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TWO NEW FLAVONE GLYCOSIDES FROM THE ROOTS OF *SOPHORA SUBPROSTRATA*[1,2]

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ABSTRACT.—Two new flavone glycosides, named sophoraflavone A (**1**) and sophoraflavone B (**2**), were isolated from the roots of *Sophora subprostrata*, together with bayin. The structures of **1** and **2** were characterized as 7,4′-dihydroxyflavone 8-C-β-D-(2-*O*-α-L-rhamnosyl) glucoside (bayin 2″-*O*-rhamnoside) and 7,4′-dihydroxyflavone 4′-*O*-β-D-glucoside by their chemical and spectral data, respectively.

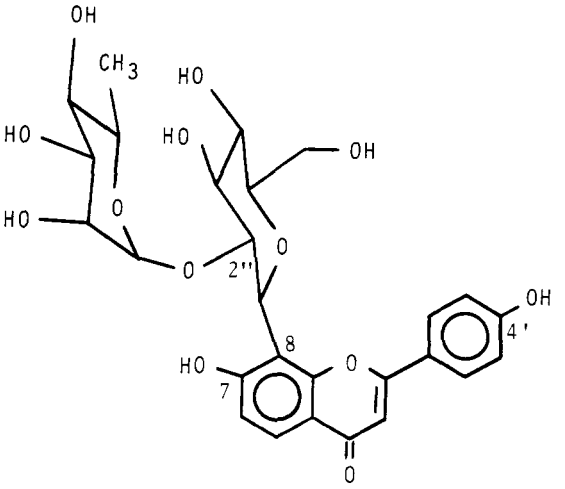
These flavonoids, having a strong fluorescence under uv light, are rare 5-deoxyflavonoids.

In an earlier paper (2), we reported the isolation and the structural elucidation of trifolirhizin-6′-monoacetate from the roots of *Sophora subprostrata* Chun et T. Chen (Leguminosae), the Chinese crude drug "Guang-Dou-Den." In our further studies on this drug, two new flavone glycosides, sophoraflavone A (**1**) and sophoraflavone B (**2**), together with bayin, have been isolated from the *n*-BuOH soluble fraction of the MeOH extract.

The dry roots of *S. subprostrata* were extracted with MeOH. The MeOH extract was treated by the usual method, as described in the experimental section, to yield a *n*-BuOH fraction. The *n*-BuOH extract was subjected to column chromatography on polyamide and silica gel to give three fractions. Each of these fractions was subjected to droplet countercurrent chromatography (dccc) and yielded compounds **1**,**2**, and bayin, respectively.

The known compound, bayin (7,4′-dihydroxyflavone 8-*C*-β-D-glucoside), was identified from its uv, ir, and ^{1}H-nmr spectral data, and this was confirmed by comparison with an authentic sample (3).

Compound **1** gave a yellow color by spraying with H_2SO_4, followed by heating.



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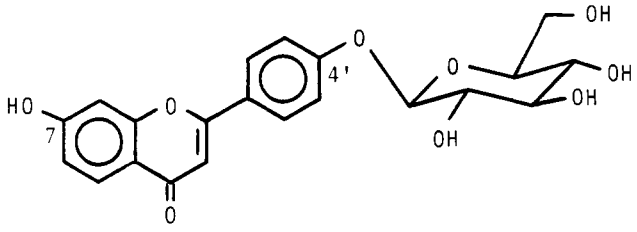
[1]Part 20 in the series "Studies on the Constituents of *Sophora* Species." For Part 19, See Y. Shirataki *et al.* (1).

[2]This paper also forms Part 8 in the series "Constituents of the Root of *Sophora subprostrata*." For Part 7, See M. Komatsu *et al.* (2).



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The uv and ir spectra were similar to those of bayin. The ^{1}H-nmr spectrum revealed the presence of bayin [δ 4.89 (1H, d, J=9.8 Hz, glucosyl H-1), δ 6.69 (1H, s, H-3), δ 6.9-8.0 (6H, H-5,6,2′,3′,5′,6′), δ 10.27 (1H, s, OH), δ 10.77 (1H, s, OH)], and rhamnose [δ 0.35 (3H, d, J=6.1 Hz, rhamnosyl Me), δ 5.0 (1H, br.s, rhamnosyl H-1)] in this structure. Acid hydrolysis of **1** gave bayin and L-rhamnose in equimolar ratio. The bathochromic shifts with diagnostic reagents in the uv absorption of **1** (4), and the presence of two phenolic hydroxy groups (7 and 4′) in the ^{1}H-nmr spectrum of **1** suggested that rhamnose must be attached to one of the glucosyl OH groups. The position of attachment of rhamnose was deduced from the ^{13}C-nmr spectral data (Table 1).

TABLE 1. ^{13}C-nmr Spectral Data for 7,4′-Dihydroxyflavone, Bayin, and Sophoraflavone A (**1**)[a]

Carbon No.	Compounds			
	7,4′-Dihydroxyflavone	Bayin	**1**	Δ **1**-Bayin
2	162.5 (s)[b]	162.5 (s)	162.5 (s)	
3	104.5 (d)	103.9 (d)	103.8 (d)	
4	176.3 (s)	176.4 (s)	176.4 (s)	
5	126.4 (d)	125.2 (d)	125.4 (d)	
6	114.7 (d)	113.9 (d)	114.0 (d)	
7	162.4 (s)[b]	160.7 (s)	160.6 (s)	
8	102.4 (d)	112.9 (s)	112.7 (s)	
9	157.4 (s)	156.1 (s)	155.9 (s)	
10	116.1 (s)	116.4 (s)	116.5 (s)	
1′	121.8 (s)	122.2 (s)	122.1 (s)	
2′,6′	128.0 (d)	128.5 (d)	128.4 (d)	
3′,5′	115.9 (d)	115.6 (d)	115.7 (d)	
4′	160.7 (s)	160.5 (s)	160.6 (s)	
1″		73.6 (d, J=143.3 Hz)	71.2 (d, J=141.1 Hz)	−2.4
2″		71.0 (d)	75.0 (d)	+4.0
3″		78.7 (d)	79.8 (d)	+1.1
4″		70.5 (d)	70.6 (d)[c]	+0.1
5″		81.9 (d)	81.9 (d)	0
6″		61.2 (t)	61.1 (t)	−0.1
1‴			100.0 (d, J=172.3 Hz)	
2‴			70.3 (d)[c]	
3‴			71.8 (d)	
4‴			70.1 (d)[c]	
5‴			67.9 (d)	
6‴			17.6 (q)	

[a]In DMSO-d_6; δ (ppm).
[b,c]Assignments may be interchanged.

The C-2″ was shifted downfield by 4.0 ppm in comparison with bayin and appeared at δ 75.0 ppm, while the adjacent anomeric carbon underwent an upfield shift of 2.4 ppm at δ 71.2 ppm (glycosylation shift). The other signals except C-3″[3] were not affected. The chemical shift values of the sugar moiety were in good agreement with reported data for 2″-*O*-rhamnosylvitexin (5). Therefore, the position of the attachment of rhamnose was proved to be at the C-2″ of **1**. α-Linkage of rhamnose was revealed by the ^{13}C-^{1}H coupling constant (J=172.3 Hz) of rhamnosyl C-1 in the ^{13}C-nmr spectrum (6). From these chemical and spectral data, the structures of **1** was established to be 7,4′-dihydroxyflavone 8-*C*-β-D-(2-*O*-α-L-rhamnosyl) glucoside (bayin 2″-*O*-rhamnoside), and it was named sophoraflavone A.

Compound **2** also gave a yellow color by spraying with H_2SO_4, followed by heating. Compound **2** yielded an aglycone and sugar in equimolar ration on acid hydrolysis. The aglycone was identified as 7,4′-dihydroxyflavone by comparison of tlc, ir, and ^{1}H-nmr spectra with an authentic sample (2). The sugar moiety was shown to be D-glucose by co-pc and co-hplc with an authentic sugar, and the β-linkage was revealed by the coupling constant (d, J=7.3 Hz) of glucose H-1 in the ^{1}H-nmr spectrum. Thus, the structure of **2** was deduced to be a 7,4′-dihydroxyflavone-β-D-glucoside. While the location of the glucose moiety may be determined by uv absorption and the bathochromic shifts with diagnostic reagents (NaOAc), we also used a comparison of the chemical shifts in ^{1}H-nmr spectra of **2**, its aglycone, and the pentaacetate of **2** (Table 2).

TABLE 2. ^{1}H-nmr Spectral Data for 7,4′-Dihydroxyflavone, Sophoraflavone B (**2**), and Pentaacetate of **2**[a]

H	Compounds				
	7,4′-Dihydroxy-flavone	**2**	Penta-acetate of **2**	Δ **2**-7,4′-Di-hydroxyflavone	Δ Penta-acetate of **2**-**2**
H-5 . . .	7.87	7.87	8.11	0	+0.24
H-6 . . .	6.91	6.91	7.29	0	+0.38
H-8 . . .	6.97	7.00	7.66	+0.03	+0.66
H-2′,6′ .	7.91	8.02	8.08	+0.11	+0.06
H-3′,5′ .	6.93	7.19	7.18	+0.26	−0.01

[a]In DMSO-d_6; δ (ppm).

It was clear that glucose was located at the 4′ position of the aglycone. Consequently, the structure of **2** was established to be 7,4′-dihydroxyflavone 4′-*O*-β-D-glucoside, and it was named sophoraflavone B.

S. Shibata *et al.* reported a markedly fluorescent spot (spot K) in the MeOH extract of the roots of *S. subprostrata* by pc (7). We studied this in the same manner, and it was proven that the spot K is a mixture of **1** and bayin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Yanagimoto MP-S3 micro melting point apparatus and are recorded uncorrected. Ir and uv spectra were taken on Nihonbunko IR-810 and UVIDEC-430 machines, respectively. ^{1}H-nmr and ^{13}C-nmr spectra were obtained on a JEOL JNM GX-270 FT nmr spectrometer at 270 MHz and 67.8 MHz, respectively, and chemical shifts are given in δ(ppm) with TMS as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Ms spectra were taken on a JEOL JMS DX-300 mass spectrometer, and

[3]The C-3″ of 2″-*O*-rhamnosylvitexin was shifted downfield by 1.0 ppm in comparison with vitexin (5).

the fabms spectra were obtained in a glycerol matrix at 3 Kv. Hplc used a Waters Model 510 pump equipped with a U6K injector and a differential refractometer, model R401 detector. Hplc were carried out with a solvent of MeCN-H_2O (4:1) using a μ-Bondapack NH_2 (Waters Ltd., 8 mm ID × 10 cm, RCSS system) at a flow rate of 1.0 ml/min and 200 psi. Dccc used a DCCC Ishii (Osaka) equipped with 250 tubes and $CHCl_3$-MeOH-H_2O (35:65:40) as solvent system in the ascending mode. Polyamide (C-200) and silica gel (C-300) for chromatography were purchased from Wako Pure Chemical Ind. Ltd., Japan. Tlc (Analtech, USA) was conducted on silica gel, and the solvent system was $CHCl_3$-MeOH-H_2O (65:35:10, lower layer). Pc was run on Toyo No. 50 paper using either *n*-BuOH-HOAc-H_2O (4:1:2) (solvent 1), *n*-BuOH-HOAc-H_2O (4:1:5) (solvent 2), or *n*-BuOH-EtOH- 0.2 N HOAc (3:1:1.5) (solvent 3) as solvent.

EXTRACTION AND SEPARATION.—The dried roots (2.5 kg) of *S. subprostrata*, obtained from China National Native Produce and Animal By-Products Import and Export Corp. and identified by Prof. T. Namba, Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University, Japan, were extracted three times with boiling MeOH, and the solvent removed in vacuo. The MeOH extract (378 g) was extracted with Et_2O and then with EtOAc.

The insoluble part was further extracted with *n*-BuOH. The combined *n*-BuOH extract was concentrated (42 g) and chromatographed on polyamide using H_2O and H_2O-MeOH (9:1-1:1) as solvents to give three fractions. Each fraction was subjected to rechromatography on silica gel using $CHCl_3$-MeOH-HOAc-H_2O (60:20:15:5) as solvent to give crude **1**, crude **2**, and crude bayin, respectively. These crude compounds were subjected to dccc to yield **1** (120 mg), **2** (28 mg), and bayin (70 mg), respectively.

SOPHORAFLAVONE A (**1**).—Pale yellow amorphous powder ($CHCl_3$/MeOH), mp 226-231° (dec); $[\alpha]^{25}D$ −32° (*c*=0.2, MeOH); *Anal.* calcd for $C_{27}H_{30}O_{13}\cdot H_2O$: C, 55.86; H, 5.56. Found: C, 55.57; H, 5.56; tlc Rf 0.22; fabms *m/z* 563 $(M+H)^+$; uv λ max (MeOH) nm (log ε) 259 sh (4.03), 313 sh (4.27), 328 (4.28); λ max (MeOH+NaOMe) nm (log ε) 254 (4.67), 326 (4.23), 392 (4.39); λ max (MeOH+$AlCl_3$) nm (log ε) 258 sh (4.03), 311 (4.27), 329 (4.28), 390 (3.53); λ max (MeOH+NaOAc) nm (log ε) 270 (4.43), 308 (4.31), 378 (4.34); ir ν max (KBr) cm^{-1} 3400 (OH), 1630 (C=O), 1610, 1580 (arom C=C); ^1H nmr (DMSO-d_6) δ 0.35 (3H, d, *J*=6.1 Hz, rhamnosyl Me), 2.8-3.8 (m, rhamnoglucosyl 9H), 4.15 (1H, t, *J*=9.2 Hz, H-2″), 4.89 (1H, d, *J*=9.8 Hz, glucosyl H-1), 5.0 (1H, br.s, rhamnosyl H-1), 6.69 (1H, s, H-3), 6.90 (2H, d, *J*=8.6 Hz, H-3′,5′), 6.96 (1H, d, *J*=8.6 Hz, H-6), 7.79 (1H, d, *J*=8.6 Hz, H-5), 8.02 (2H, d, *J*=8.6 Hz, H-2′,6′), 10.27 (1H, s, OH-4′: exchangeable in D_2O), 10.77 (1H, s, OH-7: exchangeable in D_2O); ^{13}C-nmr (Table 1).

HYDROLYSIS OF **1**.—Compound **1** (20 mg) was hydrolyzed with 5% HCl/MeOH (10 ml) for 3 h under reflux to yield bayin [mp 218-220° (dec) (mmp, co-tlc, ir, ^1H nmr)] and the sugar L-rhamnose [co-pc Rf 0.45 (solvent 1); detected with aniline hydrogen phthalate, co-hplc Rt 8.1 min].

SOPHORAFLAVONE B (**2**).—Colorless needles (MeOH), mp 297-300° (dec); $[\alpha]^{25}D$ −48° (*c*=0.2, MeOH); *Anal.* calcd for $C_{21}H_{20}O_9\cdot \frac{1}{4}H_2O$: C, 59.93; H, 4.91. Found: C, 59.92; H, 4.90; tlc Rf 0.50; fabms *m/z* 417 $(m+H)^+$; uv λ max (MeOH) nm (log ε) 256 (3.97), 316 (4.30); λ max (MeOH+NaOMe) nm (log ε) 264 (4.69), 360 (4.16); λ max (MeOH+$AlCl_3$) nm (log ε) 256 (3.97), 316 (4.30); λ max (MeOH+NaOAc) nm (log ε) 266 (4.36), 306 (4.16), 320 sh (4.07), 359 (4.14); ir ν max (KBr) cm^{-1} 3300 (OH), 1640 (C=O), 1620, 1580 (arom C=C); ^1H nmr (DMSO-d_6) δ 3.1-3.8 (m, glucosyl 6H), 5.02 (1H, d, *J*=7.3 Hz, glucosyl H-1), 6.83 (1H, s, H-3), 6.91 (1H, dd, *J*=9.2, 1.8 Hz, H-6), 7.00 (1H, d, *J*=1.8 Hz, H-8), 7.19 (2H, d, *J*=9.2 Hz, H-3′,5′), 7.87 (1H, d, *J*=9.2 Hz, H-5), 8.02 (2H, d, *J*=9.2 Hz, H-2′.6′), 10.79 (1H, s, OH-7; exchangeable in D_2O).

HYDROLYSIS OF **2**.—Compound **2** (10 mg) was hydrolyzed with 5% HCl/MeOH (5 ml) for 2 h under reflux to yield 7,4′-dihydroxyflavone (mp over 300 °, co-tlc, ir, ^1H nmr) and the sugar D-glucose [co-pc Rf 0.31 (solvent 1); detected with aniline hydrogen phthalate, co-hplc Rt 13.0 min].

ACETYLATION OF **2**.—A solution of **2** (10 mg) in a mixture of Ac_2O (1 ml) and C_5H_5N (1 ml) was allowed to stand at room temperature overnight, and the reaction mixture was worked up as usual to give a pentaacetate (12 mg) as colorless needles (MeOH), mp 238-240°; ir ν max (KBr) cm^{-1} 1750, 1230, 1210 (ester), 1640 (C=O), 1610, 1510 (arom C=C); ^1H nmr ($CDCl_3$) δ 2.05, 2.07, 2.08, 2.10, 2.37 (OAc×5), 4.0-5.4 (m, glucosyl 7H), 6.74 (1H, s, H-3), 7.12 (2H, d, *J*=9.2 Hz, H-3′,5′), 7.16 (1H, dd, *J*=8.8, 2.2 Hz, H-6), 7.41 (1H, d, *J*=2.2 Hz, H-8), 7.86 (2H, d, *J*=9.2 Hz, H-2′,6′), 8.24 (1H, d, *J*=8.8 Hz, H-5), (DMSO-d_6) δ 1.98, 2.02, 2.03, 2.35 (OAc×5), 4.1-5.8 (m, glucosyl 7H), 7.02 (1H, s, H-3), 7.18 (2H, d, *J*=8.4 Hz, H-3′,5′), 7.29 (1H, dd, *J*=8.4, 2.2 Hz, H-6), 7.66 (1H, d, *J*=2.2 Hz, H-8), 8.08 (2H, d, *J*=8.4 Hz, H-2′,6′), 8.11 (1H, d, *J*=8.4 Hz, H-5).

BAYIN.—Pale yellow needles (MeOH), mp 218-220° (dec); $[\alpha]^{25}D$ −5.0° (*c*=0.2, MeOH); *Anal.* calcd for $C_{21}H_{20}O_9\cdot H_2O$: C, 58.06; H, 5.10. Found: C, 58.12; H, 5.08; tlc Rf 0.37; fabms *m/z* 417 $(M+H)^+$. Identification was established by comparison (mmp, co-tlc, uv, ir, ^1H nmr, fabms) with an au-

thentic sample of bayin (3). The hexaacetate, colorless plates (MeOH), mp 127-128°, was also identified by direct comparison (mmp, ir, ^{1}H nmr) with an authentic sample.

INVESTIGATION OF COMPOUNDS AND SPOT K BY USING PC.—Compound **1**: Rf 0.48 (solvent 2), 0.53 (solvent 3); Compound **2**: Rf 0.63 (solvent 2), 0.62 (solvent 3); bayin: Rf 0.49 (solvent 2), 0.54 (solvent 3); spot K: Rf 0.49 (solvent 2), 0.53 (solvent 3).

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