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FLAVONE DI-*C*-GLYCOSIDES FROM *SCUTELLARIA BAICALENSIS*

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Key Word Index—*Scutellaria baicalensis*; Labiatae; di-*C*-glycosylflavones; chrysin 6-*C*-β-D-glucoside-8-*C*-α-L-arabinoside; chrysin 6-*C*-α-L-arabinoside-8-*C*-β-D-glucoside.

Abstract—From the roots of *Scutellaria baicalensis* two new di-*C*-glycosylflavones have been isolated. Their structures have been established on the basis of mass, ^{1}H and ^{13}C NMR spectroscopy as chrysin 6-*C*-glucoside-8-*C*-arabinoside and chrysin 6-*C*-arabinoside-8-*C*-glucoside.

The roots of *Scutellaria baicalensis*, widely used as an important drug in China, Korea and Japan, have been reported to contain baicalin, wogonin and other constituents [1]. In the present paper, we describe the structural elucidation of two new di-*C*-glycosylflavones obtained from the water-soluble extract of this plant.

The water extract of the roots was repeatedly chromatographed on polyamide and silica gel to afford two compounds, **1** and **2** (0.1 and 0.2%, respectively, from the dried materials).

1 showed the same UV spectrum and diagnostic shifts as chrysin (5,7-dihydroxyflavone) and the chromatographic properties of a glycoside, but gave no sugar on acid hydrolysis. A 6,8-di-*C*-glycosylchrysin structure was confirmed by the absence of signals for the two aromatic protons (H-6 and H-8) in the ^{1}H NMR spectrum. Permethylated **1** exhibited a typical mass spectrum of a PM 6,8-di-*C*-glycosylflavone [2, 3]. The molecular peak M^+ 674 agreed with a PM *C*-pentosyl-*C*-hexosylchrysin structure and the relative importance of M-175 and M-131 peaks with a 6-*C*-hexosyl-8-*C*-pentosylchrysin structure. Furthermore, since the relative intensity of the M-131 ion was higher than that of the M-119 ion and that of the M-119 was higher than that of the M-145, the pentose sugar is arabinose. The *C*-hexosyl residue must be glucose since $FeCl_3$ oxidation yielded both glucose and arabinose. Further evidence came from the ^{13}C NMR spectrum in which the signals of the *C*-glycosyl moiety exhibited 11 carbon atoms, which could be clearly distinguished from those of the flavone. These signals were analogous to those previously reported for schaftoside [4]. Thus, **1** was identified as chrysin 6-*C*-β-D-glucoside-8-*C*-α-L-arabinoside.

2 showed very similar chromatographic and spectral properties to **1**. Again no sugar was found after acid hydrolysis. The MS of PM **2** exhibited a molecular ion at *m/z* 674, identical with that of a PM *C*-pentosyl-*C*-hexosylchrysin. This structure was confirmed by the appearance of pentose fragments at M-119, M-131 and M-145. Since the relative intensity of the hexose fragment peaks M-175 was lower than the pentose fragment peaks M-131, the pentose was considered to be attached to C-6 and the hexose to C-8. Further, the relative abundance of the peaks M-131 > M-119 > M-145 favoured a 6-*C*-arabinosyl structure. The *C*-hexosyl residue was determined as glucose since $FeCl_3$ oxidation afforded both glucose and arabinose.

Since pairs of Wessely–Moser isomers frequently occur together [5], **2** could be the Wessely–Moser isomer of **1**. To substantiate this, **1** was isomerized by heating with acid and the crude mixture permethylated. TLC of the permethylation products afforded a small proportion of a constituent showing the same chromatographic behaviour and MS characteristics as PM **2**.

From the above data, **2** was assigned the structure 6-*C*-α-L-arabinosyl-8-*C*-β-D-glycosylchrysin. This is the first report of di-*C*-glycosylflavones from *Scutellaria baicalensis*.

EXPERIMENTAL

Mps are uncorr. In ^{1}H NMR and ^{13}C NMR spectra chemical shifts are given on δ (ppm) scale with TMS as the int. standard. CC was carried out with Kieselgel (Merck) and polyamide C-200 (wako) and TLC with Kieselgel $60F_{254}$ (Merck) using $FeCl_3$ and I_2 vapour as detective agents.

Plant material. The roots of *Scutellaria baicalensis* were obtained as a crude drug and identified by Dr. K. Yoneda, Faculty of Pharmaceutical Sciences, Osaka University. A voucher specimen is kept in our laboratories.

Isolation. Roots of *S. baicalensis* (10 kg) were extracted with hot H_2O at 60–70°. After standing for 2 days, the resultant ppt. was removed and the filtrate extracted with *n*-BuOH gave a brown extract (395 g), which was macerated with EtOAc. After the removal of the EtOAc-soluble flavonoids, the residue was passed through a polyamide column and successive elution with H_2O afforded a mixture of **1** and **2**, which was repeatedly chromatographed on Si gel ($CHCl_3$–MeOH, 5:1) to give **1** (10 g) and **2** (20 g).

1, pale yellow powder, mp 215–218°, $[\alpha]_D^{22}$ +96° (*c* = 1.0, MeOH–H_2O, 1:1). Found: C, 55.51; H, 5.01. $C_{26}H_{28}O_{13}$ $2/3H_2O$ requires C, 55.71; H, 5.2.. UV λ_{max}^{MeOH} nm (log ε): 216 (4.50), 249 (4.12), 275 (4.42), 316 (3.96). $\lambda_{max}^{MeOH+AlCl_3}$ nm: 220 (4.43), 255 (4.02), 290 (4.37), 333 (4.07), 390 (3.76). $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 221 (4.42), 253 (3.99), 290 (4.37), 330 (4.04), 386 (3.73). $\lambda_{max}^{MeOH+NaOAc}$ nm: 216 (4.51), 248 (4.24), 277 (4.39), 348 (3.48). $\lambda_{max}^{MeOH+NaOMe}$ nm: 248 (4.32), 260 sh (4.32), 282 (4.36), 380 (3.92). IR (KBr) ν_{CO} 1650 cm^{-1}. ^{1}H NMR (d_6–DMSO) δ ppm: 3.20–4.60 (sugar-H), 4.73 (1 H, *d*, *J* = 9.0 Hz, anomeric-H), 4.82 (1 H, *d*, *J* = 9.0 Hz, anomeric-H),

6.92 (1 H, *s*, C_3 – H), 7.53–7.63 (3 H, *m*, $C_{3'-5'}$ – H), 8.17–8.33 (2 H, *m*, $C_{2',6'}$–H). ^{13}C NMR (d_6–DMSO) δ ppm: 181.6 (C-4), 165.8 (C-2), 162.5 (C-7), 159.4 (C-9), 154.9 (C-5), 131.5 (C-4'), 131.1 (C-1'), 129.0 (C-3',5'), 126.7 (C-2',6'), 109.0 (C-6), 104.5 (C-8), 104.4 (C-3), 102.4 (C-10), 81.1, 79.0, 73.9, 70.7, 70.2, 61.0 (Glc), 75.4, 74.8, 70.9, 69.2, 69.0 (Ara). TLC: R_f 0.37 ($CHCl_3$–MeOH–H_2O, 7:3:0.5), 0.71 (MeOH–Me_2CO–H_2O–NH_4OH, 8:1.5:0,8:2). Permethyl ether: MS *m/z* (rel. int.); 674 (M^+, 20.3), 659 (M-15, 26.7), 643 (M-31, 100), 627 (M-47, 12.4), 611 (M-61, 7.1), 571 (M-103, 13.2), 543 (M-131, 11.3), 511 (M-163, 28.2), 499 (M-175, 54.1%), 485 (M-189, 7.5). TLC; R_f 0.64 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4).

2, yellow powder, mp 215–218°, $[\alpha]_D^{22}$ −31.3° (*c* = 1.0, MeOH–H_2O, 1:1). Found: C, 55.54; H, 5.45. $C_{26}H_{28}O_{13}5/6H_2O$ requires C, 55.42; H, 5.28. UV λ_{max}^{MeOH} nm (log ε): 217 (4.47), 248 (4.15), 277 (4.37), 318 (3.82). $\lambda_{max}^{MeOH+AlCl_3}$ nm: 222 (4.43), 254 (3.97), 290 (4.36), 333 (4.05), 390 (3.72). $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 222 (4.43), 254 (3.97), 290 (4.36), 331 (4.02), 386 (3.69). $\lambda_{max}^{MeOH+NaOAc}$ nm: 216 (4.51), 249 (4.18), 277 (4.37), 325 (3.77). $\lambda_{max}^{MeOH+NaOMe}$ nm: 247 (4.31), 270 (4.30), 283 (4.32), 377 (3.88). IR (KBr) ν_{CO} 1645 cm^{-1}. ^{1}H NMR (d_6-DMSO) δ ppm: 3.30–5.10 (sugar-H), 6.98 (1 H, *s*, C_3 – H), 7.55–7.70 (3 H, *m*, $C_{3'-5'}$ – H), 8.10–8.28 (2 H, *m*, $C_{2',6'}$–H). ^{13}C NMR (d_6-DMSO) δ ppm: 181.8 (C-4), 167.5 (C-2), 162.9 (C-7), 158.5 (C-9), 155.1 (C-5), 131.6 (C-4'), 131.0 (C-1'), 128.8 (C-3',5'), 126.5 (C-2',6'), 108.4 (C-6), 104.6 (C-3, 8), 103.0 (C-10), 81.6, 78.8, 73.6, 70.5, 70.0, 61.2 (Glc), 74.3, 74.1, 71.1, 69.1, 68.5 (Ara). TLC; R_f 0.37 ($CHCl_3$–MeOH–H_2O, 7:3:0.5), 0.64 (MeOH–Me_2CO–H_2O–NH_4OH, 8:1.5:0.8:2). Permethyl ether: MS *m/z*: (rel. int.): 674 (M^+, 42.4), 659 (M-15, 24.2), 643 (M-31, 100), 627 (M-47, 12.1), 613 (M-61, 18.2), 611 (M-63, 6.1), 555 (M-119, 30.3), 543 (M-131, 48.5), 529 (M-145, 10.9), 511 (M-163, 10.9), 499 (M-175, 21.2). TLC: R_f 0.58 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4).

Acid isomerization. **1** (15 mg) was heated with MeOH–4 N HCl (1:1, 2 ml) at 100° for 7 hr. After evapn and permethylation, TLC of the mixture on Si gel in $CHCl_3$–EtOAc–Me_2CO (5:1:4) gave one main band, which was eluted and cochromatographed with PM 2 in the same solvent and the MS agreed with that of a PM 6-*C*-arabinosyl-8-*C*-glucosylchrysin; *m/z* (rel. int.): 674 (M^+, 29.3%), 659 (M-15, 25.1), 643 (M-31, 100), 627 (M-47, 19.1), 613 (M-61, 20.9), 611 (M-63, 12.5), 555 (M-119, 28.1), 543 (M-131, 42.5), 529 (M-145, 11.9), 499 (M-175, 27.5).

$FeCl_3$ *oxidation.* 100 mg of **1** in aq. $FeCl_3$ (100 mg in 5 ml H_2O) was heated under reflux at 115° for 15 min and then at 125° for 6 hr, diluted with H_2O, filtered and the pale yellow filtrate passed through a column of IRC-120 [H^+] followed by IRA-50 [OH^-] to remove Fe^{3+} and Cl^- ions. The neutral soln was concd to small vol. and examined by electrophoresis to detect arabinose and glucose.

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