# Lathyrus Saponin, a New Trisaccharide Glycoside from *Lathyrus japonicus*

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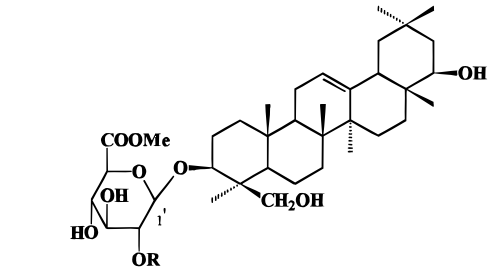
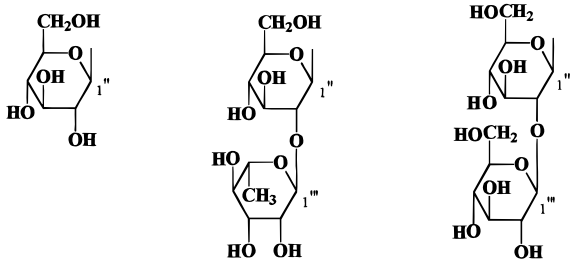
A new triterpenoid saponin, named Lathyrus saponin (**3**), was isolated from the whole plant of *Lathyrus japonicus* Willd. together with two known saponins, azukisaponins II (**1**) and V (**2**), as their methyl esters. The structure of **3** was determined to be soyasapogenol B 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucuronopyranoside on the basis of physicochemical and spectroscopic methods.

The stems and leaves of *Lathyrus japonicus* Willd. (Leguminosae = *L. maritimus* Bigel.) have been used as a folk medicine to promote digestion.[1] Although several nonprotein amino acids such as *cis*-5-hydroxy-L-pipecolic acid and *γ*-L-glutamyl-L-lathyrine have been isolated from *L. japonicus*,[2−4] no further chemical work has been reported on this plant. In a search for novel bioactive natural products from medicinal plants,[5] we have studied the extract obtained from the whole plant of *L. japonicus*. The present paper reports the isolation of flavonoids and saponins and the structure determination of a new saponin from the whole plant of *L. japonicus*.

The dried whole plant of *L. japonicus* was extracted with 80% MeOH. The residue left after evaporation of MeOH was successively fractionated with hexane, $CHCl_3$ and *n*-BuOH. The *n*-BuOH fraction was subjected to Sephadex LH-20 column chromatography to give subfractions rich in flavonoids and saponins. The subfraction rich in flavonoids was further fractionated by a combination of $SiO_2$, Sephadex LH-20, and Toyopearl HW 40F column chromatography. This fractionation resulted in the isolation of four flavonoids identified as astragalin, isoquercitrin, kaempferol 3-*O*-rutinoside, and rutin by spectral data and direct comparison with authentic samples.[6] The subfraction rich in saponins was methylated with $CH_2N_2$ and subjected to $SiO_2$ column chromatography followed by LiChroprep RP-18 purification to give **1**−**3**. The two compounds **1** and **2** were identified as known azukisaponins II and V, respectively, by spectral data and direct comparison with authentic samples.[7] Compound **3** was obtained as an amorphous powder, mp 273−279 °C. Acid hydrolysis of **3** afforded soyasapogenol B[8] as the aglycon and D-glucuronic acid and D-glucose as the sugar components.[9] The positive FAB mass spectrum of **3** showed a cationized molecular ion at *m*/*z* 995 $[M + Na]^+$ and a protonated molecular ion at *m*/*z* 973 $[M + H]^+$ as a base peak together with a fragment ion at *m*/*z* 649 $[M + H - 2 \text{ hexose}]^+$. The $^{13}C$ and DEPT NMR spectra of **3** showed 49 signals, of which 19 were assigned to the saccharide portion and 30 to the soyasapogenol B moiety. The $^1H$ NMR spectrum showed seven singlets assignable to tertiary methyl groups in the range of *δ* 0.73−1.37. It exhibited three anomeric proton signals at *δ* 5.05 (1H, d, *J* = 7.7 Hz), 5.22 (1H, d, *J* = 7.6 Hz), and 5.58 (1H, d, *J* = 7.8 Hz). The $^{13}C$ signals of **3** were superimposable onto those of azukisaponin V (**2**), except for the signals due to the sugar moiety. In the $^{13}C$ NMR spectrum the presence of three anomeric signals at *δ* 103.5, 104.7, and 106.8 confirmed the three sugar residues. Assignments of the sugar proton resonances were achieved by a $^1H-^1H$ COSY spectrum, and the $^{13}C$ data were assigned by a HMQC spectrum. Interglycosidic linkages were established by HMBC techniques. The HMBC spectrum of **3** showed cross peaks between the signals at *δ* 5.05 (glucuronic acid H-1) and 90.7 (C-3 of the aglycon), 5.58 (inner glucose H-1) and 82.2 (glucuronic acid C-2), and 5.22 (terminal glucose H-1) and 85.9 (inner glucose C-2). The anomeric configurations of the glucuronic acid and glucose were determined as *β* based on the large coupling constants ($J_{1,2}$ = 7.6−7.8 Hz) in the $^1H$ NMR spectrum. Furthermore, partial acid hydrolysis of **3** gave soyasapogenol B 3-*O*-*β*-D-glucuronopyranoside methyl ester, azukisaponin II methyl ester (**1**), and methyl glucopyranoside. Thus, the structure of saponin **3** was determined to be soyasapo-




1 Azukisaponin II 2 Azukisaponin V 3 Lathyrus saponin

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genol B 3-*O*-$\beta$-D-glucopyranosyl(1→2)-$\beta$-D-glucopyranosyl(1→2)-6′-*O*-methyl-$\beta$-D-glucuronopyranoside.

## Experimental Section

**General Experimental Procedures.** Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. The optical rotations were determined on a Rudolph Autopol III automatic polarimeter. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. The FAB mass spectrum was obtained in a 3-nitrobenzyl alcohol matrix in a positive-ion mode on a VG-VSEQ spectrometer. The NMR spectra were measured in pyridine-$d_5$ on a Bruker AMX-500 instrument, and the chemical shifts were referenced to TMS. GC analysis was performed with a Hewlett-Packard 5890 Series II gas chromatograph equipped with an $H_2$ flame ionization detector. The column was an HP-5 capillary column (30 m × 0.32 mm × 0.25 $\mu$m). Conditions: column temperature, 200 °C for TMS ethers of thiazolidine derivatives and 150 °C for TMS ethers of methyl glycopyranosides; injector and detector temperature, 290 °C; He flow rate, 30 mL/min. TLC was performed on silica gel $60F_{254}$ (Merck) and cellulose plates (Art No. 5716, Merck).

**Plant Material.** The whole plant of *L. japonicus* was collected in May 1996, Dukjeok island in Kyungki Province, Korea, and authenticated by one of us (K.B.). A voucher specimen (CNU 1146) was deposited in the Department of Pharmacognosy, Chungnam National University.

**Extraction and Isolation.** Fresh whole plant (5 kg) of *L. japonicus* was dried, and the dry plant material (1.3 kg) was extracted three times with 80% MeOH at room temperature. The MeOH extract was evaporated to dryness, and the dry residue was partitioned in succession between $H_2O$ and *n*-hexane, $CHCl_3$, and then *n*-BuOH, affording 23.6, 11.4, and 85 g of the respective extracts. A portion of the BuOH fraction (37 g) was passed through a Sephadex LH-20 column eluting with 80% MeOH to give subfractions rich in flavonoids (14.2 g) and saponins (21 g). The subfraction rich in flavonoids was subjected to $SiO_2$ column chromatography. Elution with EtOAc saturated with $H_2O$ with increasing amounts of MeOH and then MeOH gave astragalin (7 mg) and an MeOH-soluble portion. The MeOH-soluble portion was purified by a column with Sephadex LH-20 eluted with 50% MeOH to afford isoquercitrin (15 mg) and a mixture of compounds, which was further purified with Toyopearl HW 40F eluted with $H_2O$, 30% EtOH, and then MeOH to yield kaempferol 3-*O*-rutinoside (5 mg) and rutin (6 mg). The subfraction rich in saponins was methylated with $CH_2N_2$ to yield methylated saponin fraction. A portion of the methylated saponin fraction (5.7 g) was subjected column chromatography on $SiO_2$ eluted with EtOAc saturated with $H_2O$ with increasing amounts of MeOH to give compound **1** (7 mg) and a mixture of compounds **2** and **3** (100 mg). The mixture of **2** and **3** was repeatedly chromatographed on LiChroprep RP-18 with 80% MeOH as eluent to yield saponin **2** (50 mg) and then **3** (30 mg). The known compounds were identified by TLC and $^1$H and $^{13}$C NMR data as well as by direct comparison with authentic samples.[6,7]

Lathyrus saponin methyl ester (**3**) was obtained as an amorphous white powder: mp 273−279 °C; $[\alpha]^{20}_D$ +24.6° (*c* 0.53, MeOH); IR (KBr) $\nu_{max}$ 3414, 2949, 1744, 1638, 1074 cm$^{-1}$; $^1$H NMR (pyridine-$d_5$, 500 MHz) $\delta$ 0.73, 0.95, 0.98, 1.21, 1.24, 1.28, 1.37 (21H, all s, 7 × Me), 2.39 (1H, brd, $J$ = 12.8 Hz, H-18), 3.37 (1H, t-like, $J$ = 10.5 Hz, H-24), 3.43 (1H, dd, $J$ = 4.4, 7.7 Hz, H-3), 3.65 (1H, brdt, $J$ = 9.5 Hz, H-5‴), 3.74 (3H, s, OMe), 3.74 (1H, m, H-22), 3.97 (1H, m, H-5″), 4.57 (1H, d, $J$ = 10 Hz, H-5′), 4.67 (1H, t, $J$ = 9.5 Hz, H-3′), 5.05 (1H, d, $J$ = 7.7 Hz, H-1′), 5.22 (1H, d, $J$ = 7.6 Hz, H-1‴), 5.30 (1H, brs, H-12), 5.58 (1H, $J$ = 7.8 Hz, H-1″); $^{13}$C NMR (pyridine-$d_5$, 125.8 MHz) $\delta$ 90.7 (C-3), 122.4 (C-12), 144.9 (C-13), 75.6 (C-22), 63.4 (C-24), 104.7 (C-1′), 82.2 (C-2′), 77.2 (C-3′), 72.1 (C-4′), 77.2 (C-5′), 170.3 (C-6′), 52.2 (OMe), 103.5 (C-1″), 85.9 (C-2″), 77.6 (C-3″), 71.5 (C-4″), 79.5 (C-5″), 62.8 (C-6″), 106.8 (C-1‴), 77.0 (C-2‴), 77.6 (C-3‴), 69.4 (C-4‴), 78.1 (C-5‴), 61.2 (C-6‴); positive FAB MS, see text.

**Acid Hydrolysis of 3.** Saponin **3** (8 mg) was refluxed with 5% HCl in 60% aqueous dioxane (10 mL) for 2 h. The resulting solution was evaporated under reduced pressure, and the hydrolysate was extracted with ether. The ether extract was evaporated to yield soyasapogenol B, which was identified by direct comparison with an authentic sample.[8] The $H_2O$ layer was neutralized with $Ag_2CO_3$ and filtered, and the filtrate was concentrated under reduced pressure. The residue was compared with standard sugars by cellulose TLC [pyridine−EtOAc−HOAc−$H_2O$ (36:36:7:21)], which indicated the sugars to be glucuronic acid and glucose.

**Partial Acid Hydrolysis of Saponin 3.** Saponin **3** (7 mg) was refluxed with HCl (one drop) in MeOH (3.6 mL) for 10 min, and on usual workup two prosapogenins (soyasapogenol B 3-*O*-$\beta$-D-glucuronopyranoside methyl ester and **1**) were identified by TLC [$CHCl_3$−MeOH (6:1)] with authentic samples.[7] To identify sugar components, the reaction mixture was evaporated under a $N_2$ stream and dried in vacuo. The residue was dissolved in pyridine (0.05 mL), and then the solution was trimethylsilylated with TMS−HT (0.1 mL) at 60 °C for 30 min. After the addition of *n*-hexane and water, the *n*-hexane layer was removed and analyzed by GC. The retention times ($t_R$) of the peaks were 26.7 and 29.4 min for methyl glucopyranoside.

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