Phytochemical Studies of Seeds of Medicinal Plants. II.\textsuperscript{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 flavoplatyacide (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 (2R,3R)-taxifolin 7-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl-1-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl, quercetin-7-O-glucoside, rutin-7-O-glucoside, and quercetin-7-O-rutinoside (6), were also isolated.

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

Roots of Platycodon grandiflorum A. DE CANDOLLE (kiyoko in Japanese) of C. A. CANDOLLE (Campanulaceae), a Chinese crude drug (Jieseng in Chinese; kiyoko in Japanese), have been used in China as an expectorant.\textsuperscript{2)} A number of tri-terpenoid glycosides\textsuperscript{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 flavoplatyacide (1) and grandoside (2), have been isolated together with four known flavonoids, i.e., (2R,3R)-taxifolin (3),\textsuperscript{4)} quercetin-7-O-glucoside (4),\textsuperscript{5)} luteolin-7-O-glucoside (5),\textsuperscript{6)} and quercetin-7-O-rutinoside (6).\textsuperscript{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).

Flavoplatyacide (1), mp 197–200°C, [x]D = 85.0° (MeOH), showed absorption bands at 288 and 330 nm in the ultraviolet (UV) spectrum. The proton nuclear magnetic resonance (1H-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 1H- and carbon-13 nuclear magnetic resonance (13C-NMR) (Table I) spectral data\textsuperscript{8)} together with the circular dichroism (CD) behavior\textsuperscript{9)} and the aglycone of 1 was concluded to be (2R,3R)-taxifolin (3). The structure of the sugar part was determined as follows. In comparison of the 13C-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)\textsuperscript{−} at m/z 611 and two significant fragments at m/z 465 [(M−H−146 (deoxyhexose unit))] and at m/z 303 [465–]

<table>
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<th>Carbon No.</th>
<th>1</th>
<th>3</th>
<th>6</th>
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<td>Aglycone</td>
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<tr>
<td>2-C</td>
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<td>82.94 (d)</td>
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<td>5-C</td>
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<td>115.24 (d)\textsuperscript{b}</td>
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\textsuperscript{a} Assignments and multiplicities (in parentheses) were made with the aid of INEPT and 1H–13C-H COSY experiments. \textsuperscript{b–f} Assignments may be interchanged in each column.

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162 (hexose unit). In addition, on methanolsis, 1 afforded methyl rhamnside and methyl glucoside as the sugar moiety. The results indicate that 1 can be assigned as (2R,3R)-taxifolin 7-O-rhamnosylglucoside. Interglycosidic linkage in the disaccharide part of 1 was obtained as follows. In the 13C-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. Further, on the nuclear Overhauser effect correlation spectroscopy (NOESY) experiments, two significant NOE cross peaks between the anomic H (1′′-H) of rhamnose and the 6′-H of glucose and between the anomic 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 1H- and 13C-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 13C-NMR spectrum of 1, the anemic carbon (1′′-C) with a large 13C-H coupling constant (Jc−H = 169 Hz) due to terminal rhamnose was indicative of the presence of α-L-rhamnopyranosyl moiety in 1. Based on these lines of accumulated evidence, the structure for flavoplyactoside (1) is defined as (2R,3R)-taxifolin 7-O-α-L-rhamnopyranosyl(1→6)-β-d-glucopyranoside. Grandisose (2), mp 181-183°C, [α]D−39.9° (pyridine) had the molecular formula C12H22O11, based on the (M−H)− ion peak at m/z 411 in the negative ion FAB-MS. The 1H-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 anemic protons (δ 4.26, d, J = 7.6 Hz and 4.37, d, J = 7.6 Hz). On methanolsis, 2 afforded methyl glucoside and α-D-glucoside 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 13C-NMR spectrum (Table II), 2′′-C of inner glucose was shifted downfield, whereas the anemic carbon (1′′-C) and 3′′-C of inner glucose were shifted upfield compared with those reported for a usual glucosyl residue. Further, on the NOE experiments of 2, two significant NOE cross peaks between the anemic H (1′′-H) of terminal glucose and the 2′′-H of inner glucose, and between the anemic H (1′′-H) of 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 grandisose (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, 1H-NMR (400 MHz), 13C-NMR (100.5 MHz), mass spectrum, and gas liquid chromatography (GLC) data were the same as described in our previous paper. 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 keV; matrix, triethanolamine; collision gas, Xe. GLC were carried out under the following conditions: column, 5% DEGS on Chromosorb WAW DMCS (2 x 0.5 mm i.d.); hydrogen flame ionization (FID) detector; column temperature, 180°C; carrier gas nitrogen 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 x 30 cm) with H2O-MeOH or H2O-CH3CN 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 x 4) and MeOH (500 ml x 4). The residue (35 g) obtained from the MeOH extract was suspended in H2O and the aqueous suspension was extracted with n-BuOH (500 ml x 4). The residue (3 g) obtained from the n-BuOH extract 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: flavoplyactoside (1) colorless fine crystals of mp 197–200°C (MeOH), [α]D−85.0° (c = 0.16, MeOH). UV (λmax nm (log e) 280 (3.48), 330 (sh, 0.26). + NaOAc: 255, 290, 300, 360; + AICl3: 313, 390; + AcONa: 288, 330. IR (KBr) cm−1: 3400, 2900, 1630, 1575, 1070. Negative ion FAB-MS m/z (%): 611 [M(C12H22O11H2O)]−·H2O, 465 [M−H−146]−·H2O, 403 [M−H−146−2H2O]−·H2O, 81. 1H-NMR (DMSO-d6) δ: 0.86 (6H, d, J = 6.4 Hz, 2-H), 1.41 (2H, m, 2-H), 1.68 (1H, m, 3-H), 3.45, 3.80 (each 1H, m, 1-H), 4.26 (2H, d, J = 7.6 Hz, 1′′-H), 4.37 (1H, d, J = 7.6 Hz, 1′′-H). 13C-NMR given in Table I. CD (c = 0.0042) δ (mm): 1.39 x 103 (292% negative max.), ≥ 0.320, 1.40 x 103 (330% positive max.). Grandisose (2) colorless fine crystals of 181–183°C (dec.)(MeOH-acetone, [α]D−39.9° = 0.30° (c = 0.20, pyridine). IR (KBr) cm−1: 3350, 2900, 1070, 1030. Negative ion FAB-MS m/z (%): 411 [M(C12H22O11H2O)]−·H2O, 100. 1H-NMR (DMSO-d6) δ: 0.86 (6H, d, J = 6.4 Hz, 4-H, 4′-H), 1.41 (2H, m, 2-H), 1.68 (1H, m, 3-H), 3.45, 3.80 (each 1H, m, 1-H), 4.26 (2H, d, J = 7.6 Hz, 1′′-H), 4.37 (1H, d, J = 7.6 Hz, 1′′-H). 13C-NMR given in Table II. CD (c = 0.0042) δ (mm): 221–223°C (ref. 4c, 220–222°C, [α]D−35.6° (c = 0.45, MeOH)(ref. 4c, +46.2° (MeOH)). 13C-NMR given in Table I. The melting point, optical rotation, CD, IR, EI-MS, 1H- and 13C-NMR data of 3 were consistent with the published data for (2R,3R)-taxifolin. Compounds 4 (mp 248–250°C) and 5 (mp 250–253°C) were identified as quercetin 7-O-glucoside and quercetin 7-O-rutinoside, respectively, by direct comparison with authentic samples. Quercetin 7-O-rutinoside yellow fine crystals of mp 218–222°C (aq. MeOH)(ref. 9, 178–179°C), [α]D−48.5° (c = 0.65, MeOH). IR (KBr) cm−1: 3400, 1650, 1600.
107. Negative ion FAB-MS m/z (%): 609 [(M+C3H5O4H)−], 101 [(M−H−146)−], 361 [(M−H−262)−], 91. 1H-NMR (DMSO-d6) δ: 1.07 (3H, d, J=6.1 Hz, 6−H3), 4.36 (1H, brs, 1′′′-H), 5.08 (1H, d, J=7.3 Hz, 1′′′-H), 6.64 (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). 13C-NMR given in Table I.

**Methodology 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 Ag2CO3. 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 rhannoside and methyl glucoside.

**Methodology 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**


7) This compound has been isolated from *Baptisia perforilata* (Leguminosae) and from *Capparis spinosa* (Capparaceae). Thus, this is the third example of the natural occurrence of 6. Further, the 13C-NMR data (Table I) and optical rotation value of 6 are reported here for the first time.


10) With respect to the configurations of rhannose and glucose in 1 and 2, the L and D forms may be preferable from the viewpoint of natural occurrence of these sugars.
