Steroidal Saponins and Alkaloids from the Bulbs of *Lilium brownii* var. *colchesteri*

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The fresh bulbs of *Lilium brownii* var. *colchesteri* were found to contain five steroidal saponins: 26-*O*-β-D-glucopyranosylnuatigenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (6), 26-*O*-β-D-glucopyranosylnuatigenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (7), brownioside (8), deacylbrownioside (9) and 27-*O*-(3-hydroxy-3-methylglutaroyl)isonarthogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (10); and two steroidal alkaloids: β$_1$-solamargine (11) and solasodine 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (12); along with several phenolic constituents. Compounds 7, 10 and 12 are new naturally-occurring compounds.

Keywords *Lilium brownii* var. *colchesteri*; Liliaceae; steroidal saponin; steroidal alkaloid; isonarthogenin glycoside; brownioside; nuatigenin glycoside; solasodine glycoside; β$_1$-solamargine; bulb

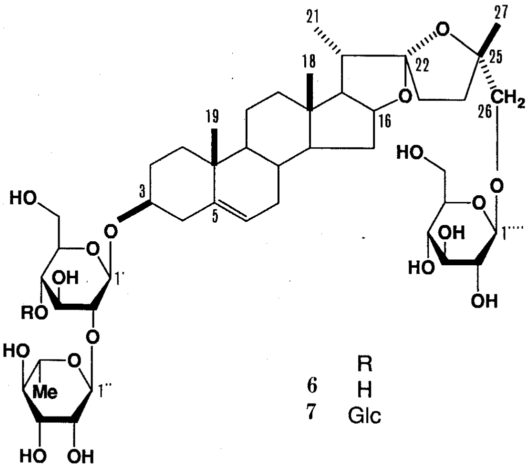
A Chinese crude drug, "Băi-hé" (百合), prepared from the bulbs of the *Lilium* species has been used as a sedative, an antitussive, an anti-inflammatory or a nutrient.[1] Internal and external anatomical studies revealed that the commercially available Bài-hé in Japan, most of which is imported from China, has its origin in the bulbs of the *Lilium brownii* var. *colchesteri* (Japanese name: Hakata-yuri).[2] *L. brownii* var. *colchesteri* is widely distributed in middle and south China at an altitude of 1500—1800 m and classified on the basis of flower shape into the subgenus *Leucolirion*.[3] No systematic chemical work appears to have been done on the plant. This paper deals with the isolation and structural elucidation of steroidal saponins and alkaloids from the bulbs of *L. brownii* var. *colchesteri*. Their confirmative structures were determined by spectroscopic and chemical means, and by comparing the data with those of known compounds.

Fresh bulbs of *L. brownii* var. *colchesteri* were extracted with hot methanol. The crude methanolic extract was suspended in water and partitioned separately against chloroform and *n*-butanol. The *n*-butanol soluble phase was subjected to silica gel, Sephadex LH-20 and DIAION HP-20 column chromatographies, and reversed-phase high performance liquid chromatography (HPLC) to give **1**—**12**.

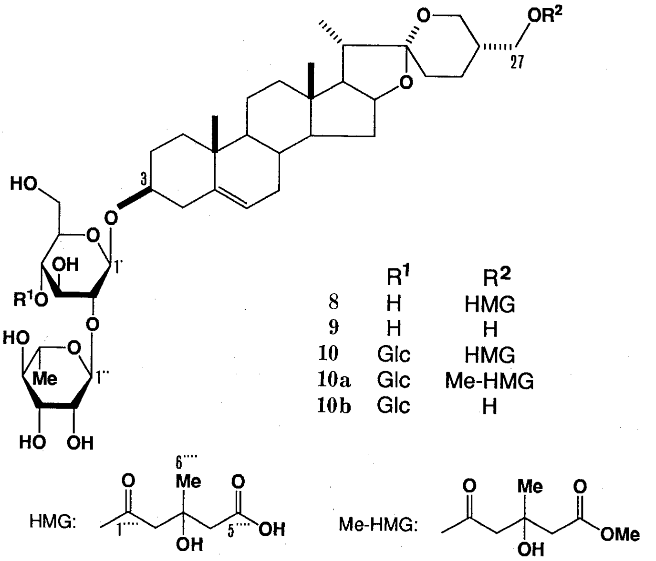
Compounds **1**—**6** and **8** were known constituents, and by spectroscopic data and direct thin-layer chromatography (TLC) comparison with authentic samples, the respective structures were identified as follows: regaloside A (**1**),[4] regaloside D (**2**),[5] 3,6′-*O*-diferuloylsucrose (**3**),[6] 1-*O*-feruloylglycerol (**4**),[7a] 1-*O*-*p*-coumaroylglycerol (**5**),[7] 26-*O*-β-D-glucopyranosylnuatigenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**6**),[8] and brownioside (**8**).[8]

The structure of **9** was elucidated as (25*S*)-spirost-5-en-3β,27-diol 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside, that is, deacylbrownioside. There has been no report on the isolation of **9** from a natural source: however, it is unclear whether **9** is a genuine compound or an artifact originated from brownioside (**8**).

Compound **7** was obtained as a white amorphous powder, $[\alpha]_D$ −90.0° (methanol). The infrared (IR) spectrum of **7** showed an absorption band of hydroxyl group(s) (3390 cm^{-1}), and the secondary ion mass spectrum (SI-MS) exhibited a $[M+Na]^+$ ion at *m*/*z* 1087. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed signals for three tertiary methyl protons at δ 1.36, 1.03 and 0.80, a secondary methyl protons at δ 1.07 (d, $J=6.9$ Hz), three anomeric protons of β-D-glucose moieties at δ 5.01 (d, $J=7.8$ Hz), 4.89 (d, $J=7.5$ Hz) and 4.84 (d, $J=7.7$ Hz), and an anomeric proton of an α-L-rhamnose moiety at δ 6.07 (br s). Acid hydrolysis of **7** with 1 N hydrochloric acid in H_2O–dioxane (1 : 1) gave D-glucose, L-rhamnose and isonuatigenin.[8] The steroidal skeleton of **7** was confirmed to be the same as that of **6** because of excellent



	R
6	H
7	Glc



	R^1	R^2
8	H	HMG
9	H	H
10	Glc	HMG
10a	Glc	Me-HMG
10b	Glc	H

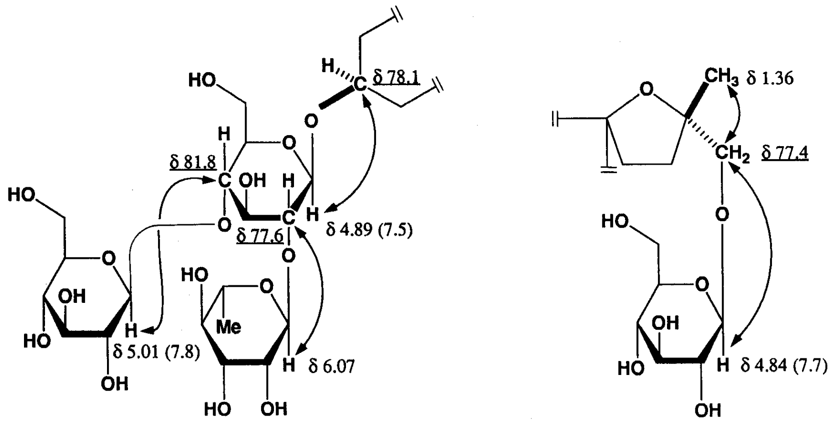
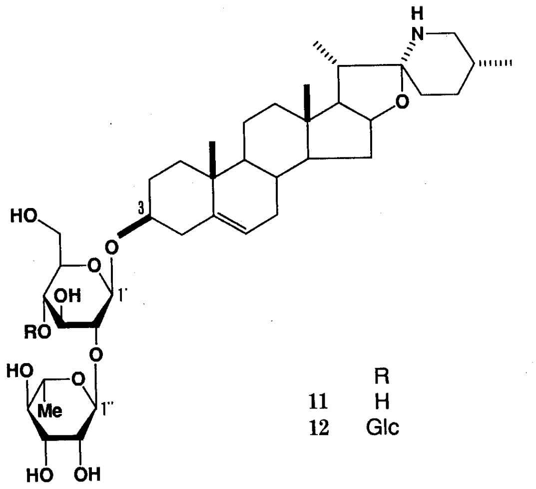


Fig. 1. ^{1}H–^{13}C Long-Range Correlations of **7** in C_5D_5N–CD_3OD (4 : 1)

J values (Hz) in the ^{1}H-NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C-NMR chemical shifts.

agreement of the spectral data of aglycon moieties between **6** and **7**. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum verified that **7** possessed a 2,4-disubstituted inner glucose moiety, two terminal glucose moieties and a terminal rhamnose moiety. The sequence and linkage positions of the sugar moieties were established by closer inspection of the ^{1}H–^{13}C correlation spectroscopy (^{1}H–^{13}C COSY) and long-range ^{1}H–^{13}C COSY (COLOC) spectra. Anomeric proton signals at δ 6.07, 5.01, 4.89 and 4.84 showed correlation with the anomeric carbon signals at δ 101.7, 105.2, 100.0 and 105.4, respectively, in the ^{1}H–^{13}C COSY spectrum. The signals at δ 5.01 and 4.84 were assigned as the anomeric protons of the terminal glucoses, and the signal at δ 4.89 as the anomeric proton of the inner glucose. The COLOC spectrum showed long-range correlation peaks between the signals at δ 6.07 and 77.6 (inner glucose C-2), δ 5.01 and 81.8 (inner glucose C-4), δ 4.89 and 78.1 (aglycon C-3), and δ 4.84 and 77.4 (aglycon C-26) as shown in Fig. 1. Thus, the α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl moiety was concluded to be attached to the C-3 hydroxyl group on the sapogenin, and the remaining β-D-glucopyranosyl moiety to the C-26 hydroxyl group. The structure of **7** was determined to be 26-*O*-β-D-glucopyranosylnuatigenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **10** was obtained as colorless needles, recrystallized from a mixed solution of chloroform and methanol, mp 220.0–230.0 °C, [α]$_D$ −90.0° (methanol). The infrared (IR) spectrum showed characteristic absorptions due to hydroxyl group(s) (3430 cm^{-1}) and carbonyl group(s) (1725 cm^{-1}). Treatment of **10** with diazomethane produced a monomethyl ester (**10a**), and alkaline hydrolysis produced 3-hydroxy-3-methylglutaric acid and a steroidal saponin (**10b**). Acetylation of **10b** with acetic anhydride in pyridine yielded a decaacetate (**10c**), and acid hydrolysis provided glucose, rhamnose and steroidal sapogenin (**10d**), identified as isonarthogenin.[8,9] Thus, **10** seemed to be isonarthogenin glycoside with a 3-hydroxy-3-methylglutaric acid as brownioside (**8**). The signals arising from the aglycon moiety of **10** were superimposable on those of **8**, and the signals from the oligoside moiety were superimposable on those of **7** in the ^{13}C-NMR spectrum. Compound **10** was determined to be 27-*O*-(3-hydroxy-3-methylglutaroyl)isonarthogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.



	R
11	H
12	Glc

Compound **11** was obtained as a white amorphous powder, [α]$_D$ −86.9° (methanol) and presented a positive Dragendorff reaction on TLC, which was indicative of an alkaloid. The SI-MS of **11** showed a quasimolecular ion peak at *m/z* 722 [M+H]$^+$. The IR spectrum showed strong absorption due to hydroxyl groups (3430 cm^{-1}). Treatment of **11** with acetic anhydride in pyridine produced an acetyl derivative (**11a**) containing six acetyl groups. The ^{13}C-NMR spectrum of **11** showed a total of 27 carbon signals arising from the aglycon moiety and 12 carbon signals due to a disaccharide. The ^{1}H-NMR spectrum showed two singlet signals at δ 1.07 and 0.88, indicating the presence of two angular methyl groups, as well as two doublet signals at δ 1.08 (*J* = 7.1 Hz) and 0.82 (*J* = 5.1 Hz) assignable to secondary methyl groups. Further, a quaternary carbon signal at δ 98.3 and olefinic carbon signals at δ 140.9 and 121.8 in the ^{13}C-NMR spectrum, and important fragment ion peaks at *m/z* 138 and 114 in the electron impact mass spectrum (EI-MS)[10] confirmed that **11** possessed a Δ^5-spirosolane skeleton. Acid hydrolysis of **11** with 1 N hydrochloric acid produced D-glucose and L-rhamnose as carbohydrate compounds together with an aglycon, identified as (22*R*,25*R*)-spirosol-5-en-3β-ol, that is, solasodine. The structure of the oligoside moiety was readily deduced to be α-L-rhamnopyranosyl-(1→2)-β-D-glucopy-

TABLE I. ^{13}C-NMR Spectral Data for **6**, **7**, Nuatigenin, **8**, **9**, **10**, **10a**, **10b**, **10d**, **11**, **11a** and **12**[a]

	6	**7**	Nuatigenin[b]	**8**	**9**	**10**	**10a**	**10b**	**10d**	**11**	**11a**	**12**
1	37.5	37.5	37.8	37.5	37.5	37.5	37.5	37.5	37.8	37.6	37.9	37.5
2	30.2	30.1	31.7	30.2	30.2	30.2	30.2	30.2	32.6	30.2	32.6[c]	30.2
3	78.2	78.2	71.3	78.2	78.3	78.2	78.2	78.2	71.3	78.2	71.3	78.2
4	39.0	38.9	43.5	39.0	39.0	38.9	39.0	39.0	43.5	39.0	43.5	39.0
5	140.9	140.8	142.0	140.9	140.9	140.8	140.9	140.9	142.0	140.9	142.0	140.8
6	121.8	121.8	120.3	121.7	121.7	121.8	121.8	121.8	121.0	121.8	121.0	121.8
7	32.2[c]	32.2[c]	32.3[c]	32.2[c]	32.3	32.2[c]	32.2[c]	32.3	32.2[c]	32.4[c]	32.3	32.4[c]
8	31.7	31.6	32.2	31.7	31.7	31.7	31.7	31.7	31.9	31.7[d]	31.8	31.7
9	50.3	50.3	50.5	50.3	50.3	50.3	50.3	50.4	50.3	50.3	50.5	50.4
10	37.2	37.1	37.0	37.2	37.2	37.2	37.2	37.2	37.1	37.2	37.1	37.2
11	21.1	21.1	21.2	21.1	21.1	21.1	21.1	21.1	21.2	21.2	21.3	21.2
12	39.9	39.8	40.0	39.9	39.9	39.8	39.9	39.9	40.0	40.1	40.1	40.1
13	40.5	40.5	40.6	40.5	40.5	40.5	40.5	40.5	40.5	40.7	40.8	40.7
14	56.5	56.5	56.6	56.6	56.7	56.6	56.7	56.7	56.8	56.7	56.8	56.7
15	32.3[c]	32.3[c]	32.6[c]	32.3[c]	32.3	32.3[c]	32.3[c]	32.3	32.3[c]	32.6[c]	32.7[c]	32.6[c]
16	81.0	80.9	81.1	81.2	81.2	81.2	81.2	81.2	81..2	78.8	79.7	78.8
17	62.5	62.5	62.6	62.8	63.0	62.8	62.8	63.0	63.0	63.6	63.4	63.6
18	16.2	16.2	16.2	16.3	16.3	16.3	16.3	16.3	16.4	16.5	16.5	16.5
19	19.4	19.4	19.6	19.4	19.4	19.4	19.4	19.4	19.6	19.4	19.5[d]	19.4
20	38.6	38.6	38.5	42.0	42.1	42.0	42.0	42.1	42.1	41.6	41.8	41.7
21	15.2	15.1	15.2	14.9	15.1	14.9	14.9	15.0	15.1	15.7	15.7	15.7
22	120.2	120.2	120.9	109.4	109.7	109.4	109.4	109.7	109.7	98.3	98.4	98.3
23	33.1	33.1	32.6	31.1	31.6	31.1	31.2	31.6	31.6	34.7	34.4	34.7
24	34.0	33.9	33.8	23.7	24.1	23.7	23.7	24.1	24.1	31.1	30.7	31.1
25	83.9	83.8	85.6	35.5	39.2	35.5	35.5	39.2	29.2	31.8[d]	31.1	31.7
26	77.5	77.4	70.1	63.0	64.1[c]	63.1	63.0	64.1[c]	64.1[d]	48.1	47.7	48.1
27	24.4	24.4	24.1	66.1	64.4[c]	66.1	66.1	64.5[c]	64.4[d]	19.8	19.6[d]	19.8
1′	100.4	100.0		100.4	100.4	100.0	100.1	100.0		100.4		100.1
2′	79.7	77.7		79.6	79.7	77.7	77.7	77.8		79.6		77.7
3′	77.9[d]	76.1		77.9[d]	77.9[d]	76.2	76.2	76.2		77.9[e]		76.2
4′	71.9	82.0		71.8	71.9	82.1	82.1	82.1		71.9		82.1
5′	78.0[d]	77.3		78.0[d]	78.0[d]	77.3	77.3	77.4		78.0[e]		77.3
6′	62.7	62.0[d]		62.7	62.7	62.0[d]	62.0[d]	62.1[d]		62.7		62.0[d]
1″	102.1	101.7		102.0	102.1	101.8	101.8	101.8		102.0		101.8
2″	72.6	72.4		72.5	72.6	72.4	72.4	72.5		72.6		72.4
3″	72.9	72.7		72.8	72.9	72.8	72.8	72.8		72.8		72.8
4″	74.2	74.1		74.2	74.2	74.1	74.1	74.2		74.2		74.2
5″	69.5	69.4		69.5	69.5	69.5	69.4	69.5		69.5		69.4
6″	18.7	18.6		18.7	18.7	18.7	18.6	18.7		18.7		18.6
1‴		105.2				105.2	105.2	105.2				105.2
2‴		74.9				75.0	74.9	75.0				75.0
3‴		78.3[e]				78.3[e]	78.3[e]	78.3[e]				78.3[e]
4‴		71.2				71.2	71.3	71.3				71.3
5‴		78.5[e]				78.5[e]	78.5[e]	78.5[e]				78.5[e]
6‴		62.1[d]				62.1[d]	62.1[d]	62.2[d]				62.1[d]
1⁗	105.5	105.4		171.6		171.6	171.4[f]					
2⁗	75.4	75.3		46.4[e]		46.4[f]	46.2[g]					
3⁗	78.4[e]	78.4		70.0		70.0	69.9					
4⁗	71.7	71.6		46.5[e]		46.5[f]	46.3[g]					
5⁗	78.5[e]	78.4		174.7		174.8	171.9[f]					
6⁗	62.7	62.7		28.3		28.3	28.3					
OMe							51.3					

a) Spectra were measured in C_5D_5N with a Bruker AM-400. Chemical shifts were expressed in ppm relative to internal standard, TMS. *b*) Spectral data were quoted from literature, *Phytochemistry*, **22**, 733 (1983). *c—g*) Assignments may be interchanged in each vertical column.

ranoside from the ^{13}C-NMR spectrum.[8,11] The above data assigned the complete structure of **11** as solasodine 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, or β_1-solamargine. β_2-Solamargine has been isolated from the berries of *Solanum khasianum*, and the structure was confirmed by ^{13}C-NMR spectrum[12]; however, the identity of pure β_1-solamargine has not been conclusively established.[13] This is the first example of unequivocal identification of β_1-solamargine on the basis of extensive spectral data and hydrolysis.

Compound **12** was obtained as a white amorphous powder and positive to the Dragendorff reagent. The SI-MS showed an $[M+H]^+$ ion at m/z 884. The spectral features of **11** and **12** were essentially analogous to one another and suggestive of the glycoside structure of the same type. Acid hydrolysis of **12** produced glucose, rhamnose and solasodine. The above result and the ^{13}C-NMR spectrum verified that **12** had the same aglycon structure as **11**, and the same oligoside linkage as **7** and **10**. The structure of **12** was formulated as solasodine 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

Compounds **7**, **10** and **12** are new constituents. The occurrence of steroidal alkaloids and their glycosides with a spirosolane skeleton seems to be limited to Solanaceae plants,[14] with the exception of the isolation of solasodine and tomatidenol from *Fritillaria camtschatcensis* (Liliaceae).[15] In this study, evidence was obtained indicating the presence of spirosolane alkaloids in the bulbs of *L. brownii* var. *colchesteri*. This is believed to be the first example of steroidal alkaloids from *Lilium* plants and the result is interesting in regard to the distribution of steroidal alkaloids in higher plants. Previously, we isolated steroidal saponins from the bulbs of *L. pardarinum*[11] and *L. brownii* var. *brownii*.[8] Distribution of the steroidal saponins may make some contribution to the chemotaxonomy of the genus *Lilium*. Further chemical examination of other *Lilium* plants is now underway in our laboratory.

Experimental

Melting points were determined with a Yazawa micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter. IR spectra were recorded on a Hitachi 260-30 spectrometer and MS on a Hitachi M-80 machine. ^{1}H- and ^{13}C-NMR spectra were taken on a Bruker AM-400 or AM-500 spectrometer. Chemical shifts are reported in ppm (δ scale) with tetramethylsilane (TMS) as an internal standard, and the following abbreviations are used: s, singlet; d, doublet; dd, doublet of doublets; dq, doublet of quartets; m, multiplet; br, broad. ^{1}H–^{13}C COSY and COLOC spectra were obtained with the usual pulse sequence and data processing was performed with standard Bruker softwear. Column chromatographies were done with Fuji Davison silica gel BW-300 (200—400 mesh, Fuji Davison Co., Ltd.), Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemicals Co., Ltd.) and DIAION HP-20 (Mitsubishi-kasei Co., Ltd.). TLC was carried out on precoated Kieselgel 60 F_{254} plates (0.25 mm thick, Merck) and preparative TLC on precoated Kieselgel 60 F_{254} (0.5 mm thick, Merck). Spots were visualized under ultraviolet (UV) light (254 nm) irradiation and by spraying a 10% H_2SO_4 solution followed by heating. Alkaloids were located using the Dragendorff reagent. HPLC was performed with the Tosoh HPLC system (pump, Tosoh CCPM; detector, Tosoh RI-8010; controller, CCP controller PX-8010) equipped with a CIG pre-packed column (Kusano Scientific Co., Tokyo, 22 i.d. × 100 mm, ODS 20 μm).

Extraction and Isolation The fresh bulbs of *L. brownii* var. *colchesteri* (4.5 kg) cultivated at Mizunami, Gifu prefecture in Japan, were extracted with hot MeOH, and the extract was concentrated under reduced pressure. The crude residue, a dark viscous syrup, was suspended in H_2O and extracted with $CHCl_3$ and then with *n*-BuOH. The concentrated *n*-BuOH phase was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH, $CHCl_3$–MeOH–H_2O and EtOAc–MeOH systems, Sephadex LH-20 with MeOH, and DIAION HP-20 with a gradient mixture of H_2O–MeOH to yield **3**, **4** and **5** as pure compounds, and **1**, **2** and **6**—**12** with a few impurities. Compounds **8**, **9** and **10** were further purified by silica gel column chromatography using $CHCl_3$–MeOH–25% NH_3 (100 : 25 : 1). Final purification of **1** and **2** was carried out by preparative TLC employing EtOAc–MeCOEt–MeOH–H_2O (10 : 10 : 1 : 1), and purification of **6**, **7**, **11** and **12** by preparative HPLC employing MeOH–H_2O (4 : 1).

Regaloside A (**1**): A pale yellow amorphous powder, 78.3 mg, $[\alpha]_D^{26}$ $-14.1°$ ($c=0.53$, MeOH).

Regaloside D (**2**): A pale yellow amorphous powder, 17.6 mg, $[\alpha]_D^{27}$ $-24.6°$ ($c=0.39$, MeOH).

3,6′-*O*-Diferuloylsucrose (**3**): A pale yellow amorphous powder, 75.5 mg.

1-*O*-Feruloylglycerol (**4**): A pale yellow syrup, 25.2 mg, $[\alpha]_D^{26}$ $\pm 0°$ ($c=0.46$, MeOH).

1-*O*-*p*-Coumaroylglycerol (**5**): A pale yellow amorphous powder, 28.2 mg, $[\alpha]_D^{28}$ $\pm 0°$ ($c=0.44$, MeOH).

26-*O*-β-D-Glucopyranosylnuatigenin 3-*O*-α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**6**): A white amorphous powder, 170 mg.

26-*O*-β-D-Glucopyranosylnuatigenin 3-*O*-α-L-Rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**7**): A white amorphous powder, 195 mg, $[\alpha]_D^{28}$ $-90.0°$ ($c=0.10$, MeOH). SI-MS *m*/*z*: 1087 $[M+Na]^+$, 902 $[M-\text{glucosyl}+H]^+$. *Anal.* Calcd for $C_{51}H_{84}O_{23}\cdot 2H_2O$: C, 55.62; H, 8.19. Found: C, 55.77; H, 7.86. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2910, 2875 (CH), 1435, 1365, 1290, 1245, 1145, 1040, 895, 875, 850, 820, 895. ^{1}H-NMR (C_5D_5N–CD_3OD, 4 : 1) δ: 6.07 (1H, br s, H-1″), 5.31 (1H, br d, $J=4.3$ Hz, H-6), 5.01 (1H, d, $J=7.8$ Hz, H-1‴), 4.89 (1H, d, $J=7.5$ Hz, H-1′), 4.84 (1H, d, $J=7.7$ Hz, H-1⁗), 4.80 (1H, dq, $J=9.5$, 6.3 Hz, H-5″), 4.67 (1H, m, H-16), 4.60 (1H, dd, $J=3.3$, 1.6 Hz, H-2″), 1.68 (3H, d, $J=6.3$ Hz, H-6″), 1.36 (3H, s, H-27), 1.07 (3H, d, $J=6.9$ Hz, H-21), 1.03 (3H, s, H-19), 0.80 (3H, s, H-18).

Acid Hydrolysis of 7 Compound **7** (20.0 mg) was hydrolyzed with 1 N HCl in H_2O–dioxane (1 : 1) on a boiling water bath for 1 h under an N_2 atmosphere. After being cooled, the reaction mixture was neutralized with 2 N NaOH and purified by the Sephadex LH-20 column chromatography with MeOH and by preparative TLC with *n*-BuOH–Me_2CO–H_2O (4 : 5 : 1) to provide L-rhamnose (1.1 mg), D-glucose (4.3 mg) and isonuatigenin (5.4 mg). L-Rhamnose: $[\alpha]_D^{25}$ $+5.5°$ ($c=0.11$, H_2O). TLC: *Rf* 0.69 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1). D-Glucose: $[\alpha]_D^{25}$ $+54.0°$ ($c=0.43$, H_2O). TLC: *Rf* 0.35 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1). Isonuatigenin: TLC: *Rf* 0.49 ($CHCl_3$–MeOH, 15 : 1); *Rf* 0.59 ($CHCl_3$–Me_2CO, 4 : 1).

Brownioside (**8**): Colorless needles ($CHCl_3$–MeOH), mp 197.0—199.5 °C, 280 mg.

Deacylbrownioside (**9**): Colorless needles ($CHCl_3$–MeOH), mp 232.0—238.0 °C, 44.9 mg. The IR and ^{1}H-NMR spectra were in agreement with those of the steroidal saponin which was obtained by alkaline hydrolysis of brownioside.[8]

27-*O*-(3-Hydroxy-3-methylglutaroyl)isonarthogenin 3-*O*-α-L-Rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**10**): Colorless needles ($CHCl_3$–MeOH), mp 220.0—230.0 °C, 251 mg. $[\alpha]_D^{28}$ $-90.0°$ ($c=0.10$, MeOH). *Anal.* Calcd for $C_{51}H_{80}O_{22}\cdot 2H_2O$: C, 56.66; H, 7.83. Found: C, 56.46; H, 7.87. SI-MS *m*/*z*: 1068 $[M+H+Na]^+$. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2930 (CH), 1725 (C=O), 1435, 1375, 1335, 1130, 1050, 985, 955, 905, 830, 805. ^{1}H-NMR (C_5D_5N) δ: 6.26 (1H, br s, H-1″), 5.30 (1H, br d, $J=3.9$ Hz, H-6), 5.14 (1H, d, $J=7.8$ Hz, H-1‴), 4.96 (1H, d, $J=6.4$ Hz, H-1′), 4.93 (1H, dq, $J=9.3$, 6.6 Hz, H-5″), 4.75 (1H, br s, H-2″), 4.59 (1H, dd, $J=9.3$, 3.1 Hz, H-3″), 3.18 (2H, s, H_2-4⁗), 3.15, 3.11 (each 1H, ABq, $J=14.1$ Hz, H_2-2⁗), 1.78 (3H, s, H-6⁗), 1.77 (3H, d, $J=6.6$ Hz, H-6″), 1.12 (3H, d, $J=6.8$ Hz, H-21), 1.05 (3H, s, H-19), 0.82 (3H, s, H-18).

Methylation of 10 Compound **10** (45.0 mg) was dissolved in MeOH and cooled at 0 °C. The CH_2N_2 in Et_2O was added to the sample solution. After being set aside 30 min at 0 °C, the reaction mixture was evaporated under reduced pressure and the crude residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (100 : 20 : 1) to yield a monomethyl ester (38.0 mg) (**10a**) as a white amorphous powder. $[\alpha]_D^{25}$ $-67.4°$ ($c=0.38$, MeOH). *Anal.* Calcd for $C_{52}H_{82}O_{22}\cdot H_2O$: C, 58.97; H, 7.80. Found: C, 57.89; H, 7.86. IR ν_{max}^{KBr} cm^{-1}: 3445 (OH), 2945 (CH), 1735 (C=O), 1445, 1430, 1375, 1340, 1260, 1200, 1155, 1055, 985, 960, 910, 810. ^{1}H-NMR (C_5D_5N) δ: 6.24 (1H, br s, H-1″), 5.30 (1H, br d, $J=4.4$ Hz, H-6), 5.12 (1H, d, $J=7.8$ Hz, H-1‴), 4.95 (1H, d, $J=6.5$ Hz, H-1′), 4.93 (1H, dq, $J=9.2$, 6.2 Hz, H-5″), 4.73 (1H, br s, H-2″), 4.57 (1H, dd, $J=9.2$, 3.3 Hz, H-3″), 3.63 (3H, s, OMe), 3.06, 3.02 (each 1H, ABq, $J=15.0$ Hz, H_2-2⁗), 3.03 (2H, s, H_2-4⁗), 1.76 (3H, d, $J=6.2$ Hz, H-6″), 1.69 (3H, s, H-6⁗), 1.12 (3H, d, $J=6.9$ Hz, H-21), 1.05 (3H, s, H-19), 0.82 (3H, s, H-18).

Alkaline Hydrolysis of 10 Compound **10** (40.1 mg) was treated with 2 N NaOH at room temperature for 2 h. The reaction mixture was passed through an Amberlite IR-120B (cation exchange resin) column and the eluate was concentrated to produce a residue, which was subjected to Sephadex LH-20 column chromatography with MeOH as the eluent, yielding 3-hydroxy-3-methylglutaric acid and a crude hydrolysate. 3-Hydroxy-3-methylglutaric acid was identified by TLC comparison with a commercially available authentic sample purchased from Aldrich Chemical Company, Inc. 3-Hydroxy-3-methylglutaric acid: TLC: *Rf* 0.64 ($CHCl_3$–MeOH–H_2O–AcOH, 40 : 20 : 2 : 1); *Rf* 0.26 (95% EtOH–H_2O–25% NH_3, 25 : 3 : 4). Spots were detected by spraying 0.05% bromocresol green solution. The crude hydrolysate was purified by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (125 : 25 : 2) to furnish a pure deacyl derivative (**10b**) (16.0 mg) of **10**. Compound **10b**: colorless needles ($CHCl_3$–MeOH), mp 280.0—285.0 °C (dec.), $[\alpha]_D^{28}$ $-82.5°$ ($c=0.08$, MeOH). SI-MS *m*/*z*: 901 $[M+H]^+$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1445, 1430, 1375, 1130, 1055, 955, 905, 810. ^{1}H-NMR (C_5D_5N) δ: 6.25 (1H, br s, H-1″), 5.30 (1H, br d, $J=4.2$ Hz, H-6), 5.13 (1H, d, $J=7.9$ Hz, H-1‴), 4.96 (1H, d, $J=6.6$ Hz, H-1′), 4.93 (overlapping with OH signal, H-5″), 4.74 (1H, br d, $J=1.9$ Hz, H-2″), 3.74 (1H, dd, $J=10.4$, 5.2 Hz, H-27a), 3.66 (1H, dd, $J=10.4$, 7.2 Hz, H-27b), 1.77 (3H, d, $J=6.2$ Hz, H-6″), 1.16 (3H, d, $J=6.9$ Hz, H-21), 1.05 (3H, s, H-19), 0.84 (3H, s, H-18).

Acetylation of 10b Compound **10b** (7.9 mg) was acetylated with acetic anhydride in pyridine and the crude acetate was chromatographed on silica gel with *n*-hexane–Me_2CO (5 : 2) to yield a decaacetate (**10c**) (9.3 mg) as a white amorphous powder. $[\alpha]_D^{25}$ $-54.1°$ ($c=0.71$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2945 (CH), 1745 (C=O), 1450, 1430, 1365, 1215, 1160, 1130, 1040, 975, 960, 905, 835, 800. ^{1}H-NMR (C_6D_6) δ: 5.88 (1H, dd, $J=10.0$, 3.3 Hz, H-3″), 5.65 (1H, dd, $J=10.0$, 10.0 Hz, H-4″), 5.54 (1H, br s, H-6), 5.51 (1H, dd, $J=9.4$, 9.4 Hz, H-3′), 5.50 (1H, dd, $J=3.3$, 1.7 Hz, H-2″), 5.45 (1H, d, $J=1.7$ Hz, H-1″), 5.35 (1H, dd, $J=9.3$, 9.3 Hz, H-3‴), 5.20 (1H, dd, $J=9.3$, 9.3 Hz, H-4‴), 5.17 (1H, dd, $J=9.3$, 7.8 Hz, H-2‴), 4.81 (1H, dq, $J=10.0$, 6.3 Hz, H-5″), 4.55 (1H, m, H-16), 4.50 (1H, dd, $J=11.9$, 1.9 Hz, H-6′a), 4.37 (1H, dd, $J=11.1$, 4.2 Hz, H-6‴a), 4.34 (1H, d, $J=7.8$ Hz H-1‴), 4.25 (1H, d, $J=7.7$ Hz, H-1′), 4.15 (1H, dd, $J=11.9$, 5.5 Hz, H-6′b), 3.86 (1H, dd, $J=11.1$, 3.2 Hz, H-6‴b), 3.83 (1H, dd, $J=9.4$, 7.7 Hz, H-2′), 3.90—3.66 (overlapping, H_2-26, H_2-27), 3.56 (1H, dd, $J=9.4$, 9.4 Hz, H-4′), 3.23 (1H, dd, $J=9.3$, 4.2, 3.2 Hz, H-5‴), 3.11 (1H, ddd, $J=9.4$, 5.5, 1.9 Hz, H-5′), 2.27, 1.91, 1.77, 1.72, 1.71, 1.69, 1.68 × 2, 1.63, 1.62 (each 3H, s, Ac), 1.45 (3H, d, $J=6.3$ Hz, H-6″), 1.19 (3H, d, $J=6.9$ Hz, H-21), 1.02 (3H, s, H-19), 0.84 (3H, s, H-18).

Acid Hydrolysis of 10b Compound **10b** (6.5 mg) was hydrolized with 1 N HCl in H_2O–dioxane (1 : 1) as for **7**. Rhamnose, glucose and isonarthogenin were detected in the reaction mixture by TLC. Isonarthogenin: TLC: *Rf* 0.50 ($CHCl_3$–MeOH, 15 : 1); *Rf* 0.40 ($CHCl_3$–Me_2CO, 4 : 1).

β_1-Solamargine (11) A white amorphous powder, 92.2 mg, $[\alpha]_D^{28}$ $-86.5°$ ($c=0.16$, MeOH). *Anal.* Calcd for $C_{39}H_{63}NO_{11} \cdot H_2O$: C, 63.31; H, 8.85; N, 1.89. Found: C, 63.30; H, 8.76; N, 1.87. EI-MS *m*/*z* (%): 721 $[M]^+$ (1.6), 547 (2.2), 396 (7.6), 282 (4.2), 208 (3), 138 (87), 114 (100). SI-MS *m*/*z*: 721 $[M+H]^+$. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2940 (CH), 1445, 1375, 1360, 1340, 1245, 1135, 1045, 910, 895, 880, 830, 810. ^{1}H-NMR (C_5D_5N) δ: 6.37 (1H, br s, H-1″), 5.31 (1H, br d, $J=4.6$ Hz, H-6), 5.04 (1H, d, $J=7.2$ Hz, H-1′), 4.99 (1H, dq, $J=9.5$, 6.3 Hz, H-5″), 4.79 (1H, br s, H-2″), 4.62 (1H, dd, $J=9.5$, 3.2 Hz, H-3″), 4.50 (1H, br d, $J=10.2$ Hz, H-6′a), 4.42 (1H, m, H-16), 4.34 (1H, dd, $J=9.5$, 9.5 Hz, H-4″), 4.33 (1H, H-6′b), 4.27 (2H, H-3′, -4′), 4.16 (1H, d, $J=9.1$, 7.2 Hz, H-2′), 3.95 (1H, m, H-3), 3.89 (1H, m, H-5′), 1.77 (3H, d, $J=6.3$ Hz, H-6″), 1.08 (3H, d, $J=7.1$ Hz, H-21), 1.07 (3H, s, H-19), 0.88 (3H, s, H-18), 0.82 (3H, d, $J=5.1$ Hz, H-27).

Acetylation of 11 Compound **11** (10.0 mg) was acetylated with Ac_2O in pyridine and the crude acetate was chromatographed on silica gel with *n*-hexane–Me_2CO (5 : 2) to yield a pure hexaacetate (**11a**) (7.4 mg) as a white amorphous powder. $[\alpha]_D^{25}$ $-39.6°$ ($c=0.48$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2945, 2870, 2845 (CH), 1755 (C=O), 1450, 1370, 1230, 1135, 1080, 1040, 980, 965, 895, 880, 835, 800. ^{1}H-NMR ($CDCl_3$) δ: 5.39 (1H, br d, $J=5.1$ Hz, H-6), 5.25 (1H, dd, $J=10.0$, 3.3 Hz, H-3″), 5.24 (1H, dd, $J=9.5$, 9.5 Hz, H-3′), 5.07 (1H, dd, $J=10.0$, 10.0 Hz, H-4″), 5.00 (1H, dd, $J=3.3$, 1.6 Hz, H-2″), 4.96 (1H, d, $J=1.6$ Hz, H-1″), 4.95 (1H, dd, $J=9.5$, 9.5 Hz, H-4′), 4.58 (1H, d, $J=7.8$ Hz, H-1′), 4.38 (1H, dq, $J=10.0$, 6.2 Hz, H-5″), 4.28 (1H, m, H-16), 4.27 (1H, dd, $J=12.2$, 4.8 Hz, H-6′a), 4.08 (1H, dd, $J=12.2$, 2.3 Hz, H-6′b), 3.71 (1H, dd, $J=9.5$, 7.8 Hz, H-2′), 3.68 (1H, ddd, $J=9.5$, 4.8, 2.3 Hz, H-5′), 3.61 (1H, m, H-3), 2.66 (1H, dd, $J=11.1$, 3.2 Hz, H-4a), 2.60 (1H, dd, $J=11.1$, 11.1 Hz, H-4b), 2.45 (1H, dd, $J=11.6$, 2.5 Hz, H-26a), 2.29 (1H, dd, $J=11.6$, 11.6 Hz, H-26b), 2.12, 2.06, 2.05, 2.02, 2.00, 1.99 (each 3H, s, Ac), 1.19 (3H, d, $J=6.2$ Hz, H-6″), 1.02 (3H, s, H-19), 0.95 (3H, d, $J=7.0$ Hz, H-21), 0.85 (3H, d, $J=6.3$ Hz, H-27), 0.81 (3H, s, H-18).

Acid Hydrolysis of 11 Hydrolysis of **11** (41.3 mg) was performed by refluxing in 1 N HCl (H_2O–dioxane) for 1 h in an N_2 atmosphere. The reaction mixture, after being cooled, was neutralized with 2 N NaOH and subjected to a silica gel column with a gradient mixture of $CHCl_3$–MeOH (19 : 1, 9 : 1, 4 : 1, 2 : 1) to yield solasodine (**11b**) (13.6 mg), L-rhamnose and D-glucose. Solasodine was identified by its physical and EI-MS, IR, ^{1}H-NMR and ^{13}C-NMR spectral data. Final purification of L-rhamnose and D-glucose was carried out by Sephadex LH-20 column chromatography with MeOH. L-Rhamnose: 6.6 mg, $[\alpha]_D^{28}$ $+9.1°$ ($c=0.33$, H_2O). TLC: *Rf* 0.65 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1). D-Glucose: 7.4 mg, $[\alpha]_D^{28}$ $+56.8°$ ($c=0.37$, H_2O). TLC: *Rf* 0.40 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1).

Solasodine 3-*O*-α-L-Rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (12) A white amorphous powder, 45.1 mg, $[\alpha]_D^{28}$ $-77.3°$ ($c=0.11$, MeOH). SI-MS *m*/*z*: 884 $[M+H]^+$, 722 $[M-\text{glucosyl}+2H]^+$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2920 (CH), 1445, 1375, 1360, 1335, 1260, 1160, 1135, 1055, 990, 910, 890, 875, 830, 810. ^{1}H-NMR (C_5D_5N) δ: 6.24 (1H, br s, H-1″), 5.29 (1H, br d, $J=4.5$ Hz, H-6), 5.12 (1H, d, $J=7.8$ Hz, H-1‴), 4.95 (1H, d, $J=6.7$ Hz, H-1′), 4.93 (1H, dq, $J=9.3$, 6.3 Hz, H-5″), 4.73 (1H, br s, H-2″), 4.57 (1H, dd, $J=9.3$, 3.3 Hz, H-3″), 4.55—4.39 (4H, H-16, -6′, -6‴a), 4.33 (2H, H-4″, -6‴b), 4.26 (1H, dd, $J=9.1$, 9.1 Hz, H-4‴), 4.24—4.17 (4H, H-2′, -3′, -4′, -3‴), 4.05 (1H, dd, $J=9.1$, 7.8 Hz, H-2‴), 3.97 (1H, m, H-5‴), 3.89 (1H, m, H-3), 3.86 (1H, m, H-5′), 1.76 (3H, d, $J=6.3$ Hz, H-6″), 1.08 (3H, d, $J=7.0$ Hz, H-21), 1.06 (3H, s, H-19), 0.87 (3H, s, H-18), 0.82 (3H, d, $J=5.3$ Hz, H-27).

Acid Hydrolysis of 12 Compound **12** (3.0 mg) was subjected to acid hydrolysis in the same way as **11**. Solasodine, rhamnose and glucose were detected in the reaction mixture by TLC. Solasodine: TLC: *Rf* 0.34 ($CHCl_3$–MeOH, 15 : 1); *Rf* 0.14 ($CHCl_3$–Me_2CO, 4 : 1).

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References

1) Jiang Su New Medical College ed., "Dictionary of Traditional Chinese Crude Drugs," Vol. 1, Shanghai Scientific Technologic Publishers, Shanghai, 1977, p. 856.
2) H. Shimomura, Y. Sashida, T. Takagishi and H. Terakado, *Shoyakugaku Zasshi*, **36**, 160 (1982).
3) M. Shimizu, "Nippon No Yuri," Seibundo-Shinkosya, Tokyo, 1976, p. 208.
4) H. Shimomura, Y. Sashida, Y. Mimaki and N. Iida, *Phytochemistry*, **27**, 451 (1988).
5) H. Shimomura, Y. Sashida and Y. Mimaki, *Shoyakugaku Zasshi*, **43**, 64 (1989).
6) H. Shimomura, Y. Sashida and Y. Mimaki, *Phytochemistry*, **25**, 2897 (1986).
7) *a*) H. Shimomura, Y. Sashida and Y. Mimaki, *Phytochemistry*, **26**, 844 (1987); *b*) R. H. Takata and P. J. Scheuer, *Lloydia*, **39**, 409 (1976).
8) Y. Mimaki and Y. Sashida, *Phytochemistry*, **29**, 2267 (1990).
9) *a*) H. Minato and A. Shimaoka, *Chem. Pharm. Bull.*, **11**, 876 (1963); *b*) G. Blunden and A. V. Patel, *Lloydia*, **49**, 687 (1986).
10) H. Budzikiewicz, C. Djerassi and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden Day, San Francisco, 1964.
11) H. Shimomura, Y. Sashida and Y. Mimaki, *Phytochemistry*, **28**, 3163 (1989).
12) S. B. Mahato, N. P. Sahu, A. N. Ganguly, R. Kasai and O. Tanaka, *Phytochemistry*, **19**, 2017 (1980).
13) L. H. Briggs, R. C. Cambie and D. M. Hyslop, *J. Chem. Soc.*, *Perkin Trans. 1*, **1975**, 2455.
14) I. W. Southon, J. Buckingham, "Dictionary of Alkaloids," Chapman and Hall Ltd., London, New York, 1989.
15) K. Kaneko, U. Nakaoka, M. Tanaka, N. Yoshida and H. Mitsuhashi, *Phytochemistry*, **20**, 157 (1981).