

Flavonoid Glucosides from *Polygonum tinctorium*

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A new flavonoid-glucoside, 3,5,4'-trihydroxy-6,7-methylenedioxy-3-O- β -D-glucopyranoside, which has an anti-platelet activity, was isolated from *Polygonum tinctorium* LOUR. and its structure was elucidated by means of carbon-13 nuclear magnetic resonance, including long range selective-proton decoupling (LSPD), and other spectroscopic methods.

Keywords *Polygonum tinctorium*; flavonoid-glucoside; anti-platelet activity; ^{13}C -NMR; LSPD

Polygonum tinctorium LOUR. (Polygonaceae, Japanese name, ai) has been cultivated as a source of indigo in Japan, and is also used as a crude drug for the alleviation of fever, as an antidote and occasionally to treat athlete's foot in folk medicine.

During our screening program of medicinal plants for various biological activities, the crude flavonoid-glycoside fraction of *P. tinctorium* showed anti-platelet activity. The active fraction of the MeOH extract of the aerial parts afforded a known compound, named Pt-2, in addition to a new flavonoid glycoside, named Pt-1. Pt-1, separated as a physiologically active constituent, inhibits human platelet aggregation induced by adenosine diphosphate (ADP). This paper deals with the structure elucidation of the new flavonoid-glucoside using the technic of long range selective-proton decoupling (LSPD) in carbon 13 nuclear magnetic resonance (^{13}C -NMR) spectroscopy.

The proton nuclear magnetic resonance (^1H -NMR) and ^{13}C -NMR spectral data (shown in Table I) suggested that Pt-1 (**1**) and Pt-2 (**2**) are of flavonoid monoglucosides. Compound **2** was identified as kaempferol-3-O- β -D-glucopyranoside by direct comparison with an authentic sample. The physical data of **2** and its acid hydrolysate, kaempferol and D-glucose, also matched those of authentic samples.

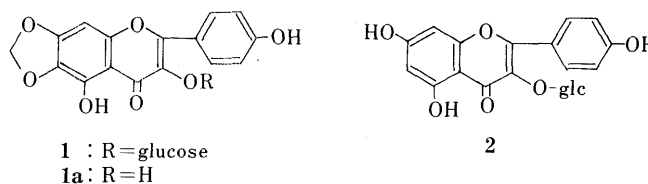
Pt-1 (**1**), was obtained as a colorless powder. $\text{C}_{22}\text{H}_{24}\text{O}_{14}$;

TABLE I. ^{13}C -NMR Spectral Data for **1**, **1a**, Irlone and **2** (δ Relative to TMS in $\text{DMSO}-d_6$)

	1	1a	Irlone	2
C-2	156.6	147.4	154.1	156.3
3	133.2	135.8	122.4	133.2
4	177.8	176.3	180.9	177.4
5	140.3	139.8	153.1	161.2
6	129.2	128.8	129.7	98.7
7	153.7	153.8	141.6	164.2
8	89.3	89.4	89.3	93.6
9	151.6	151.5	154.0	156.3
10	106.8	105.8	107.7	104.0
O-CH ₂ -O	102.8	102.6	102.7	
1'	120.6	121.4	121.4	120.9
2'6'	130.8	129.5	129.5	130.8
3'5'	115.0	115.4	115.4	115.1
4'	159.9	159.3	159.3	159.9
G-1	100.7			100.9
2	74.1			74.2
3	76.3			76.4
4	69.8			69.9
5	77.4			77.4
6	60.8			60.8

mp 294–297 °C; gave IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (OH), 1680 (C=O), 1650 (chelated carbonyl), 1610 (aromatic), 1270 (–C–O–C–); UV $\lambda_{\text{max}}^{\text{MeOH}}$ (log ϵ) nm: 217 (4.45), 278 (4.20), 341 (4.34). The UV maxima changed to 222, 297, 343 (log ϵ 4.44, 4.18, 4.37 respectively) on addition of aluminum chloride, and changed to 238, 278, 343 (log ϵ 4.28, 4.29, 4.37 respectively) on addition of sodium acetate, indicating that **1** is a flavonoid and that a hydroxyl group of **1** is located at the 5-position. This was further confirmed by other spectral data. The ^1H -NMR data of **1** were very similar to those of kanzakiflavone-2 isolated from iris.^{1,2)} A hydroxyl proton signal appeared as a low-field singlet at δ 12.40, indicating that a chelated hydroxyl group must be present at C-5.³⁾ On acid hydrolysis with 8% HCl, **1** gave a genin and D-glucose. Furthermore, the coupling constant ($J=8$ Hz) of the anomeric proton signal at δ 5.5 in the ^1H -NMR spectrum of **1** suggested that **1** is a monoglucoside and its glucoside linkage has β -configuration. The ^{13}C -NMR spectrum of **1** was compared with that of **2**. The spectra of the B-ring moieties were quite similar. The ^{13}C -NMR signals due to the flavone nucleus in the system of **1** and those of the acid hydrolysate of **1** were superimposable on each other. On comparison with the signals of **1** the signal assignable to C-3 was shifted upfield by about 3 ppm, accompanied with a downfield shift of C-2 (about 9 ppm) and C-4 (about 1.5 ppm) characteristic of the 3-O- β -D-glucopyranoside of 3-hydroxyflavonoids.⁴⁾

A singlet at δ 6.16 (2H) was typical of a methylenedioxy group. Furthermore, the ^1H -NMR spectrum clearly showed a signal at δ 6.86 due to H-8, and an A_2B_2 system centered at δ 6.91 (2H, $J=9$ Hz) due to H-3', 5' and at δ 8.08 (2H, d, $J=9$ Hz) due to H-2', 6' (*ortho* coupling). This was further substantiated by the ^{13}C -NMR signal at 102.8 ppm (triplet) due to the methylenedioxy group of **1**. The substitution position on ring A was considered to be 6,7-methylenedioxy or 7,8-methylenedioxy. In the ^{13}C -NMR spectrum of **1**, the sp^2 carbon appearing at the highest field (89.3 ppm) showed a doublet peak under off-resonance conditions. If 7,8-methylenedioxy is assumed, the ^{13}C -NMR signal of C-6 would be expected in the range from at 93 to 100 ppm, judging from several reported data.^{5,6)} This ^{13}C -NMR spectrum of C-8 was quite similar



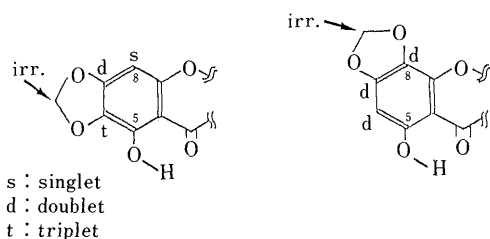


Chart 1. LSPD Coupling Pattern of **1**
Irradiation (irr.) at methylenedioxy protons.

with that of irilone (89.3 ppm).⁶⁾ From above result, the structure of **1** can be represented as 6,7-methylenedioxy, not 7,8-methylenedioxy. This conclusion was supported by LSPD.⁷⁾ When two protons of the methylenedioxy group (δ 6.16) of the genin of **1** (**1a**) were irradiated, carbons of C-6 or C-8 were coupled respectively as shown in Chart 1. If the substitution pattern is assumed to be 6,7-methylenedioxy, the C-8 carbon peak will appear as a singlet. In the case of a 7,8-methylenedioxy group, however, the C-6 carbon peak must appear as a doublet. In the LSPD, this carbon of **1a** appeared at 89.4 ppm as a sharp singlet. Therefore, the methylenedioxy group was determined to be located at the 6,7-carbons. This conclusion was further supported by the result of irradiation of the C-5 hydroxyl group. Though the assignments of the **1** and **2** carbon signals were based on literature data, the assignments of C-5 and C-7 carbons were not clear.^{5,6)} This problem was settled by irradiation of the C-5 hydroxyl group and C-8 proton in the LSPD. Finally, the above data also led us to conclude that the 153.8 ppm signal can be assigned to C-7 and 139.8 ppm signal to C-5. Irradiation of the C-8 proton enhanced the signal intensity of the C-9 carbon more than that of the C-7 carbon, which was weakly coupled with the methylenedioxy carbon. Therefore the 151.5 ppm signal was assigned to C-9 and the 153.8 ppm signal to C-7.

Experimental

General Procedures All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Jasco IRA-2 machine, and ultraviolet spectra (UV) on a Hitachi model 200-10 spectrometer. Optical rotations were determined with a Union-101 automatic digital polarimeter. NMR spectra were recorded on JEOL FX-100 and JEOL GX-270 spectrometers in dimethyl sulfoxide- d_6 (DMSO- d_6) with tetramethylsilane (TMS) as an internal standard. For column chromatography, Kieselgel 60H (Art. 7736, Merck), Lichroprep RP-18 (25–40 μ m) and Diaion HP-20 (Mitsubishi Chem. Ind. Co. Ltd., Tokyo, Japan) were used. All solvent systems for chromatography were homogeneous.

Extraction and Separation The aerial parts of *Polygonum tinctorium*,

cultivated at the Experimental Station of Medicinal Plants, Hiroshima University School of Medicine, were extracted with boiling MeOH. A suspension of the MeOH extract was fractionated using highly porous polymer, Diaion HP-20 and successively eluted with H₂O, 30%, 50%, 70%, 100% MeOH, and CHCl₃. A part of the 70% aqueous MeOH fraction (6.4 g) was separated by silica gel column chromatography with CHCl₃–MeOH (5:1). One of the biologically active fractions, frs. 6 to 8, was crystallized from CHCl₃–MeOH to give Pt-1 (**1**) (200 mg). Fraction 9 was also chromatographed on a silica gel column eluted with CHCl₃–MeOH (7:2) to give Pt-2 (**2**) (73.8 mg), which was identified as kaempferol-3-*O*- β -D-glucopyranoside by comparison of the spectral data with those of an authentic sample.

Pt-1 (1): Colorless powder from CHCl₃–MeOH, mp 294–297 °C. $[\alpha]_D^{20}$ –73.1° (c = 1.0, pyridine). IR $\nu_{\text{KBr}}^{\text{max}}$ cm^{–1}: 3400, 1680, 1650, 1610, 1550, 1480, 1340, 1270, 1170, 1100, 1050, 1010. ¹³C-NMR data for **1** are given in Table I. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 204 (4.43), 217 (4.45), 278 (4.20), 341 (4.34). UV $\lambda_{\text{max}}^{\text{MeOH-AlCl}_3}$ nm (log ϵ): 205 (4.32), 222 (4.44), 297 (4.18), 343 (4.37). UV $\lambda_{\text{max}}^{\text{MeOH-NaOAc}}$ nm (log ϵ): 219 (4.40), 238 (4.28), 278 (4.29), 343 (4.37). Anal. Calcd for C₂₂H₂₄O₁₄·2H₂O: C, 51.56; H, 4.72. Found: C, 51.90; H, 4.48.

Acid Hydrolysis of 1 **1** (103 mg) was heated with 8% HCl in H₂O–dioxane (1:1) at 80 °C for 3 h. The reaction mixture was extracted with Et₂O and the organic layer was washed with H₂O and was concentrated to give a crystalline product, which was recrystallized from MeOH to give a colorless powder, **1a** (66 mg), mp >300 °C. IR $\nu_{\text{KBr}}^{\text{max}}$ cm^{–1}: 3400, 1670, 1620, 1570, 1500, 1470, 1350, 1260, 1230, 1180, 1100, 1070, 1020. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 205 (4.49), 260 (4.27), 294 (4.27), 354 (4.46). UV $\lambda_{\text{max}}^{\text{MeOH-AlCl}_3}$ nm (log ϵ): 204 (4.47), 240 (4.29), 277 (4.36), 409 (4.49). UV $\lambda_{\text{max}}^{\text{MeOH-NaOAc}}$ nm (log ϵ): 204 (4.50), 240 (4.27), 274 (4.27), 354 (4.45). $[\alpha]_D^{16}$ –3.3° (c = 0.68, pyridine). ¹H-NMR (δ in DMSO- d_6): 6.15 (2H, s, O–CH₂–O), 6.93 (2H, d, J = 9 Hz, H-2', 6'), 6.89 (1H, s, H-8), 8.06 (2H, d, J = 9 Hz, H-3', 5'), 12.48 (1H, s, 5-OH). MS m/z : 314 (M⁺). Anal. Calcd for C₁₆H₁₀O₇·5/2 H₂O: C, 53.48; H, 4.21. Found: C, 53.42; H, 4.16.

The aqueous layer was passed through a column of Amberlite MB-3 and the eluate was evaporated to dryness. The carbohydrate hydrolysate was identified as D-glucose by thin layer chromatography.

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