Three New Isoflavonoid Glycosides from *Lupinus luteus* and *L. polyphyllus* × *arboreus*[1)]

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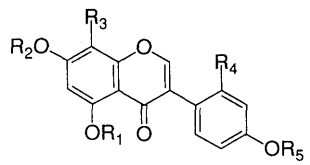
As part of our studies on leguminous plants, we examined the ingredients of *Lupinus luteus* L. and *L. polyphyllus* × *arboreus* hybrid, and three new isoflavonoid glycosides together with six known ones were isolated. They were determined to be 8-*C*-glucopyranosylgenistein 4′-*O*-glucopyranoside (1), 5-*O*-methylgenistein 4′, 7-di-*O*-glucopyranoside (2), 2′-hydroxygenistein 4′, 7-di-*O*-glucopyranoside (3) by spectroscopic and chemical methods. It was also shown that the isoflavonoid distributions in the two species were differed.

Keywords *Lupinus luteus*; *Lupinus polyphyllus* × *arboreus*; isoflavonoid glycoside; 8-*C*-glucosylgenistein 4′-*O*-glucoside; 5-*O*-methylgenistein 4′, 7-di-*O*-glucoside; 2′-hydroxygenistein 4′, 7-di-*O*-glucoside

A couple of hundred species belong to the genus *Lupinus* (Leguminosae). Among them, yellow lupine (*L. luteus*), blue lupine (*L. hirsutus*), Washington lupine (*L. polyphyllus*) and Russell lupine are cultivated worldwide. The Russell lupine is the hybrid of *L. polyphyllus* and *L. arboreus*, and is a historically improved species in horticulture.[2)]

This genus is known to contain numerous flavonoids as well as lupin alkaloids. Studies on the chemical constituents of the genus have been carried out by many researchers, and various non-polar isoflavonoids have been reported.[3)] During our studies on leguminous plants,[1)] we have also isolated many isoflavonoids including three new glycosides (**1**—**3**) from the roots of *L. luteus* and *L. polyphyllus* × *arboreus*. This paper deals with the structural elucidation and identification of these compounds, and some chemotaxonomical comparisons between the two species.

A methanolic extract of fresh roots of *L. luteus* was partitioned between 1-BuOH and H_2O. A combination of various chromatographies of the aqueous phase resulted in the isolation of compounds **1**, **2**, **4**, **5** and **6**. Similarly, the methanolic extract of the fresh roots of *L. polyphyllus* × *arboreus* gave six compounds, **3** and **5**—**9**. Compounds **4**—**9** were identified as 8-*C*-glucopyranosylgenistein (**4**),[4)] genistin (**5**),[5)] genistein 4′, 7-di-*O*-glucopyranoside (**6**),[4)] genistein 4′-*O*-glucopyranoside (**7**),[4)] 2′-hydroxygenistein (**8**),[6)] 2′-hydroxygenistein 7-*O*-glucopyranoside (**9**).[6)] This is the first report of the isolation of compounds **6**, **7** and **9** from this genus.



	R_1	R_2	R_3	R_4	R_5
1	H	H	glc	H	glc
2	Me	glc	H	H	glc
3	H	glc	H	OH	glc
4	H	H	glc	H	H
5	H	glc	H	H	H
6	H	glc	H	H	glc
7	H	H	H	H	glc
8	H	H	H	OH	H
9	H	glc	H	OH	H

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D$ −21.6° dimethyl sulfoxide (DMSO). The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed a characteristic signal at δ 8.38 ascribable to the H-2 signal of the isoflavone. Signals due to an aromatic A_2X_2 system (2H, d, J=9 Hz, δ 7.08; 2H, d, J=9 Hz, δ 7.49) and two anomeric protons (1H, d, J=10 Hz, δ 4.65; 1H, d, J=7 Hz, δ 4.90) were also observed. Methanolysis of **1** in 1 N HCl/MeOH gave 8-*C*-glucopyranosylgenistein (**4**). The negative fast atom bombardment-mass spectrum (FAB-MS) of **1** suggested that an additional hexose was attached to **4**. In the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I), the signals except for B ring were in good agreement with those of **4**, in contrast to the signals ascribable to B ring which were similar to those of **6**. Therefore, **1** was determined to be 8-*C* glucopyranosylgenistein 4′-*O*-glucopyranoside.

Compound **2**, $[\alpha]_D$ −63.5° (DMSO) showed a $[M-H]^-$ peak at m/z 607 which was larger by 14 mass units than that of **6**, indicating the addition of a methyl group to **6**. Since no phenolic proton signal was observed in the ^{1}H-NMR spectrum of **2**, the methyl group seemed to substitute for a phenol proton. In the ^{13}C-NMR spectrum (Table I), the signals due to B-ring except for C-1′ were in accord with those of **6**, while the C-4 signal showed an upfield shift (−6.7 ppm). Moreover, the methyl group showed a relationship with the H-6 signal only in the differential nuclear Overhauser effect (NOE) experiment. Consequently, the structure of **2** was elucidated to be 5-*O*-methylgenistein 4′, 7-di-*O*-glucopyranoside.

Compound **3**, $[\alpha]_D$ −19.7° (DMSO), showed an $[M-H]^-$ peak at m/z 609 in the negative FAB-MS, suggesting that it has one more hexosyl moiety than **9**. In contrast, the aglycone was identified as **8** upon acid hydrolysis. In the ^{13}C-NMR spectrum of **3** (Table I), the signals were in good agreement with those of **8**, except for a downfield shift at C-1′ (+2.7 ppm) together with the appearance of additional glucopyranosyl residues. Because one of the hydroxy signals (δ 9.57 ppm) showed NOE to H-3′ signal only, the structure of **4** was determined to be 2′-hydroxygenistein 4′,7-di-*O*-glucopyranoside.

In the meantime, obtained isoflavonoids from the two species displayed an obvious difference (Table II): *C*-

TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**—**9** (δ ppm, in DMSO-d_6)

Carbon	1	2	3	4	5	6	7	8	9
C-2	154.0	151.2	155.9	153.7	154.5	154.9	154.3	155.2	155.9
3	121.4	125.4	120.8	122.0	122.5	123.9	124.1	120.3	120.8
4	180.1	173.6	180.4	180.4	180.4	180.3	179.9	180.3	180.7
5	160.8	160.7	161.4	161.1	161.5	161.5	161.9	161.8	161.5
6	99.3	97.1	99.4	99.0	99.5	100.2	99.0	98.8	99.5
7	163.1	161.3	162.8	163.1	162.9	163.0	164.3	164.1	162.9
8	104.2	95.6	94.5	104.1	94.4	94.5	93.6	93.5	94.5
8a	157.1	158.7	157.1	157.2	157.1	157.1	157.2	157.8	157.3
4a	104.2	109.4	106.0	104.1	106.0	106.0	104.3	104.3	106.1
1′	124.1	124.4	111.2	121.2	120.9	122.1	121.8	108.5	108.4
2′	129.9	130.0	156.3	130.2	130.1	130.0	129.9	156.3	156.4
3′	115.9	115.7	103.8	115.0	115.0	116.0	115.9	102.5	102.6
4′	156.4	156.9	158.4	157.2	157.4	157.2	157.5	158.5	158.7
5′	115.9	115.7	106.5	115.0	115.0	116.0	115.9	106.1	106.3
6′	129.9	130.0	132.0	130.2	130.1	130.0	129.9	132.1	132.2
OMe		56.0							
glc C-1	73.1	100.2[a]	100.2[a]	73.1	99.8	99.7[a]	100.2		99.9
2	70.5	73.2[b]	73.1[b]	70.6	73.0	73.1[b]	73.1		73.1
3	78.7	77.2[c]	77.1[c]	78.6	77.1	77.0[c]	77.0		77.2
4	69.6	69.7[d]	69.5[d]	70.5	69.5	69.6[d]	69.6		69.6
5	81.5	76.5[e]	76.5[e]	81.6	76.3	76.5[e]	76.5		76.4
6	61.3	60.5[f]	60.5[f]	61.3	60.5	60.5[f]	60.6		60.7
glc′ C-1	100.2	99.8[a]	99.8[a]			99.6[a]			
2	73.1	73.0[b]	73.0[b]			73.0[b]			
3	76.5	77.0[c]	76.9[c]			76.9[c]			
4	69.6	69.6[d]	69.5[d]			69.5[d]			
5	76.9	76.4[e]	76.2[e]			76.3[e]			
6	60.5	60.5[f]	60.5[f]			60.5[f]			

a—*f*) In each vertical column may be interchangeable.

TABLE II. Distribution of Isoflavonoids between the Roots of *L. luteus* and Those of *L. polyphyllus* × *arboreus*

	L. luteus	*L. polyphyllus* × *arboreus*
1	+	−
2	+	−
3	−	+
4	+	−
5	+	+
6	+	+
7	−	+
8	−	+
9	−	+

glycosides were found only in *L. luteus*, whereas 2′-hydroxy derivatives were only in *L. polyphyllus* × *arboreus*. These findings appear to be significant from the standpoint of chemotaxonomy within the respective species.

Experimental

The seeds were purchased from Sakata No Tane Co., Ltd. The instruments and reagents used in this study were the same as described in an earlier paper.[7]

Extraction and Isolation Fresh roots (367 g) of *L. luteus* cultivated in the medicinal garden of our department were extracted with MeOH twice under reflux. The combined extract (40 g) was concentrated and partitioned with 1-BuOH and H_2O. The aqueous portion (24 g) was subjected to MCI gel CHP 20P column chromatography using 0%→100% MeOH to give fractions 1 to 4. These fractions were further separated by silica gel ($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2→7 : 3 : 0.5) to provide compounds **1** (0.041%), **2** (0.027%), **4** (0.065%), **5** (0.093%) and **6** (0.19%). Fresh roots (4.56 kg) of *L. polyphyllus* × *arboreus* were cultivated, collected and extracted in the same way as above. The extract (257 g) was partitioned with EtOAc and 80% MeOH. The 80% MeOH portion (208 g) was subjected to Bondapak C_{18} column chromatography using 0%→100% MeOH to give fractions 1 to 4. Fractions 1—3 were further separated by MCI gel CHP 20P (0→100% MeOH), Sephadex LH-20 (0→100% MeOH) and silica gel ($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2→7 : 3 : 0.5) to provide compounds **3** (0.0016%), **5** (0.0053%), **6** (0.0023%), **7** (0.0072%), **8** (0.0030%) and **9** (0.015%).

Compound **1**: A white amorphous powder, $[\alpha]_D^{25}$ −21.6° (c = 0.49, DMSO). UV λ_{max}^{MeOH} nm (log ε): 262 (4.32). Negative FAB-MS m/z: 593 $[M-H]^-$. ^{1}H-NMR (in DMSO-d_6) δ: 8.38 (1H, s, H-2), 6.21 (1H, s, H-6), 7.49 (2H, d, J = 9 Hz, H-2′, 6′), 7.08 (2H, d, J = 9 Hz, H-3′, 5′), 4.90 (1H, d, J = 7 Hz, 4′-*O*-glc H-1), 4.65 (1H, d, J = 10 Hz, 8-*C*-glc H-1). ^{13}C-NMR: Table I.

Solvolysis of 1 A solution of **1** in 1 N HCl/MeOH was heated under reflux for 30 min and the reaction mixture was neutralized by 3% KOH/MeOH. After filtration and concentration *in vacuo*, the concentrated filtrate was identified as including **4** (Rf 0.30) by TLC ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5).

Compound **2**: A white amorphous powder, $[\alpha]_D^{25}$ −63.5° (c = 0.34, DMSO). UV λ_{max}^{MeOH} nm (log ε): 255 (4.41). Negative FAB-MS m/z: 607 $[M-H]^-$. ^{1}H-NMR (in DMSO-d_6) δ: 8.22 (1H, s, H-2), 7.41 (2H, d, J = 9 Hz, H-2′, 6′), 7.06 (2H, d, J = 9 Hz, H-3′, 5′), 6.74 (1H, d, J = 2 Hz, H-8), 6.61 (1H, d, J = 2 Hz, H-6), 5.08 (1H, d, J = 7 Hz, 7-*O*-glc H-1), 4.90 (1H, d, J = 7 Hz, 4′-*O*-glc H-1), 3.83 (3H, s, 5-OMe). ^{13}C-NMR: Table I.

Compound **3**: A white amorphous powder, $[\alpha]_D^{25}$ −19.7° (c = 0.60, DMSO). UV λ_{max}^{MeOH} nm (log ε): 261 (4.18). Negative FAB-MS m/z: 610 $[M-H]^-$. ^{1}H-NMR (in DMSO-d_6) δ: 8.29 (1H, s, H-2), 7.11 (1H, d, J = 9 Hz, H-6′), 6.72 (1H, d, J = 2 Hz, H-8), 6.59 (1H, d, J = 2 Hz, H-3′), 6.56 (1H, dd, J = 9, 2 Hz, H-5′), 6.48 (1H, d, J = 2 Hz, H-6), 5.06 (1H, d, J = 7 Hz, 7-*O*-glc H-1), 4.82 (1H, d, J = 7 Hz, 4′-*O*-glc H-1), 12.94 (1H, s, 5-OH). ^{13}C-NMR: Table I.

Solvolysis of 3 A solution of **1** in 1 N HCl/MeOH was heated under reflux for 30 min and the reaction mixture was worked up as described for **1**. The filtrate was shown to contain **8** (Rf 0.60) by TLC ($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2).

Compound **4**: A white amorphous powder, $[\alpha]_D^{25}$ +4.7° (c = 0.52, DMSO). UV λ_{max}^{MeOH} nm (log ε): 264 (4.47). Negative FAB-MS: m/z 431 $[M-H]^-$. ^{1}H-NMR (in DMSO-d_6) δ: 8.40 (1H, s, H-2), 6.31 (1H, s, H-6), 7.39 (2H, d, J = 9 Hz, H-2′, 6′), 6.83 (2H, d, J = 9 Hz, H-3′, 5′), 4.68 (1H, d, J = 10 Hz, 8-*C*-glc H-1), 13.20 (1H, s, 5-OH). ^{13}C-NMR: Table I.

Compound **5**: A white amorphous powder, $[\alpha]_D^{20}$ −40.2° (c = 0.50,

DMSO). UV λ_{max}^{MeOH} nm (log ε): 261 (4.63). Negative FAB-MS m/z: 431 $[M-H]^-$. ^{1}H-NMR (in DMSO-d_6) δ: 8.42 (1H, s, H-2), 6.48 (1H, d, $J=2$ Hz, H-6), 6.72 (1H, d, $J=2$ Hz, H-8), 7.41 (2H, d, $J=9$ Hz, H-2′, 6′), 6.84 (2H, d, $J=9$ Hz, H-3′, 5′), 5.07 (1H, d, $J=7$ Hz, 7-*O*-glc H-1), 9.64 (1H, s, 4′-OH), 12.94 (1H, s, 5-OH). ^{13}C-NMR: Table I.

Compound **6**: A white amorphous powder, $[\alpha]_D^{20}$ $-45.5°$ ($c=0.53$, DMSO). UV λ_{max}^{MeOH} nm (log ε): 260 (4.67). Negative FAB-MS m/z: 593 $[M-H]^-$. ^{1}H-NMR (in DMSO-d_6) δ: 8.49 (1H, s, H-2), 6.49 (1H, d, $J=2$ Hz, H-6), 6.74 (1H, d, $J=2$ Hz, H-8), 7.53 (2H, d, $J=9$ Hz, H-2′, 6′), 7.12 (2H, d, $J=9$ Hz, H-3′, 5′), 5.08 (1H, d, $J=7$ Hz, 7-*O*-glc H-1), 4.93 (1H, d, $J=7$ Hz, 4′-*O*-glc H-1), 12.89 (1H, s, 5-OH). ^{13}C-NMR: Table I.

Compound **7**: A white amorphous powder, $[\alpha]_D^{20}$ $-19.7°$ ($c=0.59$, DMSO). UV λ_{max}^{MeOH} nm (log ε): 272 (4.17). Negative FAB-MS m/z: 431 $[M-H]^-$. ^{1}H-NMR (in DMSO-d_6) δ: 8.70 (1H, s, H-2), 6.24 (1H, d, $J=2$ Hz, H-6), 6.40 (1H, d, $J=2$ Hz, H-8), 7.50 (2H, d, $J=9$ Hz, H-2′, 6′), 7.10 (2H, d, $J=9$ Hz, H-3′, 5′), 4.93 (1H, d, $J=7$ Hz, 4′-*O*-glc H-1), 12.90 (1H, s, 5-OH). ^{13}C-NMR: Table I.

Compound **8**: A white amorphous powder, UV λ_{max}^{MeOH} nm (log ε): 258 (4.16). Negative FAB-MS m/z: 285 $[M-H]^-$. ^{1}H-NMR (in DMSO-d_6) δ: 8.15 (1H, s, H-2), 6.22 (1H, d, $J=2$ Hz, H-6), 6.36 (1H, d, $J=2$ Hz, H-8), 6.97 (1H, d, $J=8$ Hz, H-6′), 6.56 (1H, dd, $J=8$, 2 Hz, H-5′), 6.38 (1H, d, $J=2$ Hz, H-3′), 12.98 (1H, s, 5-OH). ^{13}C-NMR: Table I.

Compound **9**: A white amorphous powder, $[\alpha]_D^{25}$ $-34.4°$ ($c=0.57$, DMSO). UV λ_{max}^{MeOH} nm (log ε): 264 (4.19). Positive FAB-MS m/z: 449 $[M+H]^+$. ^{1}H-NMR (in DMSO-d_6) δ: 8.24 (1H, s, H-2), 6.99 (1H, d, $J=9$ Hz, H-6′), 6.71 (1H, d, $J=2$ Hz, H-8), 6.38 (1H, d, $J=2$ Hz, H-3′), 6.28 (1H, dd, $J=9$, 2 Hz, H-5′), 6.47 (1H, d, $J=2$ Hz, H-6), 5.06 (1H, d, $J=7$ Hz, 7-*O*-glc H-1), 12.97 (1H, s, 5-OH). ^{13}C-NMR: Table I.

Solvolysis of 9 A solution of **9** in 1 N HCl/MeOH was worked up as described for **1**. The filtrate was shown to contain **8** (*Rf* 0.60) by TLC ($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2).

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References and Notes

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