Phytochemical Studies of Seeds of Medicinal Plants. II.[1] A New Dihydroflavonol Glycoside and a New 3-Methyl-1-butanol Glycoside from Seeds of *Platycodon grandiflorum* A. De Candolle

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Two new glycosides, termed as flavoplatycoside (1) and grandoside (2), respectively, have been isolated from the seeds of *Platycodon grandiflorum* A. De Candolle (Campanulaceae) and their structures have been established as (2*R*,3*R*)-taxifolin 7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside and 3-methyl-1-butanol 1-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, respectively, based on chemical and spectral evidence. Four known flavonoids, (2*R*,3*R*)-taxifolin (3), quercetin 7-*O*-glucoside (4), luteolin-7-*O*-glucoside (5), and quercetin 7-*O*-rutinoside (6), were also isolated.

Keywords *Platycodon grandiflorum*; Campanulaceae; seed; dihydroflavonol glycoside; flavoplatycoside; 3-methyl-1-butanol glycoside; grandoside

Roots of *Platycodon grandiflorum* A. De Candolle (kikyo in Japanese) (Campanulaceae), a Chinese crude drug (Jieseng in Chinese; kikyo in Japanese), have been used in China as an expectorant.[2] A number of triterpenoid glycosides[3] have been identified from roots of *P. grandiflorum*, but not phytochemical study on seeds of this plant has appeared to date. This paper describes the structure elucidation of two new glycosides isolated from the seeds.

After chromatographic and high-performance liquid chromatographic (HPLC) separations of the *n*-BuOH soluble part of the MeOH extracts, two new glycosides, termed as flavoplatycoside (**1**) and grandoside (**2**), have been isolated together with four known flavonoids, *i.e.*, (2*R*,3*R*)-taxifolin (**3**),[4] quercetin-7-*O*-glucoside (**4**),[5] luteolin 7-*O*-glucoside (**5**),[6] and quercetin 7-*O*-rutinoside (**6**).[7] These known compounds were identified by direct comparison with the authentic specimens or by comparison of their physical data with those reported in the literatures (see Experimental).

Flavoplatycoside (**1**), mp 197—200 °C, $[\alpha]_D$ −85.0° (MeOH), showed absorption bands at 288 and 330 nm in the ultraviolet (UV) spectrum. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals due to five aromatic protons and two aliphatic protons [δ 4.57 and 5.06 (each 1H, d, J=11.2 Hz)] ascribable to the dihydroflavonol ring. The structure of the aglycone part of **1** was investigated by analysis of the ^{1}H- and carbon-13 nuclear magnetic resonance (^{13}C-NMR) (Table I) spectral data[4a] together with the circular dichroism (CD) behavior[4b] and the aglycone of **1** was concluded to be (2*R*,3*R*)-taxifolin (**3**). The structure of the sugar part was determined as follows. In comparison of the ^{13}C-NMR spectrum of **1** with that of **3**, a glycosylation shift was observed at C-7 and C-10 of the aglycone moiety of **1**, indicating that **1** was a 7-*O*-glycosylated compound of **3**. The negative ion fast atom bombardment mass spectrum (FAB-MS) of **1** gave a molecular ion $(M-H)^-$ at *m/z* 611 and two significant fragments at *m/z* 465 [(M−H)−146 (deoxyhexose unit)] and at *m/z* 303 [465—

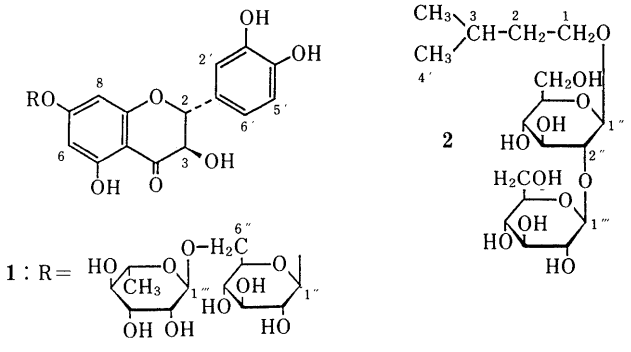

Chart 1

Table I. ^{13}C-NMR Spectral Data for **1**, **3**, and **6** (100.5 MHz, DMSO-d_6, δ_C, ppm from TMS)[a]

Carbon No.	1	3	6
Aglycone			
2-C	83.08 (d)	82.94 (d)	147.89 (s)[e]
3-C	71.57 (d)	71.45 (d)	135.96 (s)
4-C	198.41 (s)	197.62 (s)	175.96 (s)
5-C	162.80 (s)[b]	163.22 (s)	160.44 (s)
6-C	96.43 (d)	95.90 (d)	98.77 (d)
7-C	165.13 (s)	166.83 (s)	162.64 (s)
8-C	95.42 (d)	94.91 (d)	94.37 (d)
9-C	162.11 (s)[b]	162.45 (s)	155.69 (s)
10-C	101.97 (s)	100.32 (s)	104.72 (s)
1′-C	127.72 (s)	127.94 (s)	121.80 (s)
2′-C	115.32 (d)[c]	115.24 (d)[d]	115.66 (d)[f]
3′-C	144.85 (s)	144.82 (s)	144.99 (s)
4′-C	145.73 (s)	145.66 (s)	147.75 (s)[e]
5′-C	115.07 (d)[c]	115.01 (d)[d]	115.44 (d)[f]
6′-C	119.43 (d)	119.29 (d)	120.11 (d)
Glucose			
1″-C	99.38 (d)		100.06 (d)
2″-C	72.89 (d)		73.15 (d)
3″-C	76.18 (d)		76.34 (d)
4″-C	69.47 (d)		69.64 (d)
5″-C	75.41 (d)		75.64 (d)
6″-C	65.89 (t)		66.09 (t)
Rhamnose			
1‴-C	100.50 (d)		100.52 (d)
2‴-C	70.17 (d)		70.29 (d)
3‴-C	70.61 (d)		70.81 (d)
4‴-C	71.98 (d)		72.10 (d)
5‴-C	68.20 (d)		68.26 (d)
6‴-C	17.71 (q)		17.68 (q)

a) Assignments and multiplicities (in parentheses) were made with the aid of INEPT and ^{13}C–H COSY experiments. *b*—*f*) Assignments may be interchanged in each column.

TABLE II. ^{13}C-NMR Spectral Data for **2** (100.5 MHz, DMSO-d_6, δ_C, ppm, from TMS)[a]

Aglycone		Terminal glucose	
1-C	67.02 (t)	1‴-C	103.92 (d)
2-C	38.01 (t)	2‴-C	74.79 (d)
3-C	24.36 (d)	3‴-C	76.08 (d)
4-C	22.48 (q)	4‴-C	69.85 (d)
4'-C	22.48 (q)	5‴-C	76.90 (d)
Inner glucose		6‴-C	60.91 (t)
1″-C	101.29 (d)		
2″-C	82.07 (d)		
3″-C	75.97 (d)		
4″-C	69.85 (d)		
5″-C	76.52 (d)		
6″-C	60.91 (t)		

a) Assignments and multiplicities (in parentheses) were made with the aid of INEPT and ^{13}C–H COSY experiments.

162 (hexose unit)]. In addition, on methanolysis, **1** afforded methyl rhamnoside[10] and methyl glucoside[10] as the sugar moiety. The results indicate that **1** can be assigned as (2*R*,3*R*)-taxifolin 7-*O*-rhamnosylglucoside. Interglycosidic linkage in the disaccharide part of **1** was obtained as follows. In the ^{13}C-NMR spectrum (Table I), the glucosyl 6″-C of **1** resonated at 65.89 ppm was shifted downfield compared with those reported for a usual glucosyl residue.[11] Further, on the nuclear Overhauser effect correlation spectroscopy (NOESY) experiments, two significant NOE cross peaks between the anomeric H (1‴-H) of rhamnose and the 6″-H_2 of glucose and between the anomeric H (1″-H) of glucose and the protons at 6-C and 8-C of the aglycone were observed. The results revealed that the 6″-OH on the inner D-glucose was connected with the terminal L-rhamnose by an interglycosidic linkage. Finally, the anomeric configurations of each sugar unit in **1** were determined by the following ^{1}H- and ^{13}C-NMR spectra studies. The anomeric proton doublet with a large *J*-value of **1** (δ 4.97, J=7.3 Hz), due to the glucosyl part, proved the presence of β-D-glucopyranosyl moiety in **1**. While, in the ^{13}C-NMR spectrum of **1**, the anomeric carbon (1‴-C) with a large ^{13}C-H coupling constant (J_{C-H}=169 Hz) due to terminal rhamnose was indicative of the presence of α-L-rhamnopyranosyl moiety in **1**.[12] Based on these lines of accumulated evidence, the structure for flavoplatycoside (**1**) is defined as (2*R*,3*R*)-taxifolin 7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside. Grandoside (**2**), mp 181—183 °C, $[\alpha]_D$ −39.9° (pyridine) had the molecular formula $C_{17}H_{32}O_{11}$ based on the $(M-H)^-$ ion peak at *m/z* 411 in the negative ion FAB-MS. The ^{1}H-NMR of **2** showed signals due to the 3-methyl-1-butanol unit [δ 3.45 and 3.80 (each 1H, each m), 1.41 (2H, m), 1.68 (1H, m), and 0.86 (6H, d, J= 6.4 Hz)] and two anomeric protons (δ 4.26, d, J=7.6 Hz and 4.37, d, J=7.6 Hz). On methanolysis, **2** afforded methyl glucosides[10] as the sugar part. The results indicate that **2** can be assigned as 3-methyl-1-butanol glucosyl-glucoside. Interglycosidic linkage in the disaccharide part of **2** was clarified as follows. In the ^{13}C-NMR spectrum (Table II), 2″-C of inner glucose was shifted downfield, whereas the anomeric carbon (1″-C) and 3″-C of inner glucose were shifted upfield compared with those reported for a usual glucosyl residue.[11] Further, on the NOESY experiments of **2**, two significant NOE cross peaks between the anomeric H (1‴-H) of terminal glucose and the 2″-H of inner glucose, and between the anomeric H (1″-H) of inner glucose and the protons at 1-C of the 3-methyl-1-butanol unit were observed. The evidence indicates that the 2″-OH on the inner D-glucose was connected with the terminal D-glucose by an interglycosidic linkage. Finally, the anomeric protons with large *J*-values of **2** (each d, J=7.6 Hz), due to the disaccharide part, proved the presence of β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl moiety in **2**. Based on the accumulated evidence, the structure for grandoside (**2**) is defined as 3-methyl-1-butanol 1-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Experimental

The instruments used to obtain melting points, optical rotations, IR, ^{1}H-NMR (400 MHz), ^{13}C-NMR (100.5 MHz), mass spectrum, and gas liquid chromatography (GLC) data were the same as described in our previous paper.[1] The UV and CD spectrum were measured with a Shimadzu UV-3000 and a JASCO J-500 spectropolarimeter in MeOH, respectively. Melting points are uncorrected. Negative ion FAB-MS data were obtained under the following conditions; accelerating voltage, 2—3 kV; matrix, triethanolamine; collision gas, Xe. GLC were carried out under the following operating conditions: column, 1.5% SE-52 on Chromosorb WAW DMCS (2 m × 3 mm i.d.); hydrogen flame ionization (FID) detector; column temperature, 180 °C; carrier gas 35 ml/min. For column chromatography, Merck HF-254 was used and for thin layer chromatography, precoated silica gel plates (Merck HF-254) were used. Preparative HPLC was carried out on a Waters instrument with a M 6000A pump, a U6K septumless injector, a series R-401 differential refractometer and a reversed phase octadecyl silica (ODS) column (Tosoh, TSK-gel ODS-120T; 7.8 mm × 30 cm) with H_2O–MeOH or H_2O–CH_3CN as eluents.

Plant Material Seeds of *P. grandiflorum* were collected at the Medicinal Plant Garden of Setsunan University (Faculty of Pharmaceutical Sciences) in 1989.

Isolation of 1—6 The crushed seeds (674.2 g) were extracted successively with AcOEt (500 ml × 4) and MeOH (500 ml × 4). The residue (35 g) obtained from the MeOH extract was suspended in H_2O and the aqueous suspension was extracted with *n*-BuOH (500 ml × 4). The residue (18.5 g) obtained from the *n*-BuOH layer was subjected to silica gel column chromatography and the fractions containing **1**—**6** were further purified by silica gel column chromatography and reversed phase HPLC to afford **1** (19 mg), **2** (38 mg), **3** (123 mg), **4** (290 mg), **5** (15 mg), and **6** (103 mg). The physical and spectral properties for **1**—**6** are as follows. Flavoplatycoside (**1**) colorless fine crystals of mp 197—200 °C (aq. MeOH), $[\alpha]_D$ −85.0° (c=0.16, MeOH). UV λ_{max}^{MeOH} nm (log ε): 288 (3.48), 330 (sh, 0.26). +NaOMe: 255, 290, 360; +$AlCl_3$: 313, 390; +AcONa: 288, 330. IR ν (KBr) cm^{-1}: 3400, 2900, 1630, 1575, 1070. Negative ion FAB-MS *m/z* (%): 611 {$[M(C_{27}H_{32}O_{16})-H]^-$, 100}, 465 [$(M-H-146)^-$, 64], 303 [$(M-H-146-162)^-$, 81]. ^{1}H-NMR (DMSO-$d_6$) δ: 1.08 (3H, d, J=6.1 Hz, 6‴-H_3), 4.52 (1H, br s, 1″-H), 4.57 [1H, dd, J=11.2, 5.6 Hz, change to doublet (J=11.2 Hz) on D_2O addition, 3-H], 4.97 (1H, d, J=7.3 Hz, 1″-H), 5.06 (1H, d, J=11.2 Hz, 2-H), 6.08 (1H, d, J=1.5 Hz, 6-H), 6.17 (1H, d, J=1.5 Hz, 8-H), 6.76 (2H, br d, J=5.9 Hz, 5',6'-H_2), 6.90 (1H, br s, 2'-H). ^{13}C-NMR given in Table I. CD (c=0.00142) $[\theta]_{20}$ (nm): -1.39×10^4 (292)(negative max.), ±0 (320), $+1.40\times10^3$ (330)(positive max). Grandoside (**2**) colorless fine crystals of 181—183 °C (dec.)(MeOH–acetone), $[\alpha]_D$ −39.9° (c=0.30, pyridine). IR ν (KBr) cm^{-1}: 3350, 2900, 1070, 1030. Negative ion FAB-MS *m/z* (%): 411 {$[M(C_{17}H_{32}O_{11})-H]^-$, 100}. ^{1}H-NMR (DMSO-$d_6$) δ: 0.86 (6H, d, J=6.4 Hz, 4-H_3, 4'-H_3), 1.41 (2H, m, 2-H_2), 1.68 (1H, m, 3-H), 3.45, 3.80 (each 1H, m, 1-H_2), 4.26 (1H, d, J=7.6 Hz, 1″-H), 4.37 (1H, d, J=7.6 Hz, 1‴-H). ^{13}C-NMR given in Table II. (2*R*,3*R*)-taxifolin (**3**),[4] mp 221—223 °C (ref. 4*c*, 220—222 °C), $[\alpha]_D$ +35.6° (c=0.45, MeOH)[ref. 4*c*, +46.2° (MeOH)]. ^{13}C-NMR given in Table I. The melting point, optical rotation, CD, IR, EI-MS, ^{1}H- and ^{13}C-NMR data of **3** were consistent with the published data for (2*R*,3*R*)-taxifolin.[4] Compounds **4** (mp 248—250 °C) and **5** (mp 250—253 °C) were identified as quercetin 7-*O*-glucoside[5] and luteolin 7-*O*-glucoside,[6] respectively, by direct comparison with authentic samples. Quercetin 7-*O*-rutinoside (**6**),[7] yellow fine crystals of mp 218—222 °C (aq. MeOH)[ref. 9, 178—179 °C], $[\alpha]_D$ −48.5° (c=0.65, MeOH). IR ν (KBr) cm^{-1}: 3400, 1650, 1600,

1070. Negative ion FAB-MS *m/z* (%): 609 {$[M(C_{27}H_{30}O_{16})-H]^-$, 100}, 463 $[(M-H-146)^-$, 11], 301 $[(M-H-146-162)^-$, 91]. ^{1}H-NMR (DMSO-d_6) δ: 1.07 (3H, d, J=6.1 Hz, 6‴-H$_3$), 4.56 (1H, br s, 1‴-H), 5.08 (1H, d, J=7.3 Hz, 1″-H), 6.44 (1H, d, J=1.8 Hz, 6-H), 6.72 (1H, d, J=1.8 Hz, 8-H), 6.92 (1H, d, J=8.5 Hz, 5′-H), 7.55 (1H, dd, J=8.5, 1.8 Hz, 6′-H), 7.72 (1H, d, J=1.8 Hz, 2′-H). ^{13}C-NMR given in Table I.

Methanolysis of 1 A solution of **1** (3 mg) in 5% anhydrous HCl–MeOH (1.5 ml) was refluxed for 5 h. The reaction mixture was neutralized with Ag_2CO_3. The inorganic precipitate was filtered off and the filtrate was concentrated under reduced pressure to give the residue. The residue was trimethylsilylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide-pyridine, and subjected to GLC analysis to demonstrate the presence of methyl rhamnoside and methyl glucoside.

Methanolysis of 2 A solution of **2** (3 mg) in 5% anhydrous HCl–MeOH (1.5 ml) was refluxed for 5 h. The reaction mixture was worked up in the same manner as in the case of **1** to demonstrate the presence of methyl glucosides.

References and Notes

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