Studies on the Constituents of *Swertia japonica* MAKINO I. On the Structures of New Secoiridoid Diglycosides

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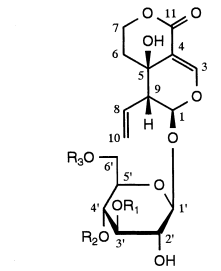
Eight new secoiridoid diglycosides, 6′-*O*-α-L-arabinopyranosylswertiamarin (1), 3′-*O*-β-D-glucopyranosylswertiamarin (2), 4′-*O*-β-D-glucopyranosylswertiamarin (3), 3′-*O*-β-D-galactopyranosylswertiamarin (4), 6′-*O*-α-D-galactopyranosylswertiamarin (5), 6′-*O*-α-D-mannopyranosylswertiamarin (6), 6′-*O*-β-D-fructofuranosylswertiamarin (7) and 5″-*O*-β-D-glucopyranosylamaroswerin (12), were isolated, together with five known compounds from the whole plants of *Swertia japonica* MAKINO. The structures of the new compounds were elucidated on the basis of chemical and spectroscopic evidence. Compounds 6 and 7 are the first naturally occuring iridoid diglycosides having an α-D-mannopyranosyl unit and β-D-fructofuranosyl unit, respectively.

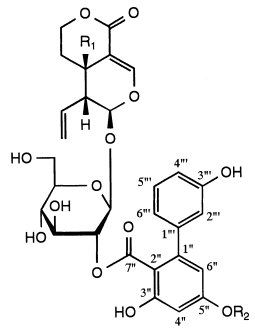
Key words *Swertia japonica*; Gentianaceae; secoiridoid diglycoside; swertiamarin biphenylcarboxylate

The Japanese crude drug, Swertia Herb (*Swertia japonica* MAKINO, Gentianaceae) has been used as a stomachic or appetite stimulant. The constituents of this crude drug have previously been investigated and were shown to contain secoiridoid glycosides,[1—5] xanthones,[6—9] flavonoids,[10] biphenyl glycosides,[11] triterpenoids[7,12] and 2,8-dioxabicyclo[3.3.1]nonanes.[13] In this paper, we describe the isolation and structural elucidation of eight new secoiridoid diglycosides, 6′-*O*-α-L-arabinopyranosylswertiamarin (**1**), 3′-*O*-β-D-glucopyranosylswertiamarin (**2**), 4′-*O*-β-D-glucopyranosylswertiamarin (**3**), 3′-*O*-β-D-galactopyranosylswertiamarin (**4**), 6′-*O*-α-D-galactopyranosylswertiamarin (**5**), 6′-*O*-α-D-mannopyranosylswertiamarin (**6**), 6′-*O*-β-D-fructofuranosylswertiamarin (**7**) and 5″-*O*-β-D-glucopyranosylamaroswerin (**12**), together with five known compounds from the whole plants of *S. japonica*. The known compounds were identified as 6′-*O*-β-D-glucopyranosylswertiamarin (**8**),[14] chironiside (**9**),[15] swertiapunimarin (**10**),[16] 6′-*O*-β-D-glucopyranosylgentiopicroside (**11**)[17] and 5″-*O*-β-D-glucopyranosylamarogentin (**13**),[18] respectively, by comparison of their spectroscopic data with those previously described in the literature. Extraction and isolation were carried out as described in the Experimental.

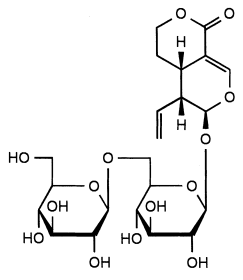
Compound **1**, $[\alpha]_D$ −92.7° (MeOH), was isolated as an amorphous powder. The molecular formula was determined to be $C_{21}H_{30}O_{14}$ by high-resolution (HR)-FAB-MS. The UV absorption maximum at 235 nm was attributed to that of an α,β-unsaturated ketone function. Acid hydrolysis of **1** gave L-arabinose and D-glucose, which were identified by gas–liquid chromatography (GLC) analysis of their thiazolizine derivatives.[19] The ^{1}H-NMR spectrum (Table 1) of the aglycone part of **1** was essentially the same as that of swertiamarin (**14**) isolated from the same plant,[1] showing signals for a vinyl group [δ_H 5.29 (1H, dd, J=9.4, 2.7 Hz, H-10A), 5.33 (1H, dd, J=16.8, 2.7 Hz, H-10B), 5.45 (1H, ddd, J=16.8, 9.4, 9.0 Hz, H-8)], an acetal methine proton [δ_H 5.68 (1H, d, J=1.5 Hz, H-1)] and a trisubstituted double bond [δ_H 7.63 (1H, s, H-3)]. Furthermore, two anomeric proton signals [δ_H 4.37 (1H, d, J=6.8 Hz, H-1″), 4.64 (1H, d, J=8.1 Hz, H-1′)] were recognized. The ^{13}C-NMR spectrum (Table 2) showed close similarity to that of **14**. However, a set of additional signals, corresponding to an α-L-arabinopyranosyl group, appeared at δ 66.9 (C-5″), 69.4 or 69.5 (C-4″), 72.4 (C-2″), 74.3 (C-3″), and 105.3 (C-1″) in the ^{13}C-NMR spectrum of **1**. This α-L-arabinopyranosyl group was involved in a glycosyl linkage at C-6′ of the inner β-D-glucopyranosyl group, because the signal due to C-6′ of the inner β-D-glucopyranosyl residue was markedly displaced downfield at δ 69.5 (+6.9 ppm), while the signal due to C-5′ was shifted upfield at δ 77.5 (−1.1 ppm), when comparing the ^{13}C-NMR spectrum of **1** with that of **14**. This was confirmed by the observation of a long-range correlation from the anomeric proton signal of the terminal α-L-arabinopyranosyl group at δ 4.37 (H-1″) to C-6′ of the inner β-D-glucopyranosyl moiety in the



1 R_1 = R_2 = H, R_3 = α-L-Ara*p*
2 R_1 = β-D-Glc*p*, R_2 = R_3 = H
3 R_1 = R_3 = H, R_2 = β-D-Glc*p*
4 R_1 = β-D-Gal*p*, R_2 = R_3 = H
5 R_1 = R_2 = H, R_3 = α-D-Gal*p*
6 R_1 = R_2 = H, R_3 = α-D-Man*p*
7 R_1 = R_2 = H, R_3 = β-D-Fru*f*
8 R_1 = R_2 = H, R_3 = β-D-Glc*p*
9 R_1 = R_2 = H, R_3 = β-D-Xyl*p*
14 R_1 = R_2 = R_3 = H



12 R_1 = OH, R_2 = β-D-Glc*p*
13 R_1 = H, R_2 = β-D-Glc*p*
16 R_1 = OH, R_2 = H



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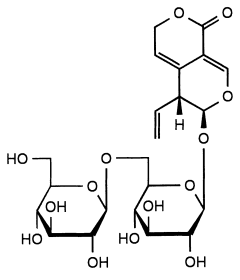

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Chart 1

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Table 1. ^{1}H-NMR Chemical Shifts of Compounds **1**—**7** and **14** (CD_3OD, 400 MHz)

Proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**
1	5.68 d (1.5)[a)]	5.74 d (1.5)	5.69 d (1.2)	5.74 d (1.5)	5.64 d (1.5)	5.61 d (1.5)	5.65 d (1.5)	5.72 d (1.5)
3	7.63 s	7.63 s	7.63 s	7.63 s	7.62 s	7.63 s	7.63 s	7.63 s
6A	1.75 br d (14.2)	1.75 br d (13.4)	1.73 br d (13.4)	1.75 br d (13.9)	1.75 br d (13.7)	1.76 br d (14.3)	1.74 br d (14.4)	1.75 br d (13.9)
6B	1.91 ddd (14.2, 12.9, 5.1)	1.91 ddd (13.4, 13.0, 5.1)	1.91 ddd (13.4, 13.0, 5.1)	1.91 ddd (13.9, 13.2, 5.2)	1.91 ddd (13.7, 12.8, 5.1)	1.93 ddd (14.3, 12.8, 5.1)	1.92 ddd (14.4, 13.2, 5.1)	1.91 ddd (13.9, 13.2, 5.1)
7A	4.34 ddd (11.0, 5.1, 1.5)	4.33 ddd (10.7, 5.1, 1.2)	4.33 ddd (10.7, 5.1, 1.5)	4.34 ddd (10.7, 5.1, 1.5)	4.33 ddd (11.0, 5.1, 1.2)	4.34 ddd (11.0, 5.1, 1.2)	4.34 ddd (11.0, 5.1, 1.7)	4.34 ddd (11.0, 5.1, 3.3)
7B	4.75 ddd (12.9, 11.0, 2.6)	4.75 ddd (13.0, 10.7, 2.4)	4.76 ddd (13.0, 10.7, 2.7)	4.76 ddd (13.2, 10.5, 2.6)	4.75 ddd (12.8, 11.0, 2.9)	4.76 ddd (12.8, 11.0, 2.7)	4.76 ddd (13.2, 11.0, 2.9)	4.76 ddd (13.9, 11.0, 2.9)
8	5.45 ddd (16.8, 9.4, 9.0)	5.45 ddd (17.1, 9.5, 9.3)	5.45 ddd (17.1, 9.8, 9.3)	5.45 ddd (17.2, 9.9, 9.3)	5.44 ddd (17.0, 9.9, 9.5)	5.39 m	5.41 ddd (16.8, 9.4, 9.0)	5.45 ddd (17.0, 9.9, 9.2)
9	2.91 dd (9.4, 1.5)	2.92 dd (9.3, 1.2)	2.91 dd (9.3, 1.2)	2.92 dd (9.3, 1.5)	2.93 dd (9.5, 1.5)	2.93 dd (9.2, 1.5)	2.96 dd (9.0, 1.5)	2.92 dd (9.2, 1.5)
10A	5.29 dd (9.4, 2.7)	5.30 dd (9.5, 2.4)	5.29 dd (9.8, 2.4)	5.30 dd (9.9, 2.2)	5.30 dd (9.9, 1.8)	5.30 dd (9.5, 2.2)	5.29 dd (9.4, 2.5)	5.29 dd (9.9, 2.2)
10B	5.33 dd (16.8, 2.7)	5.37 dd (17.1, 2.4)	5.36 dd (17.1, 2.4)	5.37 dd (17.2, 2.2)	5.37 dd (17.0, 1.8)	5.39 dd (16.9, 2.2)	5.44 dd (16.8, 2.5)	5.37 dd (17.0, 2.2)
1′	4.64 d (8.1)	4.71 d (8.1)	4.68 d (8.1)	4.71 d (8.1)	4.66 d (8.1)	4.63 d (8.1)	4.64 d (8.1)	4.64 d (8.1)
2′	3.19 dd (8.8, 8.1)	—	3.26 dd (9.0, 8.1)	—	3.21 dd (8.8, 8.1)	3.19 dd (8.8, 8.1)	3.18 dd (9.0, 8.1)	3.18 dd (8.8, 8.1)
3′	3.47 dd (8.8, 8.3)	3.59 dd (8.8, 8.3)	3.54 dd (9.0, 8.5)	—	—	—	3.37 dd (9.2, 9.0)	3.37 dd (8.8, 8.8)
4′	—[b)]	—	3.60 dd (9.3, 8.5)	—	—	—	3.47 dd (9.5, 9.2)	3.29 dd (9.5, 8.8)
5′	3.52 m	—	3.47 m	—	3.54 m	3.61 m	3.41 ddd (9.5, 4.4, 1.8)	3.31 m
6′A	3.74 dd (11.5, 5.9)	3.69 dd (12.0, 5.4)	3.86 dd (12.2, 4.1)	3.69 dd (12.0, 5.4)	—	3.78 dd (11.4, 1.8)	3.75 dd (11.0, 4.4)	3.67 dd (12.1, 5.5)
6′B	4.11 dd (11.5, 2.2)	3.90 dd (12.0, 2.2)	3.93 dd (12.2, 2.4)	3.90 dd (12.0, 2.2)	3.93 dd (11.0, 5.5)	3.93 dd (11.4, 4.4)	4.01 dd (11.0, 1.8)	3.89 dd (12.1, 1.8)
1″	4.37 d (6.8)	4.56 d (7.8)	4.40 d (8.1)	4.51 d (7.7)	4.88 d (3.7)	4.82 d (1.5)	A 3.58 d (11.7) B 3.67 d (11.7)	
2″	3.58 dd (8.8, 6.8)	3.26 dd (9.3, 7.8)	3.22 dd (9.0, 8.1)	—	—	3.84 dd (3.0, 1.5)		
3″	3.65 dd (8.8, 1.7)	—	—	—	—	3.70 dd (9.5, 3.0)	4.12 d (8.1)	
4″	3.80 ddd (5.4, 2.3, 1.7)	—	—	—	—	3.64 dd (9.5, 9.5)	4.04 dd (8.1, 7.7)	
5″A	3.66 dd (12.2, 5.4)	—	—	—	—		3.75 m	
5″B	3.87 dd (12.2, 2.3)							
6″A		3.63 dd (11.7, 6.3)	3.65 dd (12.0, 5.6)	3.69 dd (11.4, 4.4)	—	3.72 dd (11.7, 5.1)	3.68 dd (12.1, 6.6)	
6″B		3.88 dd (11.7, 2.2)	3.87 dd (12.0, 2.2)	3.77 dd (11.4, 7.7)	—	3.82 dd (11.7, 2.2)	3.74 dd (12.1, 2.9)	

a) Coupling constants (*J* in Hz) are given in parentheses. *b*) Overlapped with other signals.

Table 2. ^{13}C-NMR Chemical Shifts of Compounds **1**—**7** and **14** (CD_3OD, 100 MHz)

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**
1	99.3	99.5	99.1	99.0	99.4	99.4	99.5	99.1
3	154.8	154.6	154.7	154.6	154.8	154.7	154.8	154.8
4	109.0	109.2	109.1	109.3	109.0	109.1	109.0	109.0
5	64.3	64.5	64.3	64.5	64.3	64.4	64.3	64.3
6	33.8	33.7	33.8	33.7	33.8	33.8	33.8	33.8
7	66.0	66.0	66.0	66.0	66.0	66.0	66.0	66.0
8	133.9	133.8	133.9	133.8	134.0	133.8	133.8	133.9
9	52.1	51.9	52.1	51.9	52.1	52.1	52.0	52.0
10	121.3	121.3	121.2	121.3	121.3	121.5	121.4	121.2
11	168.0	168.0	168.0	168.0	168.0	168.0	168.0	168.0
1′	100.4	99.5	99.8	99.5	100.2	100.4	100.6	100.3
2′	74.5	73.9	74.3	73.9	74.5	74.6	74.5	74.5
3′	77.7	87.4	76.3	87.2	77.9	77.9	77.5	77.9
4′	71.4	69.9	80.4	70.0	71.7[e]	71.2	70.9	71.5
5′	77.5	77.9	77.1	78.3[c]	76.9	77.0	76.6	78.6
6′	69.5[a]	62.5	61.7	62.6[d]	67.6	66.8	61.6	62.6
1″	105.3	105.2	104.7	105.6	100.5	101.6	62.6	
2″	72.4	75.5	75.0	73.1	70.5	72.1	105.3	
3″	74.3	78.2[b]	77.9	74.8	71.6[e]	72.7	79.0	
4″	69.4[a]	71.6	71.5	70.4	71.1	68.6	77.1	
5″	66.9	78.3[b]	78.2	77.2[c]	72.5	74.5	83.7	
6″		62.7	62.5	62.7[d]	62.7	63.0	64.1	

a)—*e*) Assignments may be reversed.

^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum. On the basis of the above data, the structure of **1** was determined to be 6′-*O*-α-L-arabinopyranosylswertiamarin.

Compound **2**, $[\alpha]_D$ −114.6° (MeOH), was isolated as an amorphous powder whose molecular formula was determined to be $C_{22}H_{32}O_{15}$ by HR-FAB-MS. Acid hydrolysis of **2** gave D-glucose in the above manner. The ^{1}H- and ^{13}C-NMR spectra resembled those of **14**, except for the presence of an additional β-D-glucopyranosyl group [δ_H 4.56 (1H, d, J=7.8 Hz, H-1″); δ_C 62.7 (C-6″), 71.6 (C-4″), 75.5 (C-2″), 78.2 (C-3″ or C-5″), 78.3 (C-5″ or C-3″), 105.2 (C-1″)]. The downfield shift of C-3′ [δ_C 87.4 (+9.5 ppm)] and upfield shifts of C-2′ [δ_C 73.9 (−0.6 ppm)] and C-4′ [δ_C 69.9 (−1.6 ppm)] of **2**, relative to **14**, were ascribed to the glycosidation of the hydroxyl group at C-3′ in **14**. This was confirmed by observation of a long-range correlation from H-1″ (δ_H 4.56) to C-3′ (δ_C 87.4) in the HMBC spectrum. Based on this evidence, the structure of **2** was determined to be 3′-*O*-β-D-glucopyranosylswertiamarin.

Compound **3**, $[\alpha]_D$ −96.8° (MeOH), was isolated as an amorphous powder, and its molecular formula, $C_{22}H_{32}O_{15}$, was determined by HR-FAB-MS. Acid hydrolysis gave D-glucose in the above manner. The ^{1}H- and ^{13}C-NMR spectra closely resembled those of **14**, except for the presence of an additional β-D-glucopyranosyl group [δ_H 4.40 (1H, d, J=8.1 Hz, H-1″); δ_C 62.5 (C-6″), 71.5 (C-4″), 75.0 (C-2″), 77.9 (C-3″), 78.2 (C-5″), 104.7 (C-1″)] and a difference in the chemical shifts at C-3′ [δ_C 76.3 (−1.6 ppm)], C-4′ [δ_C 80.4 (+8.9 ppm)] and C-5′ [δ_C 77.1 (−1.5 ppm)]. These data indicated that the addtional β-D-glucopyranosyl group in **3** was attached to the hydroxyl group at C-4′ in **14**. This finding was supported by the observation of a long-range correlation from H-1″ (δ_H 4.40) to C-4′ (δ_C 80.4) in the HMBC spectrum. Thus, the structure of **3** was determined to be 4′-*O*-β-D-glucopyranosylswertiamarin.

Compound **4** was isolated as an amorphous powder, $C_{22}H_{32}O_{15}$ (HR-FAB-MS), $[\alpha]_D$ −88.2° (MeOH). Acid hydrolysis of **4** gave D-galactose and D-glucose in the above manner. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **14**, except for the presence of an additional β-D-galactopyranosyl group [δ_H 4.51 (1H, d, J=7.7 Hz, H-1″); δ_C 62.6 or 62.7 (C-6″), 70.4 (C-4″), 73.1 (C-2″), 74.8 (C-3″), 77.2 or 78.3 (C-5″), 105.6 (C-1″)] and difference in the chemical shifts at C-2′ [δ_C 73.9 (−0.6 ppm)], C-3′ [δ_C 87.2 (+9.5 ppm)] and C-4′ [δ_C 70.0 (−1.5 ppm)], suggesting that the addtional β-D-galactopyranosyl group in **4** is attached to the hydroxyl group at C-3′ in **14**. This was confirmed by the presence of a cross peak between H-1″ (δ 4.51) and C-3′ (δ 87.2) in the HMBC spectrum. Accordingly, the structure of **4** was determined to be 3′-*O*-β-D-galactopyranosylswertiamarin.

Table 3. Comparison of ^{1}H- and ^{13}C-NMR Chemical Shifts of the Sugar Moiety of Compounds **7** and **15** (CD_3OD)

	7		**15**	
No.	^{1}H[a]	^{13}C[b]	^{1}H	^{13}C
1′	4.64 d (8.1)[c]	100.6	4.18 d (8.1)	105.7
2′	3.18 dd (9.0, 8.1)	74.5	3.16 dd (9.2, 8.1)	75.1
3′	3.37 dd (9.2, 9.0)	77.5	3.34 dd (9.2, 9.2)	77.8
4′	3.47 dd (9.5, 9.2)	70.9	3.44 dd (9.5, 9.2)	71.1
5′	3.41 ddd (9.5, 4.4, 1.8)	76.6	3.34 m	76.6
6′A	3.75 dd (11.0, 4.4)	61.6	3.77 dd (11.0, 4.0)	61.8
6′B	4.01 dd (11.0, 1.8)		3.97 dd (11.0, 1.8)	
1″A	3.58 d (11.7)	62.6	3.58 d (11.6)	62.2
1″B	3.67 d (11.7)		3.67 d (11.6)	
2″		105.3		105.2
3″	4.12 d (8.1)	79.0	4.10 d (8.1)	79.2
4″	4.04 dd (8.1, 7.7)	77.1	4.03 dd (8.1, 7.7)	76.6
5″	3.75 m	83.7	3.74 m	83.7
6″A	3.68 dd (12.1, 6.6)	64.1	3.68 dd (12.1, 7.0)	64.1
6″B	3.74 dd (12.1, 2.9)		3.74 dd (12.1, 2.6)	

a) Measured at 400 MHz. *b*) Measured at 100 MHz. *c*) Coupling constants (J in Hz) are given in parentheses.

Table 4. ^{1}H- and ^{13}C-NMR Chemical Shifts of Compound **12** (CD_3OD)

No.	^{1}H[a)]	^{13}C[b)]	No.	^{1}H	^{13}C
1	5.59 d (1.5)[c)]	99.4	1″		148.2
3	7.30 s	153.8	2″		106.5
4		109.4	3″		165.9
5		64.3	4″	6.60 d (2.6)	104.4
6A	1.71 br d (13.6)	33.6	5″		162.8
6B	1.79 ddd (13.6, 12.8, 5.2)		6″	6.45 d (2.6)	113.3
7A	4.28 ddd (11.0, 5.2, 1.5)	65.8	7″		171.9
7B	4.72 ddd (12.8, 11.0, 2.6)		1‴		146.1
8	5.33 ddd (16.9, 8.8, 8.4)[d)]	133.3	2‴	6.73 dd (2.2, 1.5)	116.6
9	2.85 dd (8.8, 1.5)	51.9	3‴		157.7
10A	5.26 dd (8.4, 3.7)	121.5	4‴	6.81 ddd (8.1, 2.2, 1.5)	114.9
10B	5.33 dd (16.9, 3.7)[d)]		5‴	7.21 dd (8.1, 7.7)	129.7
11		167.5	6‴	6.75 ddd (7.7, 2.2, 1.5)	121.1
1′	4.32 d (8.1)	98.6	1⁗	5.05 d (7.3)	100.9
2′	4.71 dd (9.2, 8.1)	75.3	2⁗	—[i)]	74.9[e)]
3′	2.81 dd (9.2, 9.2)	74.9[e)]	3⁗	—	78.6[g)]
4′	3.24 dd (9.5, 9.2)	71.4[f)]	4⁗	—	71.5[f)]
5′	3.11 ddd (12.1, 6.2)	78.5[g)]	5⁗	—	77.9
6′A	3.62 dd (12.1, 6.2)	62.4[h)]	6⁗A	3.67 dd (12.5, 6.2)	62.7[h)]
6′B	3.84 dd (12.1, 2.2)		6⁗B	3.89 dd (12.5, 2.2)	

a) Measured at 400 MHz. *b*) Measured at 100 MHz. *c*) Coupling constants (*J* in Hz) are given in parentheses. *d*, *e*) Signals overlapped. *f*—*h*) Assignments are interchangeable. *i*) Overlapped with other signals.

Compound **5** was isolated as an amorphous powder, $C_{22}H_{32}O_{15}$ (HR-FAB-MS), $[\alpha]_D$ −46.0° (MeOH). Acid hydrolysis gave D-galactose and D-glucose in the above manner. The ^{1}H- and ^{13}C-NMR spectra of **5** were closely related to those of **14**, except for the presence of an additional α-D-galactopyranosyl group [δ_H 4.88 (1H, d, J=3.7 Hz, H-1″); δ_C 62.7 (C-6″), 70.5 (C-2″), 71.1 (C-4″), 71.6 or 71.7 (C-3″), 72.5 (C-5″), 100.5 (C-1″)], and differences in the chemical shifts at C-5′ [δ_C 76.9 (−1.7 ppm)] and C-6′ [δ_C 67.6 (+5.0 ppm)]. These findings indicated that the addtional α-D-galactopyranosyl group in **5** is attached to the hydroxyl group at C-6′ in **14**. This was confirmed by a cross peak observed between H-1″ (δ 4.88) and C-6′ (δ 67.6) in the HMBC spectrum. Therefore, the structure of **5** was determined to be 6′-*O*-α-D-galactopyranosylswertiamarin.

Compound **6** was isolated as an amorphous powder, $C_{22}H_{32}O_{15}$ (HR-FAB-MS), $[\alpha]_D$ −28.1° (MeOH). Acid hydrolysis of **6** gave D-glucose and D-mannose in the above manner. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **14**, except for the presence of an additional α-D-mannopyranosyl group [δ_H 4.82 (1H, d, J=1.5 Hz, H-1″); δ_C 63.0 (C-6″), 68.6 (C-4″), 72.1 (C-2″), 72.7 (C-3″), 74.5 (C-5″), 101.6 (C-1″)], and differences in the chemical shifts at C-5′ [δ_C 77.0 (−1.6 ppm)] and C-6′ [δ_C 66.8 (+4.2 ppm)], indicating that the addtional α-D-mannopyranosyl group in **6** is attached to the hydroxyl group at C-6′ in **14**. This finding was supported by the nuclear Overhauser effect correlation spectroscopy (NOESY) cross peak observed between H-1″ (δ 4.82) and H-6′B (δ 3.93). Consequently, the structure of **6** was determined to be 6′-*O*-α-D-mannopyranosylswertiamarin. Compound **6** is the first naturally occuring iridoid diglycoside having an α-D-mannopyranose.

Compound **7** was isolated as an amorphous powder, $C_{22}H_{32}O_{15}$ (HR-FAB-MS), $[\alpha]_D$ −80.2° (MeOH). Acid hydrolysis gave D-fructose and D-glucose. The ^{1}H- and ^{13}C-NMR spectra resembled those of **14**, except for the presence of an additional β-D-fructofuranosyl group [δ_H 3.58 (1H, d, J=11.7 Hz, H-1″A), 3.67 (1H, d, J=11.7 Hz, H-1″B); δ_C 62.6 (C-1″), 64.1 (C-6″), 77.1 (C-4″), 79.0 (C-3″), 83.7 (C-5″), 105.3 (C-2″)]. This β-D-fructofuranosyl group was involved in a glycosyl linkage at C-6′ of the inner β-D-glucopyranosyl group, because the HMBC spectrum showed a correlation between H_2-6′ (δ 3.75, 4.01) and C-2″ (δ 105.3). This was confirmed by comparison with the ^{1}H- and ^{13}C-NMR data (Table 3) for authentic methyl β-D-fructofuranosyl-(2→6)-β-D-glucopyranoside (**15**).[20)] On the basis of this evidence, the structure of **7** was determined to be 6′-*O*-β-D-fructofuranosylswertiamarin. Compound **7** is the first naturally occuring iridoid diglycoside having a β-D-fructofuranose.

Compound **12** was isolated as an amorphous powder, $[\alpha]_D$ −40.4° (MeOH), and the molecular formula was determined to be $C_{35}H_{40}O_{19}$ by HR-FAB-MS. Acid hydrolysis gave D-glucose. The ^{1}H- and ^{13}C-NMR spectra (Table 4) were closely related to those of amaroswerin (**16**) isolated from the same plant,[2)] except for the presence of an additional β-D-glucopyranosyl group [δ_H 5.05 (1H, d, J=7.3 Hz, H-1⁗); δ_C 62.4 or 62.7 (C-6⁗), 74.1 or 71.5 (C-4⁗), 74.9 (C-2⁗), 77.9 (C-5⁗), 78.5 or 78.6 (C-3⁗), 100.9 (C-1⁗)]. The NOESY spectrum determined the position of this β-D-glucopyranosyl group to be at C-5″, by showing correlations between H-1⁗ (δ 5.05) and H-4″ (δ 6.60); and H-1⁗ and H-6″ (δ 6.45). Accordingly, the structure of **12** was determined to be 5″-*O*-β-D-glucopyranosylamaroswerin.

Experimental

General Procedures Optical rotations were determined using a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL JNM-LA 400 (400, 100 MHz, respectively) spectrometer. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane as an internal standard. The HR-FAB-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230—400 mesh). Preparative HPLC was carried out on a Tosoh HPLC system (pump, CCPM; detector, UV-8020 or RI-8020); Condition A, Column, Cosmosil 5C18AR, 10 mm i.d.×25 cm (Nacalai Tesque); mobile phase, MeOH–H_2O (1 : 8); flow rate, 1.0 ml/min; RI detector; Condition B, Column, Cosmosil 5SL, 10 mm i.d.×25 cm (Nacalai Tesque); mobile phase, CH_2Cl_2–MeOH–H_2O (30 : 10 : 1); flow rate, 1.0 ml/min; UV detector, 225 nm. GLC was carried out on a Shimadzu GC-7A gas chromatograph.

Plant Material The dried whole plants of *S. japonica* were purchased from Uchida Wakanyaku Co., Ltd., Tokyo, Japan, in 2002.

Extraction and Isolation The dried whole plants of *S. japonica* (2.0 kg) were extracted with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure. The MeOH extract (474 g) was suspended in water, and this suspension was extracted with $CHCl_3$, Et_2O, AcOEt, *n*-BuOH and H_2O. The aqueous layer was passed through a Mitsubishi Diaion HP-20 column, and the adsorbed material was eluted with H_2O and MeOH. The MeOH eluate-fraction from the Diaion HP-20 column was concentrated. The residue (20.8 g) was chromatographed on a silica-gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1), and the eluate was separated into 12 fractions (frs. 1—12) . Fraction 3 was purified by preparative HPLC (Condition A) to give **1** (1.8 mg). Fraction 5 was purified by preparative HPLC (Condition B) to give **2** (2.3 mg), **3** (2.2 mg), **4** (0.5 mg), **5** (0.9 mg), **6** (0.8 mg), **7** (0.7 mg), **8** (0.9 mg), **9** (27.1 mg), **10** (12.5 mg) and **11** (33.6 mg). Fraction 11 was purified by preparative HPLC (Condition B) to give **5** (0.8 mg). Fraction 10 was purified by preparative HPLC (Condition B) to give **12** (12.7 mg) and **13** (8.8 mg).

6′-*O*-α-L-Arabinopyranosylswertiamarin (**1**): Amorphous powder. $[\alpha]_D^{27}$ −92.7° (*c*=0.2, MeOH). UV λ_{max} MeOH nm (log ε): 235 (4.0). FAB-MS *m/z*: 529 $[M+Na]^+$, 507 $[M+H]^+$. HR-FAB-MS *m/z*: 529.1536 ($[M+Na]^+$, Calcd for $C_{21}H_{30}O_{14}Na$; 529.1533). ^{1}H-NMR (400 MHz, CD_3OD): see Table 1. ^{13}C-NMR (100 MHz, CD_3OD): see Table 2.

3′-*O*-β-D-Glucopyranosylswertiamarin (**2**): Amorphous powder. $[\alpha]_D^{25}$ −114.6° (*c*=0.2, MeOH). UV λ_{max} MeOH nm (log ε): 235 (4.0). HR-FAB-MS *m/z*: 559.1636 ($[M+Na]^+$, Calcd for $C_{21}H_{32}O_{15}Na$; 559.1639). ^{1}H-NMR (400 MHz, CD_3OD): see Table 1. ^{13}C-NMR (100 MHz, CD_3OD): see Table 2.

4′-*O*-β-D-Glucopyranosylswertiamarin (**3**): Amorphous powder. $[\alpha]_D^{25}$ −96.8° (*c*=0.2, MeOH). UV λ_{max} MeOH nm (log ε): 234 (4.0). HR-FAB-MS *m/z*: 559.1659 ($[M+Na]^+$, Calcd for $C_{21}H_{32}O_{15}Na$; 559.1639). ^{1}H-NMR (400 MHz, CD_3OD): see Table 1. ^{13}C-NMR (100 MHz, CD_3OD): see Table 2.

3′-*O*-β-D-Galactopyranosylswertiamarin (**4**): Amorphous powder. $[\alpha]_D^{27}$ −88.2° (*c*=0.05, MeOH). UV λ_{max} MeOH nm (log ε): 235 (4.1). HR-FAB-MS *m/z*: 559.1646 ($[M+Na]^+$, Calcd for $C_{21}H_{32}O_{15}Na$; 559.1639). ^{1}H-NMR (400 MHz, CD_3OD): see Table 1. ^{13}C-NMR (100 MHz, CD_3OD): see Table 2.

6′-*O*-α-D-Galactopyranosylswertiamarin (**5**): Amorphous powder. $[\alpha]_D^{27}$ −46.0° (*c*=0.09, MeOH). UV λ_{max} MeOH nm (log ε): 235 (3.9). HR-FAB-MS *m/z*: 559.1620 ($[M+Na]^+$, Calcd for $C_{21}H_{32}O_{15}Na$; 559.1639). ^{1}H-NMR (400 MHz, CD_3OD): see Table 1. ^{13}C-NMR (100 MHz, CD_3OD): see Table 2.

6′-*O*-α-D-Mannopyranosylswertiamarin (**6**): Amorphous powder. $[\alpha]_D^{25}$ −28.1° (*c*=0.09, MeOH). UV λ_{max} MeOH nm (log ε): 234 (3.8). HR-FAB-MS *m/z*: 559.1639 ($[M+Na]^+$, Calcd for $C_{21}H_{32}O_{15}Na$; 559.1639). ^{1}H-NMR (400 MHz, CD_3OD): see Table 1. ^{13}C-NMR (100 MHz, CD_3OD): see Table 2.

6′-*O*-β-D-Fructofuranosylswertiamarin (**7**): Amorphous powder. $[\alpha]_D^{25}$ −80.2° (*c*=0.08, MeOH). UV λ_{max} MeOH nm (log ε): 235 (3.9). HR-FAB-MS *m/z*: 559.1637 ($[M+Na]^+$, Calcd for $C_{21}H_{32}O_{15}Na$; 559.1639). ^{1}H-NMR (400 MHz, CD_3OD): see Table 1. ^{13}C-NMR (100 MHz, CD_3OD): see Table 2.

5″-*O*-β-D-Glucopyranosylamaroswerin (**12**): Amorphous powder. $[\alpha]_D^{25}$ −40.4° (*c*=1.3, MeOH). UV λ_{max} MeOH nm (log ε): 217 (4.7), 258 sh (4.2), 307 (3.9). HR-FAB-MS *m/z*: 787.2081 ($[M+Na]^+$, Calcd for $C_{35}H_{40}O_{19}Na$; 787.2062). ^{1}H-NMR (400 MHz, CD_3OD): see Table 4. ^{13}C-NMR (100 MHz, CD_3OD): see Table 4.

Determination of Absolute Structures of Sugar Moieties in 1—6 and 12 Each of compounds **1**—**6** and **12** (*ca.* 0.5 mg) was refluxed with 5% HCl for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The solution was concentrated *in vacuo* and dried to give a glycosyl residue which was subjected to preparation of the corresponding thiazolidine derivative, followed by trimethylsilylation and GLC analysis, according to the reported procedure. GLC conditions: column, G-column 1.2 mm i.d.×40 m; column temp., 245 °C; carrier gas, N_2 (32 ml/min); detector, FID. L-Arabinose t_R 22.4 min, D-glucose t_R 41.8 min, D-mannose t_R 42.0 min, D-galactose t_R 44.6 min (ref.: L-arabinose t_R 22.4 min, D-arabinose t_R 24.2 min; D-glucose t_R 41.8 min, L-glucose t_R 44.0 min; D-galactose t_R 44.6 min, L-galactose t_R 48.0 min).

Acid Hydrolysis of 7 Compound **7** (0.5 mg) was refluxed with 2% HCl for 1 h in a water bath. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The solution was concentrated *in vacuo* and dried to give a monosaccharide. The composition was determined by GLC analysis to be D-fructose and D-glucose as their TMSi derivatives. GLC condition: column, 3% SE-52 on Chromosorb W (AW) 2 mm i.d.×1.5 m; column temp., 160 °C; carrier gas, N_2 (40 ml/min); detector, FID. D-Fructose t_R 9.4 min, D-glucose t_R 12.9 and 20.5 min.

Preparation of Methyl β-D-Fructofuranosyl-(2→6)-β-D-glucopyranoside The reaction mixture containing 0.2 g of sucrose, 0.5 g of methyl β-D-glucose, and β-fructofuranosidase (6 unit, Funakoshi, from *Candida* sp.) in 1.75 ml of 50 mM phosphate buffer (pH 6.5) was incubated for 10 h at 40 °C. The reaction solution was subjected to preparative HPLC [column, TSKgel Amide-80 (Tosoh), 7.8 mm i.d.×30 cm; mobile phase, CH_3CN–H_2O (4 : 1); RI detector; column temperature, 40 °C; flow rate, 1.5 ml/min] to give methyl β-D-fructofuranosyl-(2→6)-β-D-glucopyranoside.[20] The structure of the product was determined by FAB-MS (*m/z*: 379 $[M+Na]^+$), ^{1}H-NMR (Table 3), ^{13}C-NMR (Table 3) and HMBC data.

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