using 20% solvent B ($MeOH-HOAc-H_2O$, 18 : 1 : 1) in solvent A ($HOAc-H_2O$, 1 : 19) and increasing the proportion of B by 2% per min., with a flow rate of 1 ml/min and a constant temp. of 25°. $R_t$s for *Zea mays* pigments and authentic markers in parentheses: Cy 3-glucoside 10.09 (10.05), Cy 3-malonylglucoside 14.41 (14.39) and Cy 3-dimalonylglucoside 15.69 min. (15.70 min.). For details of other techniques used, see ref. [9].

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# ACYLATED LUTEOLIN GLUCOSIDES FROM *SALIX GILGIANA*

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**Key Word Index**—*Salix gilgiana*; Salicaceae; luteolin 7-(6″-*trans*-cinnamoyl)glucoside; luteolin 7-(6″-feruloyl)glucoside; luteolin 7-(6″-acetyl)glucoside; luteolin 7-(6″-*p*-coumaroyl)glucoside; apigenin 7-glucoside.

**Abstract**—Besides apigenin and luteolin 7-glucoside, four novel luteolin glucosides acylated with acetic, *trans*-cinnamic, *p*-coumaric and ferulic acids, respectively were isolated from the leaves of *Salix gilgiana*. The position of the acyl groups was determined to be at C-6″ by the $^{13}C$ NMR spectral data.

## INTRODUCTION

About 40 species of *Salix* are native to Japan. They are all dioecious and produce hybrids easily by cross pollination. Many are deciduous and have no leaves in the flowering period, which sometimes makes differentiation difficult. In continuation of a chemotaxonomic study of the genus, the leaf flavonoids of *S. gilgiana*, which is natival to Japan, Korea and N.W. China [1], were investigated. Seven flavonoids including three new acylated flavone glucosides were isolated and characterised.

## RESULTS AND DISCUSSION

From the ethyl acetate soluble portion of a methanolic extract of the leaves of *S. gilgiana*, seven compounds **1**–**7** were isolated, which all gave a positive Shinoda and Molish–Udransky reactions except for **1**. The compounds are numbered in their order of polarity on thin layer chromatography.

Compounds **1**, mp 350–352°, **2**, mp 265–266°, and **3**, mp 239–240° were identified as luteolin, luteolin 7-glucoside [2] and apigenin 7-glucoside, respectively, from their spectroscopic properties.

In the EIMS spectrum of **4**, a fragment at $m/z$ 286 suggested the presence of four hydroxy groups in the aglycone. Further fragments at $m/z$ 153, 152 and 134 indicated that two hydroxy groups were attached to both the A and B rings. The UV spectrum in MeOH showed an absorption at 269 (band II) and 350 nm (band I). A bathochromic shift with increasing intensity of band I with sodium methoxide indicated a hydroxy group at C-4′. Furthermore, a shift with sodium acetate/boric acid (24 nm) showed an *ortho*-diphenol system in the B ring. A bathochromic shift of band I (38 nm) with aluminium trichloride/hydrochloric acid indicated a hydroxy group at C-5. The absence of a sodium acetate shift indicated that the 7-hydroxyl was not free. In the $^{13}C$ NMR spectrum, the chemical shifts of the sugar moiety showed the presence of $\beta$-D-glucopyranose. Compound **4** was, therefore, considered to be a derivative of luteolin 7-glucoside **2**. In the $^1H$ NMR spectrum (Table 1), in addition to the protons of **2**, a double doublet at 7.32 and two doublets at 7.31 and

Table 1. $^1$H NMR spectral data for compounds **3**–**6**

| | **3** | **4** | **5** | **6** |
|---|---|---|---|---|
| 3 | 6.71 *s* | 6.68 *s* | 6.75 *s* | 6.71 *s* |
| 6 | 6.49 *d* (2.0) | 6.48 *d* (1.7) | 6.44 *d* (2.1) | 6.47 *d* (2.1) |
| 8 | 6.78 *d* (2.0) | 6.74 *d* (1.7) | 6.77 *d* (2.1) | 6.76 *d* (2.1) |
| 2′ | 7.42 *d* (2.0) | 7.39 *d* (1.7) | 7.42 *d* (2.1) | 7.42 *d* (1.5) |
| 5′ | 6.90 *d* (8.1) | 6.87 *d* (8.1) | 6.91 *d* (8.6) | 6.89 *d* (8.1) |
| 6′ | 7.43 *dd* (2.0, 8.1) | 7.40 *dd* (1.7, 8.1) | 7.44 *dd* (2.1, 8.6) | 7.44 *dd* (1.5, 8.1) |
| 2‴ | 7.54 *d* (7.3) | 7.18 *d* (1.7) | | 7.37 *d* (8.6) |
| 3‴ | 7.35 *br t* (7.2, 7.3) | | | 6.67 *d* (8.6) |
| 4‴ | 7.28 *br d* (7.2) | | | |
| 5‴ | 7.35 *br t* (7.2, 7.3) | 6.68 *d* (8.1) | | 6.67 *d* (8.6) |
| 6‴ | 7.54 *d* (7.3) | 6.94 *dd* (1.7, 8.1) | | 7.37 *d* (8.6) |
| α | 6.58 *d* (15.8) | 6.41 *d* (15.8) | | 6.33 *d* (15.8) |
| β | 7.59 *d* (15.8) | 7.49 *d* (15.8) | | 7.48 *d* (15.8) |
| $CH_3O$ | | 3.76 *s* | | |
| $CH_3CO$ | | | 2.01 *s* | |

All spectra were taken in DMSO-$d_6$ on a JEOL-GX 270 instrument. Chemical shifts are shown in δ values, and coupling constants (*J* in parentheses) are given in Hz.

7.34 ppm, which corresponded to a mono-substituted benzene, and two doublets at 6.53 and 7.59 ppm (each 1H), in a large *J* value (15.8 Hz), which is attributable to *trans*-α,β-unsaturated ester, were observed. These signals indicated a *trans*-cinnamoyl group attached to **2**. The IR spectrum (1690 cm$^{-1}$) and the EIMS (*m/z* 149, 148, 132 and 131) also supported the presence of a cinnamoyl group. Finally it was considered to be a *trans*-cinnamoyl group esterified with a hydroxy group of the glucose moiety. Table 2 shows the $^{13}$C NMR spectral data. The position of the cinnamoyl group on the glucose molecule was determined by the differences in chemical shifts between **2** and **4**. The glucose carbons adjacent to the cinnamoyl group showed downfield shift due to the acylation (C-6″ + 3.1 ppm, C-5″ − 3.3 ppm, C-4″ + 0.5 ppm) [3]. Consequently, it was concluded that **4** was luteolin 7-*O*-(6″-*trans*-cinnamoyl)-β-D-glucopyranoside.

The EIMS, $^1$H NMR and UV spectra of **5** showed that the aglycone was luteolin, and the $^{13}$C NMR indicated the sugar was β-D-glucopyranose. In the EIMS spectrum, the fragments of 194 and 177 suggested the presence of a feruloyl group. In the $^1$H NMR spectrum, a singlet (3H) at 3.76 ppm corresponded to a methoxy and the three signals at 6.68, 6.94 and 7.18 ppm also showed feruloyl protons. The position of the ester at C-6″ was decided in the same way as above. On the basis of these data, **5** was determined as luteolin 7-*O*-(6″-feruloyl)-β-D-glucopyranoside.

From its EIMS, $^1$H NMR, $^{13}$C NMR and UV spectra, **6** was also considered to be a luteolin derivative. A singlet at 2.0 ppm (3H) in the $^1$H NMR spectrum and two signals at 20.5 and 170.1 ppm in the $^{13}$C NMR suggested the presence of an acetyl group, which was confirmed by the fragment of *m/z* 43. The site of the acetyl group was deduced by the chemical shifts of the glucose carbons. From these data, **6** was identified as luteolin 7-*O*-(6″-acetyl)-β-D-glucopyranoside.

From the spectral data compound **7** was also considered to be a luteolin derivative. The IR spectrum (1690 cm$^{-1}$), the $^1$H NMR data (two doublets at 6.67 and 7.37 ppm) and the $^{13}$C NMR data (130 and 115.6 ppm) were attributed to the ester moiety. In the EIMS the fragments *m/z* 164 and 147 implied the presence of a *p*-coumaroyl group. After consideration of the $^{13}$C NMR spectrum (Table 2) **7** was concluded to be luteolin 7-*O*-(6″-*p*-coumaroyl)-β-D-glucopyranoside. This glycoside was previously isolated from the leaves of *Catalpa bignonioides* and named bignonoside [4], but the present study has added detailed spectral data to confirm its identity.

Alkaline hydrolyses of **4**–**7** gave *trans*-cinnamic acid, ferulic acid, acetic acid and *p*-coumaric acid, respectively, identified by direct comparison with authentic markers.

Recently, a number of acylated flavonoid glycosides have been isolated; for example, luteolin 7-glucuronide-3′-feruloylglucoside and luteolin 7,4′-diglucuronide-3′-feruloylglucoside from *Riccia fluitans* [5], apigenin 7-(4″-*p*-coumaroyl)glucoside from *Salix alba* [6] and isorhamnetin 3-(6″-acetyl)glucoside from *S. viminalis* [7]. In our present study of *Salix gilgiana*, four novel acylated flavone glucosides were identified and may prove useful markers in the chemotaxonomy of the genus *Salix*. An investigation of the flavonoid glycosides of other *Salix* species is already in progress.

## EXPERIMENTAL

Mps are uncorr. MS were measured at 70 eV and the $^1$H and $^{13}$C NMR spectra in DMSO-$d_6$ on JEOL JNM-FX100, JNM-GX270 and Varian XL-300 spectrometers.

*Plant material.* The leaves of *Salix gilgiana* Seemen were collected in September, 1985 at Kawauchi, Sendai-city, Miyagi-prefecture, Japan, and a voucher specimen deposited in the Herbarium of Gifu Pharmaceutical University, Gifu-city, Japan.

*Extraction and isolation.* The dried leaves of *S. gilgiana* (1.6 kg) were extracted with twice MeOH (15 l) at 80° for 30 hr, and the combined extracts were concd. The residue was suspended in $H_2O$, and extracted successively with EtOAc and *n*-BuOH. The EtOAc fraction (100 g) was chromatographed on silica gel with $CHCl_3$, EtOAc and MeOH. The EtOAc eluent gave a mixture of compounds **1**–**7**. The eluent was rechromatographed on silica gel using $CHCl_3$–MeOH–$H_2O$. Recryst. gave **1** (50 mg), **2** (5 g), **3** (100 mg), **4** (1.8 g), **5** (80 mg), **6** (90 mg), and **7** (320 mg), respectively.

*Luteolin 7-O-(6″-trans-cinnamoyl)-β-D-glucopyranoside* (**4**).

Table 2. $^{13}$C NMR spectral data for compounds 2–6

| | 2* | 3† | 4‡ | 5‡ | 6‡ |
|---|---|---|---|---|---|
| 2 | 164.4 | 164.4 | 164.4 | 164.4 | 164.3 |
| 3 | 103.1 | 103.1 | 102.9 | 103.1 | 102.9 |
| 4 | 182.9 | 181.8 | 181.8 | 181.8 | 181.7 |
| 5 | 161.1 | 161.2 | 161.1 | 161.0 | 161.1 |
| 6 | 99.4 | 99.5 | 99.4 | 99.4 | 99.4 |
| 7 | 162.8 | 162.7 | 162.6 | 162.6 | 162.6 |
| 8 | 94.7 | 95.4 | 94.6 | 94.6 | 94.5 |
| 9 | 156.8 | 156.9 | 156.7 | 156.8 | 156.7 |
| 10 | 105.2 | 105.4 | 105.2 | 105.3 | 105.2 |
| 1′ | 121.2 | 121.3 | 121.1 | 121.1 | 121.2 |
| 2′ | 113.4 | 113.5 | 113.4 | 113.4 | 113.4 |
| 3′ | 145.7 | 145.8 | 145.6 | 145.7 | 145.7 |
| 4′ | 148.8 | 149.9 | 149.8 | 149.8 | 149.8 |
| 5′ | 115.9 | 117.6 | 115.8 | 115.8 | 115.9 |
| 6′ | 119.1 | 119.1 | 119.0 | 119.0 | 119.0 |
| 1″ | 99.6 | 99.5 | 99.5 | 99.3 | 99.4 |
| 2″ | 73.0 | 73.0 | 72.9 | 72.9 | 72.9 |
| 3″ | 76.3 | 76.2 | 76.1 | 76.1 | 76.1 |
| 4″ | 69.5 | 70.0 | 69.7 | 69.7 | 69.9 |
| 5″ | 77.1 | 73.8 | 73.7 | 73.7 | 73.7 |
| 6″ | 60.6 | 63.7 | 63.3 | 63.2 | 63.4 |
| 1‴ | | 133.8 | 125.3 | | 124.8 |
| 2‴ | | 121.1 | 110.9 | | 130.0 |
| 3‴ | | 128.1 | 149.2 | | 115.6 |
| 4‴ | | 130.4 | 147.7 | | 159.7 |
| 5‴ | | 128.8 | 115.3 | | 115.6 |
| 6‴ | | 128.1 | 122.9 | | 130.0 |
| $\underline{\mathrm{C}}\mathrm{H{=}CH{-}CO}$ | | 144.7 | 145.1 | | 144.8 |
| $\mathrm{CH{=}}\underline{\mathrm{C}}\mathrm{H{-}CO}$ | | 116.0 | 114.0 | | 113.6 |
| $\mathrm{CH{=}CH{-}}\underline{\mathrm{C}}\mathrm{O}$ | | 166.0 | 166.4 | | 166.4 |
| $\mathrm{CH_3O}$ | | | 55.4 | | |
| $\underline{\mathrm{C}}\mathrm{H_3CO}$ | | | | 20.5 | |
| $\mathrm{CH_3}\underline{\mathrm{C}}\mathrm{O}$ | | | | 170.1 | |

Spectra were taken in DMSO-$d_6$ on
*JEOL JNM FX 100,
†JEOL JNM GX 270, and
‡Varian XL-300 spectrometers. Chemical shifts are shown in $\delta$ values.

Yellow needles (MeOH), mp 225°. EIMS (*m/z*): 286, 153, 152, 149, 148, 130. IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 1690 (CH=CH-$\underline{\mathrm{CO}}$), 1650, 1610. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 258, 269, 280 sh, 350; + NaOMe: 269, 400; + AlCl$_3$: 275, 423; + AlCl$_3$/HCl: 276, 366, 388; + NaOAc: 260 (sh), 267, 350; + NaOAc/H$_3$BO$_3$: 265, 374. Alkaline hydrolysis with 2N NaOH at room temp. for 4 hr, followed by acidification and extraction with EtOAc gave luteolin and *trans*-cinnamic acid identified on TLC using CHCl$_3$–MeOH, 3:1.

*Luteolin* 7-O-(6″-*feruloyl*)-*β*-D-*glucopyranoside* (5). A pale yellow powder (MeOH), mp 201–203°. EIMS (*m/z*): 286, 194, 177, 153, 152. IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 1690, 1650, 1610. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 252, 269, 300 (sh), 337; + NaOMe: 268, 390; + AlCl$_3$: 275, 300 (sh), 325, 360 (sh), 423; + AlCl$_3$/HCl: 277, 298 sh, 325, 358; + NaOAc: 265 (sh), 296 (sh), 335, 408; + NaOAc/H$_3$BO$_3$: 258, 296 (sh), 335, 376.

*Luteolin* 7-O-(6″-*acetyl*)-*β*-D-*glucopyranoside* (6). Yellow needles (MeOH), mp 224–227°. EIMS (*m/z*): 286, 153, 152, 60, 43. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 257, 266 (sh), 350; + NaOMe: 275, 410; + AlCl$_3$: 274, 300 (sh), 320 (sh), 370 (sh), 426; + AlCl$_3$/HCl: 263 (sh), 273 (sh), 295 (sh), 317 (sh), 364; + NaOAc: 262, 364 (sh), 410; + NaOAc/H$_3$BO$_3$: 260, 374.

*Luteolin* 7-O-(6″-*p*-*coumaroyl*)-*β*-D-*glucopyranoside* (7). A colourless powder (MeOH), mp 273–275°. EIMS (*m/z*): 286, 164, 153, 152, 147. IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 1690, 1650, 1610. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 258 (sh), 270, 302 (sh), 320; + NaOMe: 269, 300 (sh), 310, 370; + AlCl$_3$: 276, 298 (sh), 315 (sh), 370 (sh), 428; + AlCl$_3$/HCl: 278, 298, 316 (sh), 364; + NaOAc: 268, 300 (sh), 318, 410; + NaOAC/H$_3$BO$_3$: 265, 300(sh), 315, 378.

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