Constituents of Seeds of *Ailanthus altissima* SWINGLE. Isolation and Structures of Shinjuglycosides A, B, C, and D

Shin YOSHIMURA, Masami ISHIBASHI, Takahiko TSUYUKI, Takeyoshi TAKAHASHI,* and Kazuhiro MATSUSHITA†

Department of Chemistry, Faculty of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113

†*Application Center, Scientific Instrument Project, JEOL Co. Ltd., Nakagami, Akishima, Tokyo 196*

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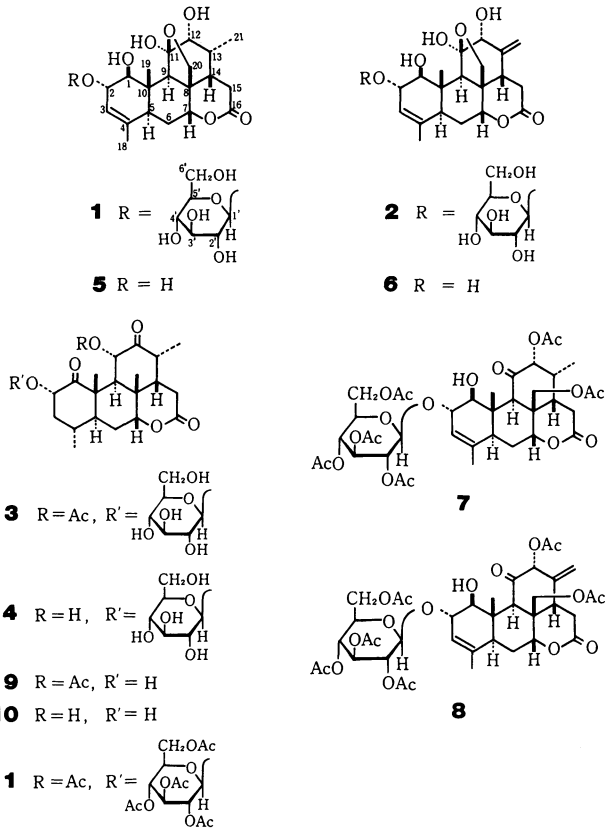
Four bitter quassinoid glycosides, shinjuglycosides A, B, C, and D were isolated from seeds of *Ailanthus altissima* SWINGLE and their structures were established to be 2-β-D-glucopyranosides of chaparrin, shinjulactone A, amarolide 11-acetate, and amarolide, respectively, from spectral data and enzymatic or acid-catalyzed hydrolysis.

Recently several quassinoid glycosides have been isolated from Simaroubaceous plants[1] and some of them have been shown to exhibit useful biological activities.[2] In our continuous studies of constituents of Simaroubaceous plants, we have investigated *Ailanthus altissima* SWINGLE (Japanese name: Shinju or Niwaurushi), and reported several new quassinoids isolated from the stem and root bark of the plant.[3,4] Now we examined bitter constituents in seeds of the plant. This paper describes isolation and structure determination of four new quassinoid glycosides named shinjuglycosides A, B, C, and D (**1**, **2**, **3**, and **4**). Although many quassinoids have been obtained from *A. altissima*, the isolation of their glycosides from the plant has not been reported yet.

Methanol extracts of seeds of *A. altissima* were defatted with hexane and partitioned between dichloromethane and water. The aqueous layer was subjected to separation by Dowex 1×2 anion exchange chromatography eluted with acetic acid in concentration of 0—2 M (1 M=1 mol dm^{-3}) linear gradient. A fraction eluted with about 0.2 M acetic acid was further purified by DEAE-Sephadex A-25 anion exchange chromatography (eluted with 0—0.5 M ammonium hydrogencarbonate linear gradient) and Sephadex G-10 gel filtration chromatography (eluted with water) to give a mixture of bitter substances. It was shown to consist of two compounds by ^{1}H NMR spectral measurement, but they could not be separated from each other by silicagel chromatography or reversed phase chromatography. However, they were separated by repetition of cellulose column chromatographies to give two new quassinoid glycosides, named shinjuglycoside A (**1**; *ca.* 0.015%) and shinjuglycoside B (**2**; *ca.* 0.03%).

The structure of shinjuglycoside A (**1**), mp 162—164 °C (decomp) was determined as follows. The molecular formula ($C_{26}H_{38}O_{12}$) was suggested by secondary ion mass spectrometry (SIMS) which showed the MH^+ peak at *m/z* 543. An abundant fragment ion at *m/z* 363 (MH^+-180) together with an ion at *m/z* 380 (M^+-162) observed in the EI mass spectrum indicated the presence of a hexose moiety. The structure of shinjuglycoside A (**1**) was inferred to be a β-D-glucoside of chaparrin (**5**)[5] from ^{1}H NMR spectrum. The sugar part was identified to be a D-glucose by the GLC examination after methanolysis and trimethylsilylation. Shinjuglycoside A (**1**) was hydrolyzed with β-glucosidase in 0.1 M acetate buffer (pH 5.0) at 37 °C for 2 weeks to afford chaparrin (**5**), which was identified by the comparison of mp, $[\alpha]_D$, IR, TLC, and ^{1}H NMR spectra with those of an authentic sample. The ^{13}C NMR spectrum of shinjuglycoside A (**1**) revealed that a signal due to C-2 of the aglycone part shifted to lower field by 9.8 ppm than that of chaparrin (**5**), indicating that the D-glucose must be attached at the C-2 position. In the ^{1}H NMR of **1**, an anomeric proton appeared at δ 5.22 as a doublet signal (J=7.6 Hz) and the coupling constants $J_{2',3'}$, $J_{3',4'}$, and $J_{4',5'}$ of the sugar part are all 8.9 Hz. Thus the structure of shinjuglycoside A (**1**) was determined to be 2-*O*-β-D-glucopyranosylchaparrin.[6]

When treated with acetic anhydride in pyridine at room temperature for 24 h, **1** gave a hexaacetyl deriva-



tive (**7**), mp 144—145 °C, which still showed the presence of hydroxyl(s) in the IR spectrum. SIMS of **7** showed an MH^+ ion at m/z 795 and a tetra-*O*-acetylhexose oxonium ion at m/z 331. In the ^1H NMR spectrum of **7**, six singlet signals due to the acetoxyl groups appeared at δ 2.02, 2.03, 2.04, 2.10, 2.17, and 2.18, and the signal of 12-H (δ 6.18, d, J=4.3 Hz) was observed at much lower field in comparison with that of **1** (δ 3.94, d, J=4.3 Hz). These findings indicate that **7** is 2′,3′,4′,6′,12,20-hexa-*O*-acetyl derivative of **1**.

The structure of shinjuglycoside B (**2**), mp 194—196 °C (decomp) was determined by the same chemical and spectral methods as those developed in **1**. Shinjuglycoside B (**2**) gave an MH^+ peak at m/z 541 in SIMS, indicating the molecular formula of $C_{26}H_{36}O_{12}$, and the presence of a hexose moiety was shown by fragment ions at m/z 378 ($M^+ - 162$) in EI mass spectrum and at m/z 361 ($MH^+ - 180$) in SIMS. Enzymatic hydrolysis of **2** gave shinjulactone A (**6**).[3] Glycosylation shift ($\Delta\delta$ 9.7) of the signal due to C-2 was observed in the ^{13}C NMR spectrum of **2**. The ^1H NMR spectrum of **2** showed an anomeric proton at δ 5.20 as a doublet signal (J=7.6 Hz) and the coupling constants of the sugar part are 8.6 Hz. Thus the structure of shinjuglycoside B (**2**) was determined to be 2-*O*-β-D-glucopyranosylshinjulactone A.

Under the same conditions as in the case of **1**, **2** also gave a hexaacetyl derivative (**8**), mp 145—148 °C. The structure of **8**, therefore, has been established to be 2′,3′,4′,6′,12,20-hexa-*O*-acetyl derivative of **2** from spectral data.

Bitter principles in the dichloromethane layer obtained by the partition (*vide supra*) were then investigated. The organic layer was separated by silica-gel column chromatography to afford four known quassinoids, amarolide,[7] chaparrinone,[8] ailanthone,[3] and chaparrin[5] in very low yields from less polar fractions. The fractions eluted with more than 20% methanol in chloroform were further subjected to separation by silica-gel column chromatography, silicic acid partition chromatography, and Sephadex G-10 gel filtration chromatography. Two new quassinoid glycosides named shinjuglycosides C (**3**) and D (**4**) were isolated in 0.005% and 0.002% yield, respectively.

Shinjuglycoside C (**3**), mp 180—185 °C, showed the presence of two secondary methyls, two tertiary

Table 1. ^1H-NMR spectra at 400 MHz of shinjuglycosides A and B (**1** and **2**)[a,b]

	1		**2**	
	δ	J	δ	J
1-H	4.00 d	7.6	4.07 d	7.9
2-H	4.57 m		4.57 m	
3-H	5.77 br s		5.80 br s	
5-H	2.55 br d	11.0	2.57 br d	12.8
6α-H	1.99 ddd	14.5, 2.7, 2.1	2.01 ddd	15, 2.5, 2.5
6β-H	1.83 ddd	14.5, 14.5, 2.7	1.85 ddd	15, 12.8, 2.5
7-H	4.46 t	2.7	4.57 t	2.5
9-H	3.00 s		3.21 s	
12-H	3.94 d	4.3	4.53 s	
13-H	2.42 m			
14-H	1.86 m		2.80 dd	13.6, 5.3
15α-H	3.22 dd	18.7, 14.0	3.55 dd	18.5, 13.6
15β-H	2.85 dd	18.7, 5.2	2.90 dd	18.5, 5.3
4-CH_3	1.45 br s		1.46 br s	
10-CH_3	1.58 s		1.56 s	
13-CH_3	1.10 d	7.3		
20-H	3.73 d	8.5	3.65 d	8.2
20-H′	4.15 d	8.5	4.13 d	8.2
21-H			5.20 d	1.5
21-H′			5.26 d	1.5
1′-H	5.22 d	7.6	5.20 d	7.6
2′-H	4.10 dd	7.6, 8.9	4.07 dd	7.6, 8.6
3′-H	4.23 t	8.9	4.21 t	8.6
4′-H	4.27 t	8.9	4.23 t	8.6
5′-H	3.99 m		3.97 m	
6′-H	4.41 dd	11.9, 5.2	4.39 dd	11.9, 5.2
6′-H′	4.58 dd	11.9, 2.4	4.53 dd	11.9, 2.4

a) δ and J are expressed in ppm and Hz, respectively. b) Measured in C_5D_5N.

Table 2. ^{13}C-NMR spectra of shinjuglycoside A (**1**), B (**2**), chaparrin (**5**) and shinjulactone A (**6**)[a,b]

Assignment No. of C	**1**	**5**	**2**	**6**[c]
1	83.9	83.8	83.9	83.6
2	82.6	72.8	82.4	72.7
3	123.8	127.0	123.8	127.1
4	136.0	134.9	135.9	134.8
5	41.5	41.9	41.5	41.9
6	25.9	26.2	25.8	26.2
7	78.9	79.2	78.9	79.2
8	46.2	46.3	45.6	45.8
9	42.7	42.9	47.9	48.0
10	41.7	41.8	41.9	41.9
11	110.6	110.7	110.4	110.5
12	79.6	79.7	80.6	80.7
13	30.5	31.8	147.7	148.0
14	44.3	44.6	44.6	44.9
15	31.7	30.6	35.3	35.4
16	170.4	170.5	169.5	169.6
18	21.1	21.2	21.1	21.2
19	10.5	10.7	10.5	10.6
20	71.8	71.8	72.6	72.7
21	13.2	13.3	118.2	118.0
1′	106.2		106.2	
2′	76.0		76.1	
3′	78.5		78.5	
4′	71.6		71.6	
5′	78.4		78.4	
6′	62.7		62.8	

a) Measured in C_5D_5N. b) Chemical shifts are expressed in δ-value. c) Chemical shifts and assignment described in Ref. 3 were found to be revised as shown in this table. Chemical shifts of the signals of C-5, C-10, and C-14 were assigned to be δ 49.6, 44.9, and 41.9, respectively, in Ref. 3.

TABLE 3. ^{1}H-NMR SPECTRA OF SHINJUGLYCOSIDE A HEXAACETATE (**5**) AND SHINJUGLYCOSIDE B HEXAACETATE (**6**)[a,b]

	5		**6**	
	δ	J	δ	J
1-H	4.44 d	8.0	4.42 d	8.0
2-H	4.10 m		4.06 dd	8.0, 4.6
3-H	5.75 br s		5.79 br s	
5-H	2.58[c]		2.64 br d	13.0
6α-H	d)		1.99 ddd	14.0, 2.4, 2.4
6β-H	1.79 ddd	15.0, 11.0, 2.5	1.81 ddd	14.0, 13.0, 2.4
7-H	4.49 t	2.5	4.58 t	2.4
9-H	3.02 s		3.27 s	
12-H	6.18 d	4.3	6.77 s	
13-H	2.58[c]			
14-H	d)		2.97 dd	13.8, 5.2
15α-H	2.97 dd	18.5, 14.0	3.30 dd	18.0, 13.8
15β-H	2.81 dd	18.5, 5.3	2.85 dd	18.0, 5.2
4-CH_3	1.46 br s		1.47 br s	
10-CH_3	1.30 s		1.31 s	
13-CH_3	0.80 d	7.3		
20-H	3.90 d	8.5	3.79 d	8.2
20-H'	4.24 d	8.5	4.24 d	8.2
21-H			5.35 s	
21-H'			5.45 s	
1'-H	5.34 d	7.9	5.36 d	7.6
2'-H	5.57 dd	7.9, 9.8	5.57 dd	7.6, 9.5
3'-H	5.53 t	9.8	5.54 t	9.5
4'-H	5.77 t	9.8	5.78 t	9.5
5'-H	4.10 m		4.11 ddd	9.5, 4.6, 2.4
6'-H	4.42 dd	12.2, 2.4	4.43 dd	12.2, 2.4
6'-H'	4.66 dd	12.2, 4.6	4.60 dd	12.2, 4.6
OAc	2.02, 2.03, 2.04, 2.10, 2.17, 2.18		2.02, 2.03, 2.04, 2.05, 2.18×2	

a) δ and J are expressed in ppm and Hz, respectively. b) Measured in C_5D_5N. c) Signals are overlapped. d) Signals are not assignable.

methyls, and one acetoxyl group in the 400 MHz ^{1}H NMR spectrum. Most of other protons including those attributed to a D-glucose residue were assigned (see Table 4). The molecular formula ($C_{28}H_{40}O_{12}$) was suggested by SIMS which showed the MH^+ peak at m/z 569, and an abundant fragment ion at m/z 407 ($MH^+ - 162$). The molecular formula was also supported by an intense peak observed at m/z 406 ($M^+ - 162$) in the EI mass spectrum. These spectral data suggest that shinjuglycoside C (**3**) consists of amarolide 11-acetate (**9**)[7] as an aglycone and β-D-glucopyranose as a sugar part. Acid hydrolysis of **3** with 1.5 M sulfuric acid-methanol (1:2) yielded D-glucose, and amarolide (**10**) concomitant with hydrolysis of 11-acetate moiety. The former was identified by GLC as its trimethylsilyl derivative, and the latter identified in comparison with an authentic sample by IR, ^{1}H NMR and TLC.

TABLE 4. ^{1}H-NMR SPECTRA OF SHINJUGLYCOSIDES C AND D (**3** AND **4**)[a,b]

	3		**4**	
	δ	J	δ	J
2-H	5.39 dd	11.3, 7.6	5.48 dd	11.3, 7.6
7-H	4.37 br s		4.30 t	3.7
9-H	3.40 d	13.4	3.09 d	12.8
11-H	5.62 d	13.4	4.67 d	12.8
13-H	3.13 quin	6.4	3.08 quin	6.4
15α-H	c)		2.28 dd	18.6, 12.5
15β-H	2.74 dd	18.6, 6.7	2.67 dd	18.6, 6.7
4-CH_3	0.97 d	6.7	1.00 d	6.7
8-CH_3	1.30 s[d]		1.45 s[d]	
10-CH_3	1.49 s[d]		1.50 s[d]	
13-CH_3	0.63 d	6.4	0.63 d	6.4
11-OAc	2.19 s			
1'-H	4.85 d	7.6	4.97 d	7.6
2'-H	4.10 dd	7.6, 8.6	4.09 dd	7.6, 8.9
3'-H	4.18 dd	8.6, 8.9	4.15 dd	8.9, 8.5
4'-H	4.23 dd	8.9, 8.9	4.24 dd	9.6, 8.5
5'-H	3.89 ddd	8.9, 5.5, 2.4	3.76 ddd	9.6, 5.2, 2.4
6'-H	4.38 dd	11.9, 5.5	4·35 dd	11.9, 5.2
6'-H'	4.58 dd	11.9, 2.4	4.50 dd	11.9, 2.4

a) δ and J are expressed in ppm and Hz, respectively. b) Measured in C_5D_5N. c) Signals are not assignable. d) The assignments of these signals may be reversed.

Shinjuglycoside D (**4**) was crystallized from methanol as colorless needles, mp 282—284 °C (decomp). The molecular formula ($C_{26}H_{38}O_{11}$) was given by SIMS which showed the $[M+Na]^+$ peak at m/z 549. The structure of **4** was suggested to be amarolide 2-β-D-glucopyranoside by the 400 MHz ^{1}H NMR spectrum (see Table 4). In the ^{13}C NMR, a glycosylation shift was observed between the signals due to C-2 of **4** and that of amarolide (**10**). Acid-hydrolysis of **4** yielded amarolide (**10**) and D-glucose, each of which was identified by the same method as described for **3**.

Acetylation of **3** and **4** afforded the same compound (**11**), mp 238 °C (from methanol-diethyl ether). In the IR spectrum of **11**, no hydroxyl was detected. In the mass spectrum (EI), the fragment ions were observed at m/z 676 (M^+—AcOH) and at m/z 331 corresponding to a tetra-*O*-acetylhexose oxonium ion. In the ^{1}H NMR spectrum four methyl signals appeared at δ 0.92 (d, J=7.7, 13-CH_3), 1.05 (d, J=6.4, 4-CH_3), 1.29 (s, tertiary CH_3), and 1.54 (s, tertiary CH_3) together with five resonances due to the acetoxyl groups δ 2.01, 2.02, 2.07, 2.11, and 2.19.

Thus, the structure of **3** proves to be amarolide 11-acetate 2-β-D-glucopyranoside, and the structure of **4** to be amarolide 2-β-D-glucopyranoside.

Experimental

General Procedures. All melting points were measured on a Mel-temp capillary melting apparatus (Laboratory

devices) and uncorrected. Optical rotations were determined on a JASCO polarimeter DIP-181. Infrared (IR) spectra were measured on a Hitachi 260-30 spectrometer. Low and high resolution mass spectra (MS and HRMS, respectively) were run on a JEOL JMS-D300 mass spectrometer operating at 70 eV and secondary ion mass spectra (SIMS) on a Hitachi M-80 mass spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra (400 MHz) were taken using a JEOL JMN GX-400 spectrometer and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra (22.5 MHz) JEOL FX 90Q. Chemical shifts were expressed in ppm downfield from tetramethylsilane as an internal standard (δ value) and coupling constants in Hz. Thin-layer chromatography (TLC) was carried out on Kieselgel 60 GF_{254} coated in 0.25 mm or on TLC plates Kieselgel 60/Kieselgur F_{254} (E. Merck). Wakogel C-200 (Wako), Silicic acid AR (Mallincrodt), Dowex 1×2 (Dow Chemical), DEAE-Sephadex A-25, Sephadex G-10 (Pharmacia Fine Chemicals), or Cellulose microcrystalline (E. Merck) was used for column chromatography. Commercialized β-glucosidase available from Sigma Chemical Company was used.

Plant Materials. Seeds with pericarps of *Ailanthus altissima* SWINGLE were air-dried, and grinded to afford 3.7 kg of materials.

Extraction and Separation. Seeds (3.7 kg) was extracted with methanol (18 L×2) for 24 h. Extracts were concentrated, defatted with hexane, and partitioned between dichloromethane and water.

A half of the aqueous layer was concentrated to give brown tar (90 g), which was dissolved in distilled water (2 L). The aqueous solution was charged to Dowex 1×2 (OH^--form; 4.0×40 cm) and the resin was then washed with distilled water (2 L). The Dowex 1 column was then eluted with acetic acid (4 L) with 0—2 M linear gradient and 260 fractions (each 15 mL) were collected. Fractions 41—52 were combined and lyophilized to give a yellow powder (fraction A; 4.6 g).

A part of fraction A (3.6 g) was dissolved in a small amount of water and then charged to a column of DEAE-Sephadex A-25 (HCO_3^--form; 2.6×90 cm). Elution was performed with an aqueous ammonium hydrogencarbonate solution (4 L) with 0.01—0.5 M linear gradient and 400 fractions (each 10 mL) were collected. The fractions 113—157 were combined and lyophilized to give white powder (fraction B; 1.8 g). The fraction exhibited a bitter taste and was shown to be a complex mixture by TLC examination (Kieselgel 60/Kieselgur F_{254} developed with 2-propanol-conc. aqueous ammonia-water 6:1:1).

Fraction B was devided into three portions. Each portion was dissolved in a small amount of water and charged to a column of Sephadex G-10 (1.2×130 cm). Elution was performed with water, and 50 fractions (each 5 mL) were col-

TABLE 5. ^{13}C-NMR SPECTRA OF SHINJUGLYCOSIDE C(**3**), D(**4**), AMAROLIDE 11-ACETATE (**9**), AND AMAROLIDE (**10**)[a,b]

Assignment No. of C	**3**	**9**	**4**	**10**
1	211.8	213.5	213.1	215.9
2	75.6	70.8	75.7	70.8
3	45.1	49.2	45.2	48.9
4	29.0	29.1	29.1	29.0
5	36.8	37.0	40.1	40.4
6	26.7	26.8	26.9	27.9
7	75.6	75.5	75.7	74.8
8	50.5	50.2	50.7	50.4
9	47.2	47.3	47.5	47.9
10	35.5	35.6	35.4	35.5
11	81.7	81.8	82.0	82.0
12	203.4	203.6	210.3	210.5
13	47.6	48.1	48.0	47.9
14	43.0	43.0	42.7	42.7
15	29.0	29.1	29.1	29.0
16	170.1	170.1	168.9	168.9
18	18.4	18.5	18.4	18.6
19	12.6	12.9	12.9	13.1
20	10.5	10.6	10.6	10.7
21	21.3	21.5	21.5	21.6
11-OAc	168.7	168.6		
	21.0	20.5		
1′	104.1		103.1	
2′	77.0		76.0	
3′	78.8		78.6	
4′	71.2		71.5	
5′	78.4		78.2	
6′	62.7		62.8	

a) Measured in C_5D_5N. b) Chemical shifts are expressed in δ-value.

TABLE 6. ^{1}H-NMR SPECTRUM OF 11-*O*-ACETYL-2-*O*-(2′,3′,4′,6′-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSYL)-AMAROLIDE (**11**)[a,b]

	δ	J		δ	J
2-H	4.90 dd	11.6, 7.6,	4-CH_3	1.05 d	7.7
5-H	1.48 ddd	14.9, 12.8, 3.4	13-CH_3	0.92 d	6.4
6β-H	1.80 ddd	14.9, 14.9, 1.8	8-CH_3	1.29 s[c]	
7-H	4.31 br s		10-CH_3	1.54 s[c]	
9-H	3.02 d	12.5	OAc	2.01, 2.02, 2.07, 2.11, 2.19	
11-H	5.28 d	11.5	1′-H	4.46 d	7.6
13-H	3.02 m		2′-H	5.02 dd	9.5, 7.6
14-H	2.38 ddd	12.5, 7.4, 4.6	3′-H	5.04 dd	9.5, 9.5
			4′-H	5.13 dd	9.8, 9.5
			5′-H	3.54 ddd	9.8, 4.9, 2.8
			6′-H	4.12 dd	12.2, 2.8
				4.21 dd	12.2, 4.9

a) δ and J are expressed in ppm and Hz, respectively. b) Measured in $CDCl_3$. c) The assignments of these signals may be reversed.

lected. The fractions 19—27, showing a bitter taste and two spots on TLC, were combined and lyophilized to give white powder (fraction C; 1.5 g).

Fraction C was applied to a Cellulose microcrystalline column (2.6×70 cm) and was eluted with acetone–water (9:1), and 50 fractions (each 15 mL) were collected. Fractions 22—27 were combined and evaporated to give shinjuglycoside A (**1**; 151 mg) and fractions 43—50 to give shinjuglycoside B (**2**; 119 mg). Fractions 28—42 (808 mg) were a mixture of shinjuglycosides A and B. A part of these fractions (578 mg) was separated by the same method to give **1** (50 mg), **2** (177 mg), and a mixture (350 mg) of **1** and **2**. This mixture was found to contain **1** and **2** in a ratio of *ca.* 1:2 by ^{1}H NMR spectral measurement.

A part (16.5 g) of the dichloromethane extract (*ca.* 60 g) obtained by the partition was separated by silica-gel column chromatography (C-200, 825 g; column A) eluted with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (each 1 L), 20, 40, 60% (each 2 L) methanol in chloroform, successively. Fractions of 500 mL each were collected. Fractions 25—32 (5.3 g) were combined and further separated by silica-gel column chromatography (C-200, 380 g; column B) eluted with the lower layer of a solvent mixture of chloroform–methanol–water (50:12:3). Fractions of 200 mL each were collected. Fractions 5—7 (1.4 g) of column B were subjected to separation by the silicic acid partition chromatography. Silicic acid (350 g) was mixed with water (235 mL) and packed in a column with chloroform (column C). Elution was performed with 0 (2 L), 3, 6, 9, 12, 15, 18, and 21% (each 1 L) ethanol in water-saturated chloroform. Fractions of 300 mL each were collected and monitored by TLC (ethyl acetate–methanol, 7:3). Fractions 8—13 (1.5 g) of column B were combined and also separated by silicic acid partition chromatography (silicic acid 400 g containing water 270 mL; column D). Elution was performed with the same solvent as that for column C and the effluent was collected in 200 mL-fractions.

Fractions 16—19 (175 mg) of column C were separated by column chromatography on Sephadex G-10 prepared by the same procedure as before to give shinjuglycoside C (140 mg). Fractions 27—30 (175 mg) of column D were separated to give shinjuglycoside D (80 mg) and a mixture (75 mg) of shinjuglycosides A and B by column chromatography on Sephadex G-10 prepared before.

Shinjuglycoside A (1). Amorphous solid, mp 162—164 °C (decomp); $[\alpha]_D^{21}$ +66.5° (*c* 3.79, MeOH); IR(KBr) 3400, 1720, and 1040 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); MS(SIMS) *m/z* 543 (MH^+) and 363; HRMS (EI) Found: *m/z* 380.1868. Calcd for $C_{20}H_{28}O_7$ ($M-C_6H_{10}O_5$): 380.1835.

Shinjuglycoside A Hexaacetate (7). Shinjuglycoside A (20 mg) was acetylated with pyridine (1 mL) and acetic anhydride (1 mL) for 50 h at room temperature. The usual work-up and silica-gel column chromatography afforded an amorphous solid (28 mg), mp 144—145 °C; IR (Nujol) 3550, 1750, 1240, and 1040 cm^{-1}; ^{1}H NMR (Table 3); MS (SIMS) *m/z* 795 (MH^+), 447, 405, 387, 345, 331, and 289; HRMS (EI) Found: *m/z* 674.2603. Calcd for $C_{34}H_{42}O_{14}$ ($M-C_4H_8O_4$): 674.2575.

Enzymatic Hydrolysis of 1. A mixture of **1** (64 mg) and β-glucosidase (20 mg) in 0.1 M acetate buffer (5 mL; pH 5.0) was allowed to stand for 2 weeks at 37 °C. The mixture was heated in boiling water for 10 min. After being cooled, the mixture was filtered and the filtlate was passed through a short column of Dowex 50 W (H^+). The effluent was evaporated to dryness *in vacuo*. The residue was purified by silica-gel column chromatography (C-200, 40 g, chloroform–methanol 7:3) to yield a crystalline chaparrin (18 mg).

Methanolysis of 1 and GLC Examination. A small amount of **1** in a tube was dried *in vacuo*, and 3% anhydrous HCl–MeOH (0.5 mL) was added. The tube was sealed and heated for 16 h at 80 °C. After being evaporated, the residue was trimethylsilylated by the usual method. Trimethylsilyl derivatives of methyl α-D-glucoside (t_R=6.2 min) and methyl β-D-glucoside (t_R=7.48 min) were deteacted by the GLC examination (GC-4BM, 1.5% OV-17, 1.0 m, 70 mL/min N_2, 156—245 °C, 4 °C/min).

Shinjuglycoside B (2). Amorphous solid, mp 194—196 °C; $[\alpha]_D^{20}$ +48.0° (*c* 2.77, MeOH); IR (KBr) 3400, 1720, and 1040 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); MS (SIMS) *m/z* 541 (MH^+) and 361; HRMS (EI) Found: *m/z* 378.1662. Calcd for $C_{20}H_{26}O_7$ ($M-C_6H_{10}O_5$): 378.1679.

Shinjuglycoside B Hexaacetate (8). Acetylation of **2** (35.3 mg) was carried out under the same conditions as described for **1** to give an amorphous solid (39.3 mg), mp 145—148 °C; IR (Nujol) 3550, 1740, 1220, and 1040 cm^{-1}; ^{1}H NMR (Table 3); MS (SIMS) *m/z* 793 (MH^+), 445, 403, 385, 343, 331, and 289; HRMS (EI) Found: *m/z* 672.2404. Calcd for $C_{34}H_{40}O_{14}$ ($M-C_4H_8O_4$): 672.2419.

Enzymatic Hydrolysis of 2. A mixture of shinjuglycoside B (126 mg) and β-glucosidase (50 mg) in 0.1 M acetate buffer (10 mL; pH 5.0) was treated by the same procedure as before. Shinjulactone A (**6**) was isolated and identified as the sole aglycone.

Methanolysis of **2** and GLC examination gave the same result as that obtained for **1**.

Shinjuglycoside C (3). Amorphous solid, mp 180—185 °C (decomp); $[\alpha]_D^{23}$ −37° (*c* 6.1, MeOH); IR (KBr) 3450, 1730, 1640, 1080, and 1040 cm^{-1}; ^{1}H NMR (Table 4); ^{13}C NMR (Table 5); MS (SIMS) *m/z* 569 (MH^+) and 407; HRMS (EI) Found: *m/z* 406.2005. Calcd for $C_{22}H_{30}O_7$ ($M-C_6H_{10}O_5$): 406.1992.

Acid Hydrolysis of 3. A solution of **3** (28 mg) in 1.5 M sulfuric acid–methanol (1:2, 10 mL) was refluxed for 7 h and then water (10 mL) was added. The mixture was concentrated *in vacuo* and extracted with dichloromethane three times. The combined dichloromethane layer was dried over anhydrous magnesium sulfate and evaporated to give a residue (7 mg), which was identified to be amarolide (**10**) by the comparison of IR, ^{1}H NMR and TLC with an authentic sample.

The aqueous layer obtained from the hydrolysis was passed through a short column of anion exchange resin (Amberlite IRA-400, OH^- form) and evaporated *in vacuo* to give a residue which was identified as D-glucose by GLC after trimethylsilylation.

Shinjuglycoside D (4). Colorless needles crystallized from methanol, mp 282—284 °C (decomp); $[\alpha]_D^{20}$ −42° (*c* 1.0, MeOH) IR (KBr) 3450, 1735, 1720, 1640, 1240, 1080, and 1040 cm^{-1}; ^{1}H NMR (Table 4); ^{13}C NMR (Table 5); MS (SIMS) *m/z* 549 ($[M+Na]^+$); HRMS (EI) Found: *m/z* 364.1908. Calcd for $C_{20}H_{28}O_6$ ($M-C_6H_{10}O_5$): 364.1886.

Acid Hydrolysis of 4. Hydrolysis of **4** with 1.5 M sulfuric acid-methanol (1:2) led to the isolation and identification of amarolide (**10**) and D-glucose.

11-O-Acetyl-2-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-amarolide (11) from 3 and 4. Shinjuglycoside C (17.8 mg) was acetylated with pyridine (1 mL) and acetic anhydride (1 mL) for 24 h at room temperature. The usual work-up and silica-gel column chromatography (C-200, 12g, ethyl acetate–dichloromethane 2:1) yielded, after crystallization from methanol–diethyl ether, a pentaacetate (**11**; 18 mg), as colorless needles, mp 238 °C; IR (Nujol) 1735, 1715, 1240, and 1040 cm^{-1}; ^{1}H NMR (Table 6); MS (EI) *m/z* (%) 676 (M^+−AcOH, 0.4), 616 (2), 556 (3), 543 (1), 514 (1), 501 (5), 483 (2), 454 (0.8), 441 (3), 390 (2), 375 (4), 348 (6), 331 (28), 271 (18), 169 (100), 109 (54), and 60 (64). HRMS (EI) Found: *m/z* 676.2741. Calcd for $C_{34}H_{44}O_{14}$ ($M-C_2H_4O_2$): 676.2732.

Under the same conditions, shinjuglycoside D was acetylated to give a crystalline residue. Recrystallization from

methanol–diethyl ether afforded **11** as colorless needles which was identified by mp, IR, ^{1}H NMR, MS, and TLC.

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