indicates that this sugar unit is a pentose with carbon signals at δ105.90, 75.44, 78.07, 70.78, 67.35 (Table 5), characteristic of a β-D-xylopyranosyl,[4,5] which was assigned as an arabinopyranosyl unit in the previous paper (**I**).[1] Its anomeric proton signal at δ5.12 (1H, d, $J = 7.4$ Hz) also confirmed its β-configuration. In general, $^{3}J_{H1,H2} > 5$ Hz for a β-configuration and $^{3}J_{H1,H2} < 5$ Hz for an α-configuration.[5–12] A ROESY spectrum in which the anomeric proton showed cross-peaks to H-3 (δ4.12,

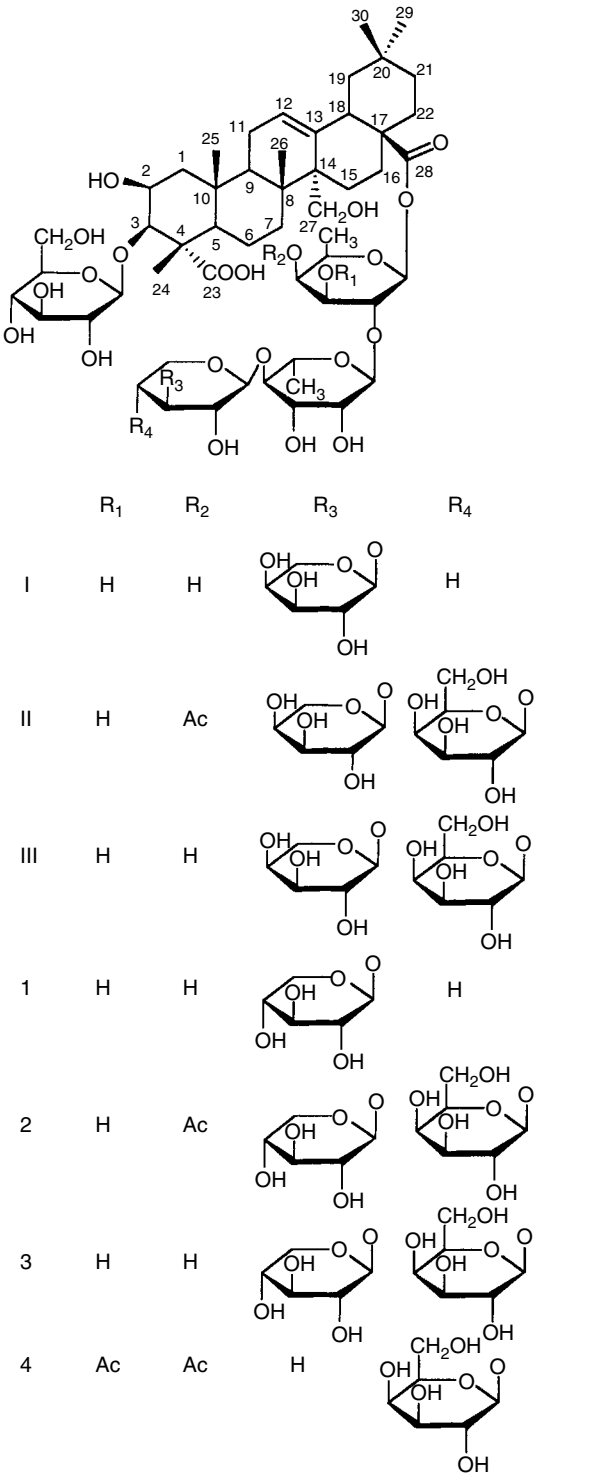

Figure 1. Structures of Arillatanosides A–C in Ref. 1 (**I–III**) and revision (**1–3**) and polygalasaponin XXXV (**4**).

1H, m) and H-5 (δ3.61, 1H, t, J = 10.9 Hz; δ4.23, 1H, m) further confirmed the β-configuration. *Gluco-*, *galacto-* and *manno-pyranose* configurations can be distinguished by the TOCSY technique, using a relatively short spin-lock time (τ_m = 60 ms).[5–12] Thus, this unit could be assigned as β-D-xylopyranose because it shows correlation peaks from the anomeric proton to H-5 in the TOCSY spectra (τ_m = 60 ms). Furthermore, the anomeric proton H-1 shows five correlation peaks from the anomeric carbon C-1 to C-5 in an

Table 1. ^{13}C NMR data for the aglycone of saponins **1–4** (125 MHz; δ in pyridine-d_5)

C	1	2	3	4
1	44.40	44.32	44.50	44.34
2	70.47	70.39	69.93	70.39
3	86.52	86.10	87.50	86.01
4	53.15	52.99	53.42	52.95
5	52.59	52.57	52.44	52.53
6	21.61	21.48	21.88	21.55
7	33.98	34.00	33.93	33.95
8	41.30	41.26	41.25	41.23
9	49.47	49.40	49.50	49.38
10	37.08	37.10	36.84	37.08
11	23.67	23.76	23.34	23.77
12	127.95	127.91	128.09	127.86
13	139.17	139.07	139.10	138.99
14	47.11	47.14	47.10	47.13
15	24.64	24.58	24.93	24.56
16	24.29	24.03	24.64	24.00
17	48.26	48.15	48.34	48.04
18	42.20	42.17	42.13	41.95
19	45.52	45.47	45.35	45.45
20	30.89	30.88	30.78	30.83
21	33.88	33.73	33.93	33.58
22	32.47	32.52	32.32	32.45
23	182.38	181.29	182.28	180.83
24	14.44	14.29	14.63	14.28
25	17.64	17.61	17.61	17.56
26	19.00	18.90	18.97	18.82
27	64.51	64.51	64.23	64.51
28	176.86	176.79	176.62	176.49
29	33.23	33.17	33.14	33.12
30	24.04	24.03	23.81	24.00

HMQC–TOCSY spectrum, which also confirmed it to be β-D-xylopyranose.[10–12] The absence of arabinose in Arillatanoside A (**I**) was also confirmed by acid hydrolysis, indicating the presence of only glucose (Glc), fucose (Fuc), rhamnose (Rha), and xylose (Xyl).

Interglycosidic linkages were confirmed by an HMBC spectrum that showed the following key correlations: H-1 (δ6.03, 1H, d, J = 7.9 Hz) of Fuc and C-28 (δ176.85) of aglycone, C-1 (δ101.20) of Rha and H-2 (δ4.7, 1H, t, J = 9.2 Hz) of Fuc, H-1 (δ5.04, 1H, d, J = 7.2 Hz) of inner ^{1}Xyl and C-4 (δ85.35) of Rha, H-1 (δ5.12, 1H, d, J = 7.44 Hz) of terminal ^{2}Xyl and C-3 (δ87.90) of inner ^{1}Xyl, H-1 (δ5.02, 1H, d, J = 7.3 Hz) of Glc and C-3 (δ86.52) of aglycone. The latter four linkages were also confirmed by a ROESY spectra (Table 5). Thus, the revised structure of Arillatanoside A (**1**) can be proposed as 28-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl presenegenin-3-*O*-β-D-glucopyranoside.

Arillatanoside B (**2**): white amorphous, $[\alpha]_D^{19}$ + 5.42° (*c* 0.32, MeOH). The HR-FAB-MS spectrum that showed m/z 1439.6381 $[M - H]^-$ established the molecular formula $C_{66}H_{104}O_{34}$ (calc. for $C_{66}H_{103}O_{34}$: 1439.6331). The FAB-MS also showed following peaks at m/z (rel. int.): 1440 $[M]^-$

Table 2. ^{1}H NMR data for the aglycones of **1–4** (500 MHz; δ in pyridine-d_5; J, Hz)

H	1	2	3	4
1	2.25 (m); 1.30 (m)	2.27 (d, 13.60); 1.31 (m)	2.24 (m); 1.34 (m)	2.26 (m); 1.31 (m)
2	4.68 (m)	4.69 (m)	4.53 (m)	4.69 (m)
3	4.54 (br.s)	4.56 (br.s)	4.43 (br.s)	4.57 (br.s)
5	2.18 (m)	2.17 (m)	2.36	2.16 (m)
6	1.96 (m); 1.74 (m)	1.96 (m); 1.74 (m)	1.97 (m); 1.76 (m)	1.97 (m); 1.72 (m)
7	1.23 (m); 1.11 (t, $J = 14.11$)	1.30 (m); 1.11 (m)	1.22 (m); 1.13 (m)	1.27 (m); 1.11 (m)
9	2.30 (t, 9.16)	2.30 (m)	2.43 (m)	2.31 (m)
11	2.11 (m); 2.04 (m)	2.10 (m); 2.04 (m)	1.99 (m); 1.93 (m)	2.10 (m); 2.04 (m)
12	5.80 (t)	5.80 (t)	5.87 (t)	5.77 (t)
15	2.12 (m)	2.13 (m)	2.18 (m)	2.15 (m)
16	1.98 (m); 1.75 (m)	2.10 (m); 1.70 (m)	2.05 (m); 1.78 (m)	2.03 (m); 1.75 (m)
18	3.19 (dd, 12.97, 3.81)	3.20 (br.d, 12.22)	3.23 (dd, 12.72, 3.52)	3.18 (br.d, 13.32)
19	1.70 (m); 1.29 (m)	1.74 (m); 1.29 (m)	1.76 (m); 1.33 (m)	1.72 (m); 1.28 (m)
21	2.16 (m); 1.75 (m)	2.13 (m); 1.68 (m)	2.25 (m); 1.89 (m)	2.09 (m); 1.65 (m)
22	1.96 (m); 1.64 (m)	2.05 (m); 1.69 (m)	1.95 (m); 1.75 (m)	2.03 (m); 1.65 (m)
24	1.92 (s)	1.93 (s)	1.89 (s)	1.93 (s)
25	1.52 (s)	1.54 (s)	1.53 (s)	1.52 (s)
26	1.11 (s)	1.11 (s)	1.14 (s)	1.08 (s)
27	4.12 (m); 3.82 (d, 10.97)	4.06 (m); 3.80 (d, 5.13)	4.18 (m); 3.75 (m)	4.03 (m); 3.79 (d, 6.06)
29	0.77 (s)	0.78 (s)	0.80 (s)	0.77 (s)
30	0.85 (s)	0.89 (s)	0.80 (s)	0.91 (s)

(100), 1308 $[M-132]^-$ (19), 1278 $[M-162]^-$ (13), 1145 $[M-162-132]^-$ (4), 982 $[M-162-132-162]^-$ (2), 679 [aglycone $-$ H $+$ 162]$^-$ (3). The ^{1}H NMR spectrum showed six anomeric proton signals at δ4.90 (1H, d, $J = 7.69$ Hz), δ4.97 (1H, d, $J = 7.49$ Hz), δ5.00 (1H, d, $J = 8.48$ Hz), δ5.29 (1H, d, $J = 6.70$ Hz), δ6.05 (1H, d, $J = 7.10$ Hz), δ6.25 (1H, br.s) and one acetyl methyl signal at δ1.96 (3H,s). The ^{13}C NMR spectrum exhibited six anomeric carbon signals at δ94.73, δ101.86, δ103.36, δ105.40 (2 $\times$ C), δ106.21 and one additional carbonyl carbon at δ171.24 apart from two carbonyl carbon atoms of the aglycone. So **2** was assumed to contain six sugar units and one acetyl group. Compared with the ^{13}C NMR data of polygalasaponin XXXIV,[2] **2** exhibited one more sugar unit than polygalasaponin XXXIV. The HMQC–TOCSY spectrum shows this unit as a pentose with carbon signals at δ105.40, δ74.74, δ77.2, δ70.54, δ66.85, characteristic of β-D-xylopyranose.[4] The anomeric proton signal at δ5.29 (1H, d, $J = 6.70$ Hz) also confirmed the β-configuration. The similarity of the ^{13}C and ^{1}H NMR data of this sugar with the terminal β-D-xylopyranose of **1** further confirmed this deduction. The ^{1}H and ^{13}C NMR signal assignments were obtained by HMQC–TOCSY, HMQC, DQF H–H COSY and subsequently confirmed by HMBC. The results are summarized in Tables 3 and 4.

The downfield shift of C-3 of inner ^{1}Xyl from 84.6 ppm to 76.7 ppm and the upfield shift of C-4 of inner ^{1}Xyl from 78.3 ppm to 71.6 ppm in **2**, relative to polygalasaponin XXXIV, suggested that outer ^{2}Xyl should be connected to C-3 of inner ^{1}Xyl. This was also confirmed by an HMBC spectrum, which showed long-range correlation between H-1 (δ5.29, 1H, d, $J = 6.70$ Hz) of outer ^{2}Xyl and C-3 (δ84.22) of inner ^{1}Xyl. So the structure of Arillatanoside

Table 3. ^{13}C NMR data for sugar moieties of **1–4** (125 MHz; δ in pyridine-d_5)

C	1	2	3	4
3-*O*-Glc-1	105.34	105.40	105.30	105.43
2	75.32	75.28	75.33	75.29
3	78.33	78.35	78.10	78.39
4	71.60	71.62	71.47	71.64
5	78.23	78.35	77.84	78.39
6	62.69	62.73	62.50	62.78
28-*O*-Fuc-1	94.95	94.73	94.91	94.26
2	73.59	74.44	73.43	72.95
3	76.91	74.09	77.29	74.67
4	73.35	74.89	72.69	71.28
5	72.59	70.68	72.49	70.14
6	17.07	16.62	17.00	16.55
Ac at 3				20.70
				170.17
Ac at 4		20.90		20.47
		171.24		170.89
Rha-1	101.20	101.86	100.99	102.13
2	71.83	71.62	71.78	71.41
3	72.59	72.55	72.69	72.42
4	85.35	84.79	86.22	84.65
5	68.12	68.45	67.61	69.04
6	18.59	18.75	18.35	18.68
^{1}Xyl-1 (inner)	106.83	106.21	106.57	106.82
2	75.44	76.01	76.01	75.64
3	87.90	84.22	86.55	76.67

Table 3. (*Continued*)

C	1	2	3	4
4	68.92	71.62	70.78	78.26
5	67.01	66.19	66.34	65.07
^{2}Xyl-1 (outer)	105.90	105.40	106.05	
2	75.44	74.74	75.33	
3	78.07	77.27	77.60	
4	70.78	70.54	69.77	
5	67.35	66.85	67.17	
Gal-1		103.36	103.19	104.48
2		70.39	69.93	71.85
3		74.74	74.58	75.12
4		69.88	69.93	70.14
5		77.58	77.60	77.33
6		62.30	62.37	62.30

B was revised to be 28-*O*-*β*-D-galactopyranosyl-(1 → 4)-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-xylopyranosyl-(1 → 4)-*α*-L-rhamnopyranosyl-(1 → 2)-[4-*O*-acetyl]-*β*-D-fucopyranosyl presenegenin-3-*O*-*β*-D-glucopyranoside.

Arillatanoside C (**3**): white amorphous, $[\alpha]_D^{19}$+8.02° (*c* 0.41, MeOH). The HR-FAB-MS exhibited a quasi-molecular ion at *m/z* 1397.6255 $[M - H]^-$ corresponding to the molecular formula $C_{64}H_{102}O_{33}$ (calc. for $C_{64}H_{101}0_{33}$: 1397.6225). FAB-MS also showed peaks at *m/z* (rel. int.): 1398 $[M]^-$ (100), 1266 $[M - 132]^-$ (13), 1236 $[M - 162]^-$ (12), 679 [aglycone − H + 162]$^-$ (5). Comparing the ^{1}H and ^{13}C NMR data of **3** with **2**, all of the signals overlapped with each other except that **3** was lacking on acetyl group. The ^{1}H and ^{13}C NMR signal assignments were obtained by HMQC–TOCSY, HMQC spectra and comparison with **2**. The results were confirmed by HMBC and are summarized in Tables 3 and 4. Determination of sugar linkages was

Table 4. ^{1}H NMR data for sugar moieties of **1–4** (500 MHz; *δ* in pyridine-d_5; *J*, Hz)

H	1	2	3	4
3-*O*-Glc-1	5.02 (d, 7.34)	5.00 (d, 8.48)	4.98 (d, 7.24)	5.01 (d, 7.87)
2	4.09 (m)	3.96 (m)	4.06 (m)	3.89 (m)
3	4.19 (m)	4.09 (m)	4.22 (m)	4.12 (m)
4	4.14 (m)	4.12 (m)	4.19 (m)	4.12 (m)
5	3.89 (m)	3.88 (m)	3.86 (m)	3.88 (m)
6	4.42 (br.s, 12.21); 4.26 (dd, 5.15, 12.21)	4.41 (m); 4.24 (m)	4.43 (m); 4.28 (m)	4.43 (m); 4.25 (m)
28-*O*-Fuc-1	6.03 (d, 7.92)	6.05 (d, 7.10)	5.98 (d, 8.41)	6.13 (d, 8.07)
2	4.68 (t, 9.16)	4.53 (m)	4.74 (m)	4.53 (m)
3	4.20 (m)	4.35 (m)	4.22 (m)	5.53 (d, 6.46)
4	3.98 (m)	5.52 (br.s)	3.95 (m)	5.55 (br.s)
5	3.92 (m)	4.04 (m)	3.95 (m)	4.08 (m)
6	1.48 (d, 5.72)	1.25 (d, 4.53)	1.47 (d, 5.87)	1.16 (br.s)
Ac at 3				2.02 (s)
Ac at 4		1.96 (s)		2.02 (s)
Rha-1	6.47 (br.s)	6.25 (br.s)	6.58 (br.s)	5.66 (br.s)
2	4.79 (br.s)	4.74 (br.s)	4.75 (br.s)	4.51 (m)
3	4.66 (dd, 3.43, 9.54)	4.59 (d, 12.22)	4.59 (t, 9.59)	4.42 (m)
4	4.30 (t, 9.54)	4.23 (m)	4.23 (m)	4.20 (m)
5	4.44 (t, 10.11)	4.44 (m)	4.44 (m)	4.28 (m)
6	1.64 (d, 6.10)	1.70 (d, 5.32)	1.62 (d, 5.87)	1.73 (br.s)
^{1}Xyl-1 (inner)	5.04 (d, 7.15)	4.97 (d, 7.49)	4.88 (m)	4.95 (d, 8.07)
2	3.90 (m)	3.96 (m)	3.94 (m)	3.95 (m)
3	3.97 (m)	4.11 (m)	3.97 (m)	4.00 (m)
4	4.01 (m)	4.37 (m)	4.44 (m)	4.25 (m)
5	4.22 (m); 3.47 (t, 10.59)	4.42 (m); 3.46 (t, 9.27)	4.36 (m); 3.47 (d, 11.15)	4.26 (m); 3.42 (d, 12.11)
^{2}Xyl-1 (outer)	5.12 (d, 7.44)	5.29 (d, 6.70)	5.05 (d, 7.37)	
2	4.02 (m)	4.01 (m)	4.04 (m)	
3	4.12 (m)	4.07 (m)	4.10 (t, 8.22)	
4	4.12 (m)	4.04 (m)	4.07 (m)	
5	4.23 (m); 3.61 (t, 10.97)	4.35 (m); 3.61 (t, 9.27)	4.27 (m); 3.52 (d, 10.17)	
Gal-1		4.90 (d, 7.69)	4.89 (d, 7.83)	4.91 (d, 8.68)
2		4.47 (m)	4.41 (m)	4.41 (m)
3		4.02 (m)	4.03 (m)	4.05 (m)
4		4.43 (m)	4.48 (m)	4.44 (m)
5		3.95 (m)	3.95 (m)	4.09 (m)
6		4.38 (m); 4.28 (m)	4.32 (m); 4.19 (m)	4.40 (m); 4.30 (m)

Table 5. Some 2D NMR data for **1–4**

	1	2	3	4
^{1}H signals				
HMQC–TOCSY (^{13}C signals)				
Glc-1	Glc-1, 2, 3, 4, 5, 6	Glc-1, 2, 3, 4, 5, 6	Glc-1, 2, 3, 4, 5, 6	Glc-1, 2, 3, 4, 5, 6
Fuc-1	Fuc-1, 2, 3, 4	Fuc-1, 2, 3, 4	Fuc-1, 2, 3, 4	Fuc-1, 2, 3, 4
Fuc-6	Fuc-5, 6	Fuc-5, 6	Fuc-5, 6	Fuc-5, 6
Rha-2	Rha-1, 2, 3, 4, 5, 6	Rha-1, 2, 3, 4, 5, 6	Rha-1, 2, 3, 4, 5, 6	Rha-1, 2, 3, 4, 5, 6
^{1}Xyl-1	^{1}Xyl-1, 2, 3, 4, 5	^{1}Xyl-1, 2, 3, 4, 5	^{1}Xyl-1, 2, 3, 4, 5	^{1}Xyl-1, 2, 3, 4, 5
^{2}Xyl-1	^{2}Xyl-1, 2, 3, 4, 5		^{2}Xyl-1, 2, 3, 4, 5	^{2}Xyl-1, 2, 3, 4, 5
Gal-1		Gal-1, 2, 3, 4	Gal-1, 2, 3, 4	Gal-1, 2, 3, 4
Gal-6		Gal-5, 6	Gal-5, 6	Gal-5, 6
HMBC (^{13}C signals)				
Glc-1	Glc-3, 5, C-3 (aglycone)	Glc-5, C-3 (aglycone)	Glc-5, C-3 (aglycone)	C-3 (aglycone)
Fuc-1	Fuc-3, 5, C-28 (aglycone)	Fuc-3, 5, C-28 (aglycone)	Fuc-3, 5, C-28 (aglycone)	Fuc-3, 5, C-28 (aglycone)
Rha-1	Rha-3, 5	Rha-3, 5	Rha-3, 5, Fuc-2	Rha-3, 5
^{1}Xyl-1	^{1}Xyl-2, 5, Rha-4	^{1}Xyl-5, Rha-4	^{1}Xyl-2, 5, Rha-4	Rha-4
^{2}Xyl-1	^{2}Xyl-2, 5, ^{1}Xyl-3		^{2}Xyl-3, ^{1}Xyl-3	
Gal-1		Gal-2, 3, ^{1}Xyl-4	Gal-3, 5, ^{1}Xyl-4	^{1}Xyl-4
ROESY (^{1}H signals only for **1**)				
Glc-1	Glc-3, 5, H-3 (aglycone)			
Fuc-1	Fuc-2, 3, 5, Fuc-6			
Rha-1	Rha-2, Fuc-2, 3			
^{1}Xyl-1	^{1}Xyl-3, 5, Rha-4			
^{2}Xyl-1	^{2}Xyl-3, 5, ^{1}Xyl-3			
TOCSY ($\tau_m = 60$ ms) (^{1}H signals only for **1**)				
Glc-1	Glc-2, 3, 4, 5, 6			
Fuc-1	Fuc-2, 3, 4			
Rha-1	Rha-2			
^{1}Xyl-1	^{1}Xyl-2, 3, 4, 5			
^{2}Xyl-1	^{1}Xyl-2, 3, 4, 5			
^{13}C signals				
HMQC–TOCSY (^{1}H signals)				
Glc-6	Glc-1, 2, 3, 4, 5, 6	Glc-1, 2, 3, 4, 5, 6	Glc-1, 2, 3, 4, 5, 6	Glc-1, 2, 3, 4, 5, 6
Fuc-1	Fuc-1, 2, 3, 4	Fuc-1, 2, 3, 4	Fuc-1, 2, 3, 4	Fuc-1, 2, 3, 4
Fuc-6	Fuc-5, 6	Fuc-5, 6	Fuc-5, 6	Fuc-5, 6
Rha-1	Rha-1, 2	Rha-1, 2	Rha-1, 2	Rha-1, 2
Rha-6	Rha-3, 4, 5, 6	Rha-3, 4, 5, 6	Rha-3, 4, 5, 6	Rha-3, 4, 5, 6
^{1}Xyl-3	^{1}Xyl-1, 2, 3, 4, 5	^{1}Xyl-1, 2, 3, 4, 5	^{1}Xyl-1, 2, 3, 4, 5	^{1}Xyl-1, 2, 3, 4, 5
^{2}Xyl-5	^{2}Xyl-1, 2, 3, 4, 5		^{2}Xyl-1, 2, 3, 4, 5	^{2}Xyl-1, 2, 3, 4, 5
Gal-1		Gal-1, 2, 3, 4	Gal-1, 2, 3, 4	Gal-1, 2, 3, 4
Gal-6		Gal-5, 6	Gal-5, 6	Gal-5, 6
HMBC (^{1}H signals)				
Glc-1	H-3 (aglycone), Glc-5	H-3 (aglycone), Glc-5	H-3 (aglycone), Glc-5	Glc-5
Fuc-1	Fuc-2, 5	Fuc-2, 5	Fuc-2, 5	Fuc-2, 5
Rha-1	Fuc-2, Rha-2, 5	Fuc-2	Fuc-2, Rha-2	Fuc-2
^{1}Xyl-1	Rha-4, ^{1}Xyl-5$_b$, 3	Rha-4, ^{1}Xyl-5$_{a,b}$, 3	Rha-4, ^{1}Xyl-5$_{a,b}$, 3	
^{2}Xyl-1	^{1}Xyl-3, ^{2}Xyl-5$_b$, 2		^{1}Xyl-3, ^{2}Xyl-5$_b$, 2	^{1}Xyl-3
Gal-1		^{1}Xyl-4, Gal-2, 3	^{1}Xyl-4, Gal-2, 5	^{1}Xyl-4

Subscripts a, b show upfield and downfield proton of methylene respectively.

achieved by HMBC (Table 5). The revised structure of Arillatanoside C (**3**) is found to be 28-*O*-*β*-D-galactopyranosyl-(1 → 4)-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-xylopyranosyl-(1 → 4)-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-fucopyranosyl presenegenin-3-*O*-*β*-D-glucopyranoside.

To sum up, the terminal arabinopyranosyl unit of Arillatanosides A–C that were reported previously[1] were corrected to a xylopyranosyl unit on the basis of 2D NMR data and chemical methods.

Another saponin, polygalasaponin XXXV (**4**), which has been isolated from *Polygala fallax Hemsl.* by Zhang,[2] was also investigated. The complete assignments of the ^{1}H and ^{13}C spectra of saponins **1–4** were achieved using 2D NMR spectra and are summarized in Tables 1–4. The assignments of ^{2}Xyl C-4 and ^{1}Xyl C-2, C-3, C-4 were corrected by comparison with the chemical shifts of those in the previous paper.[1] The complete assignments of the ^{1}H NMR spectrum of saponins **1–4** have been reported for the first time.

EXPERIMENTAL

General procedures

Optical rotations were recorded on a HORIBA SEPA-300 digital polarimeter using a sodium lamp. FAB-MS spectra were carried out on a VG Autospect 3000 spectrometer. All NMR experiments were recorded on a Bruker DRX-500 MHz spectrometer, operating at 500 MHz and 100 MHz for ^{1}H and ^{13}C respectively, equipped with an inverse detection 5 mm probe (BBI probe, ^{1}H 90° pulse width: 9.5 μs) operating at room temperature. Samples **1** (40 mg), **2** (25 mg), **3** (35 mg), **4** (40 mg) were dissolved in pyridine-d_s (0.4 ml) to record NMR spectra using the low-field signals of pyridine-d_s δ8.71 and δ149.9 for the ^{1}H and ^{13}C spectra as an internal reference.

The ^{1}H and ^{13}C NMR spectra were acquired under standard conditions. The NMR conditions for all compounds were as follows: 1D spectra were acquired using 64k data points and spectral widths of 5000 Hz and 27 500 Hz for ^{1}H and ^{13}C respectively; 32k data points were used for the processing with no window function for ^{1}H spectra and an exponential function (LB = 4) for ^{13}C spectra.

Standard pulse sequences were used for 2D spectra. Spectral widths of 5000 Hz and 27 500 Hz were used for ^{1}H and ^{13}C respectively. Relaxation delays of 1.5 or 2 s were used for all 2D NMR experiments. 2D spectra used 1024 × 256 (gs-COSY, gs-HMQC, gs-ROESY and HMQC-TOCSY) and 2048 × 256 (gs-HMBC) data-point matrices and then were zero filled to 1024 × 512 and 2048 × 512 respectively. A non-shifted sine window function was used along the f_1 and f_2 axes for gs-COSY, gs-HMQC, gs-HMBC and a 90°-shifted sine window function was used along the f_1 and f_2 axes for gs-ROESY and HMQC–TOCSY. The HMQC–TOCSY experiment utilized 180 ms as a mixing time to obtain total correlation. ROESY used 300 ms as a mixing time. HMBC experiments used a 62 ms delay to obtain ^{1}H and ^{13}C long-range correlation. Z-PFG was used to obtain HMQC, HMBC and DQF H–H COSY spectra. Data processing was carried out on an SGI Indy workstation computer with Bruker XWINNMR programs.

Extraction and isolation

See previous paper.[1]

De-ionization of 1–3

Samples dissolved in water with a little methanol were loaded to a column of cation-exchange resin. After washing with water, the sample was eluted with methanol.

Acid hydrolysis of 1–3

The samples **1–3** (2 mg) were heated with 5% H_2SO_4–MeOH (10 ml) under reflux for 6 h. The reaction was extracted with AcOEt after being diluted with H_2O. The aqueous layer was then neutralized with $NaHCO_3$ and concentrated under reduced pressure. The residue was compared with standard sugars by co-thin layer chromatography ($CHCl_3$–MeOH–H_2O–HOAC, 7 : 3 : 0.5 : 1; detection with spray agent: 4% *α*-naphthol–EtOH–5% H_2SO_4). Glucose, fucose, rhamnose and xylose were detected in **1**, and the same four sugars in addition to galactose were detected in **2** and **3**.

Spectral data

Polygalasaponin XXXV (**4**): $[\alpha]_D^{19}$+8.02° (*c* 0.41, MeOH). HR-FAB-MS *m/z*: 1397.625-459 $[M - H]^-$ (calc.: 1397.6225). FAB-MS *m/z* (rel. int.): 1398 $[M]^-$ (100), 1266 $[M - 132]^-$ (13), 1236 $[M - 162]^-$ (12), 679 [aglycone-H + 162]$^-$ (5).

For the ^{1}H and ^{13}C NMR data, see Tables 1–4.

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