Phenolic Compound from the Roots of *Cirsium japonicum* DC.

YUKINORI MIYAICHI, MIKIKO MATSUURA and TSUYOSHI TOMIMORI*

Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa 920-11, Japan

(Received July 18, 1994)

From the roots of *Cirsium japonicum* DC., a new flavone glycoside (1) was isolated, together with linarin, syringin, sinapylaldehyde 4-O-β-D-glucopyranoside, ferulylaldehyde 4-O-β-D-glucopyranoside, chlorogenic acid (5-O-cafeoylquinic acid), 1,5-di-O-cafeoylquinic acid, tachioside and uridine. The structure of 1 was shown to be 5,7,4'-trihydroxy-6-methoxyflavone 7-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside on the basis of the chemical and spectral data.

**Keywords**—*Cirsium japonicum*; Compositae; phenolic compound; flavonoid; structure elucidation

*Phenolic Compound from the Roots of *Cirsium japonicum* DC.*  

*Phenolic Compound from the Roots of *Cirsium japonicum* DC.* (Japanese name: Noazami) is a perennial herb of the family Compositae, whose dried root is one of the botanical origins of the crude drug, “Wazokudan” (和統断) in Japan and has been used for the treatment of neuralgia and rheumatism. From the root, two polyolefines and ten polyacetylenes have been isolated.

In the present paper, we describe the isolation and identification of a new flavone glycoside (1) and eight known compounds (2-8) from an n-BuOH-soluble fraction of the root.

Compound 1 was obtained as yellow needles, mp 190-192°C (dec.), and was positive to the Mg-HCl test. The infrared (IR) spectrum gave absorption bands corresponding to hydroxyl and conjugated carbonyl groups and aromatic rings. The ultraviolet (UV) spectrum of 1 showed a bathochromic shift by addition of AlCl3-HCl indicating the presence of a free hydroxyl at the C-5 position.

The proton nuclear magnetic resonance (1H-NMR) spectrum of 1 showed the presence of one methoxyl (δ 3.77), one phenolic hydroxyl (δ 10.40), one chelated hydroxyl (δ 12.98), one C-3 proton (δ 6.86) and sugar protons including two anomeric protons (δ 1.10, 3H, d, J = 5.8 Hz, δ 3.16-3.76, 10H; δ 5.27, 1H, brs, δ 5.34, 1H, d, J = 7.7 Hz). In the aromatic region of the spectrum, the remaining five protons appeared as a singlet (δ 7.03, 1H) and a pair of doublets (δ 6.94 and δ 7.95, each 2H, d, J = 8.8 Hz).

On acid hydrolysis, 1 gave 5,7,4'-trihydroxy-6-methoxyflavone (hispidulin) and a sugar portion, which was shown to consist of D-glucose and L-rhamnose by thin layer chromatography (TLC). 1 was partially hydrolyzed with acid to give homoplantaginin (hispidulin 7-O-β-D-glucopyranoside, 1a).

In the 1H- and 13C nuclear magnetic resonance (13C-NMR) spectra of 1, a nuclear Overhauser effect (NOE) was observed at the C-2 proton (δ 3.61, t, J = 8.0 Hz) of glucosyl on irradiating the C-1 proton of rhamnose. Besides, the C-2 carbon of glucosyl was shifted downfield by 4.4 ppm compared with that of 1a (Table I). Thus, rhamnose is attached to C-2 of the inner glucosyl moiety. The α-configuration of rhamnosyl linkage was indicated by comparison of the 13C-NMR spectrum of 1 with that of methyl-a-L-rhamnopyranoside.

From these results, the structure of 1 was determined to be 5,7,4'-trihydroxy-6-methoxyflavone 7-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (hispidulin 7-O-neohesperidoside).

As regards the absolute configurations (D/L) of the sugars, Mihashi et al. have recently reported that the pairs of nine aldose enantiomers were separated by gas-liquid chromatography (GLC) as the trimethylsilyl ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(R)-carboxylate. In the present work, we established a more simple analytical method for the determination of D/L of glucose and rhamnose by TLC instead of GLC. The standard samples of thiazolidine derivatives of D- and L-glucoses and D- and L-rhamnoses, i.e. methyl 2-(D-glucopentahydroxypentyl)-thiazolidine-4(R)-carboxylate, methyl 2-(L-glucopentahydroxy­pentyl)-thiazolidine-4(R)-carboxylate, methyl 2-(D-rhamnootetrahydroxypentyl)-thiazolidine-4(R)-carboxylate, methyl 2-(L-rhamnootetrahydroxypentyl)-thiazolidine-4(R)-carboxylate were prepared by the method given in the literature.

As described in the experimental part, 10 and 13 each showed two clearly separated spots on TLC, Rf: 0.49 and 0.41; 0.65 and 0.58, respectively, due to the C-2 epimers of thiazolidine derivatives. On the other hand, 11 and 12 gave single spots of Rf: 0.45 and 0.62, respectively. This method was used to determine the absolute configurations of component sugars of 1. The hydrolysate of 1 was treated as in the case of the standard sugar samples and

---

Natural Medicines 49(1), 92-94 (1995)
Table 1. $^{13}$C Chemical Shifts of Compounds 1 and 1a

<table>
<thead>
<tr>
<th>C</th>
<th>1</th>
<th>1a</th>
<th>1&quot;</th>
<th>1</th>
<th>1a</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>164.3</td>
<td>164.3</td>
<td>1&quot;</td>
<td>97.9</td>
<td>100.2</td>
</tr>
<tr>
<td>3</td>
<td>102.7</td>
<td>102.7</td>
<td>2&quot;</td>
<td>77.6</td>
<td>73.2</td>
</tr>
<tr>
<td>4</td>
<td>182.3</td>
<td>182.3</td>
<td>3&quot;</td>
<td>75.8</td>
<td>76.7</td>
</tr>
<tr>
<td>5</td>
<td>152.5</td>
<td>152.4</td>
<td>4&quot;</td>
<td>69.7</td>
<td>69.6</td>
</tr>
<tr>
<td>6</td>
<td>132.7</td>
<td>132.5</td>
<td>5&quot;</td>
<td>77.2</td>
<td>77.3</td>
</tr>
<tr>
<td>7</td>
<td>155.8</td>
<td>156.5</td>
<td>6&quot;</td>
<td>60.5</td>
<td>60.6</td>
</tr>
<tr>
<td>8</td>
<td>94.4</td>
<td>94.3</td>
<td>1&quot;&quot;</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>152.0</td>
<td>152.1</td>
<td>2&quot;&quot;</td>
<td>70.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>105.7</td>
<td>105.7</td>
<td>3&quot;&quot;</td>
<td>70.3</td>
<td></td>
</tr>
<tr>
<td>1&quot;</td>
<td>121.1</td>
<td>121.1</td>
<td>4&quot;&quot;</td>
<td>71.9</td>
<td></td>
</tr>
<tr>
<td>2&quot;</td>
<td>128.6</td>
<td>128.6</td>
<td>5&quot;&quot;</td>
<td>68.5</td>
<td></td>
</tr>
<tr>
<td>3&quot;</td>
<td>116.0</td>
<td>116.0</td>
<td>6&quot;&quot;</td>
<td>18.1</td>
<td></td>
</tr>
<tr>
<td>4&quot;</td>
<td>161.4</td>
<td>161.4</td>
<td>OMe</td>
<td>60.3</td>
<td>60.3</td>
</tr>
</tbody>
</table>

examined by TLC. By the comparison of the RF values with those of the standard samples, the sugars were determined to be D-glucose and L-rhamnose.

Compounds 2, 3, 6, and 9 were identified as linarin (2),$^{9}$ syringin (3),$^{9,11,12}$ chlorogenic acid (5-O-cafeoylquinic acid, 6),$^{9,13}$ and uridine (9),$^{14}$ respectively, by direct comparisons with authentic specimens. Compounds 4, 5, 7, and 8 were identified as sinapylaldehyde 4-O-β-D-glucopyranoside (4),$^{11,13}$ ferulaldehyde 4-O-β-D-glucopyranoside (5),$^{11,13}$ 1,5-di-O-cafeoylquinic acid (7),$^{13}$ and tachioside (8),$^{12,14}$ by the comparison of respective spectral and physical data with those described in the literature.

**EXPERIMENTAL**

**General procedures** All melting points were determined on a Yanagimoto micromelting point apparatus and are recorded uncorrected. UV spectra were taken in MeOH on a Shimadzu dual-wavelength/ doublebeam recording spectrophotometer UV-3000. IR spectra were taken in KBr disk on a Hitachi 270-30 infrared spectrophotometer. NMR spectra were taken in DMSO-d$_6$ on a JEOL JNM-DX-300 mass spectrometer, and optical rotations on a JASCO DIP-370 digital polarimeter. TLC was carried out on Kieselgel 60F-254 layer was concentrated and the residue (25.6 g) was chromatographed on ODS by using H$_2$O-MeOH (10 : 1 → 1 : 0) as a eluent to give four fractions, fr. 1-4, in the order of elution. Fraction 1 was rechromatographed on silica gel [CHCl$_3$-MeOH-H$_2$O (8 : 1 → 2 : 1 → 0.1)] to give 9 and 8. Fraction 2 gave 6. Fraction 3 was subjected to rechromatography on silica gel [CHCl$_3$-MeOH-H$_2$O (10 : 1 → 2 : 1 → 0.1)] to give 4, 5, and 3. Fraction 4 was subjected to rechromatography on silica gel [AcOEt-acetone-H$_2$O (1 : 0 → 0 → 4 : 1)] to give two fractions, fr. 5 and 6, in the order of elution. Fraction 5 gave 7. Fraction 6, containing a mixture of two flavonoids, was passed through a silica gel column [CHCl$_3$-MeOH-H$_2$O (10 : 1 → 2 : 1 → 0.1)] to give 2 and 1. Yields: 1 (34 mg), 2 (65 mg), 3 (1.3 g), 4 (52 mg), 5 (16 mg), 6 (70 mg), 7 (82 mg), 8 (13 mg), 9 (12 mg).

1 [5, 7, 4'-Trihydroxy-6-methoxy flavone 7-O-α-D- rhamnopyranosyl-(1→2)-β-D-glucopyranoside (hispidulin 7-O-neohesperidoside)] Yellow needles (AcOEt-acetone, mp 190–192°C (dec.), [α]$_D$ = -82.5° (c = 0.11, MeOH). IR (KBr): 3392 cm$^{-1}$ (OH), 1662 (conjugated CO), 1606 (arom. C=C). UV $\lambda_{max}$ nm (log $\epsilon$): 273 (4.38), 332 (4.51); $\lambda_{max}$ MeOH nm (log $\epsilon$): 273 (3.41), 304 (4.12), 358 (4.47), 383 (4.53); $\lambda_{max}$ MeOH-AcOH nm (log $\epsilon$): 280 (4.34), 300 (4.35), 359 (4.54); $\lambda_{max}$ AcOEt-AcOH nm (log $\epsilon$): 284 (4.36), 298 (4.39), 350 (4.52); $\lambda_{max}$ MeOH-NaOAc nm (log $\epsilon$): 270 (4.33), 388 (4.51); $\lambda_{max}$ MeOH-NaOAc(H$_2$O) nm (log $\epsilon$): 272 (4.38), 333 (4.50). HydNMR: 1.10 (3H, d, $J = 5.8$ Hz, 6"-H$_3$), 3.18 (1H, t, $J = 9.5$ Hz, 4"'-H), 3.22 (1H, t, $J = 8.8$ Hz, 4'-H), 3.36 (1H, m, 5'-H), 3.40 (1H, dd, $J = 5.5, 12.0$ Hz, 6'-H), 3.47 (1H, dd, $J = 3.3, 9.5$ Hz, 3'-H), 3.51 (1H, brt, $J = 8.2$ Hz, 3"'-H), 3.61 (1H, t, $J = 7.8$ Hz, 2"'-H), 3.68-3.76 (3H, m, 6", 2", 5"'-H), 3.77 (3H, s, OMe), 5.27 (1H, brs, 1"'-H), 5.34 (1H, d, $J = 7.7$ Hz, 1"-H), 6.86 (1H, s, H), 6.94 (2H, d, $J = 8.8$ Hz, 3', 5'-H), 7.03 (1H, s, H), 7.95 (2H, d, $J = 8.8$ Hz, 2', 6'-H), 10.40 (1H, brs, 4'-OH), 12.98 (1H, s, 5-OH). 13C-NMR: TABLE I.

**Hydrolysis of 1** 1 (10 mg) in 2 N H$_2$SO$_4$ (2 ml) was heated under reflux for 4 h. The precipitate deposited during the reaction was filtered off, washed with water, dried and recrystallized from MeOH to obtain yellow needles (3.4 mg), mp 291°C. This product was identified as hispidulin$^{b}$ by direct comparisons (TLC, UV, IR, 1H- and 13C-NMR). The filtrate was neutralized with Ba(OH)$_2$. After centrifugation of the precipitate, the supernatant was washed with n-BuOH and the water layer was concentrated in vacuo to give a sugar fraction, which was shown to contain glucose ($R_f$: 0.06, TLC-1) and rhamnose ($R_f$: 0.17, TLC-1).

**Determination of the absolute configurations of the sugars of 1** Pyridine solution (100 μl) of the above sugar fraction and L-cysteine methyl ester hydrochloride (3.0 mg) were mixed and warmed at 60°C for 1 h in the same way as described in the literature.$^{21}$ After removal of the solvent, the residue was dissolved in water.
(40 µl) and extracted with n-BuOH (100 µl). The organic layer was washed with water (20 µl) and concentrated in vacuo to give a syrup, which was shown to contain methyl 2-(D-glucopentahydroxypentyl)-thiazolidine-4(R)-carboxylate and methyl 2-(L-rhamno-tetrahydroxypentyl)-thiazolidine-4(R)-carboxylate by TLC-2 [methyl 2-(D-glucopentahydroxypentyl)-thiazolidine-4(R)-carboxylate, Rf: 0.49, 0.41 (C-2 epimers of thiazolidine); methyl 2-(L-glucopentahydroxypentyl)-thiazolidine-4(R)-carboxylate, Rf: 0.45; methyl 2-(D-rhamno-tetrahydroxypentyl)-thiazolidine-4(R)-carboxylate, Rf: 0.62; methyl 2-(L-rhamno-tetrahydroxypentyl)-thiazolidine-4(R)-carboxylate, Rf: 0.65, 0.58 (C-2 epimers of thiazolidine)].

Partial hydrolysis of 1 (10 mg) in 2 N-H2SO4 (2 ml) was heated at 80°C on a water bath for 30 min. The reaction mixture was neutralized with Ba(OH)2. After centrifugation of the precipitate, the supernatant was washed with Et2O and the water layer was concentrated to give the residue, which was chromatographed on silica gel using CHCl3-MeOH-H2O (100:12:1) as an eluent to give yellow needles, mp 258°C (dec.). This product was identified as homoplantaginin (la) by direct comparisons (TLC, UV, IR, 1H- and 13C-NMR) with an authentic specimen obtained from Plantago asiatica L.51

Identification of 2-9

2 (mp 265°C), 3 (mp 193°C), 6 (mp 208°C) and 9 (mp 165°C) were identified as linarin (2),8 l syringin (3),9,11,12 l chlorogenic acid (5-O-caffeoylquinic acid, 6)8,13 l and uridine (9),10 l respectively, by direct comparisons with authentic specimens (1H- and 13C-NMR, IR and UV) and physical data with those described in the literature.

Acknowledgements: We are grateful to Miss K. Yakubo of Hokuriku University for MS measurement.

REFERENCES