Journal of Natural Products
Vol. 51, No. 1, pp. 180-183, Jan-Feb 1988

A NEW PHENOLIC GLYCOSIDE FROM *PHTHEIROSPERMUM JAPONICUM*

YOSHIO TAKEDA

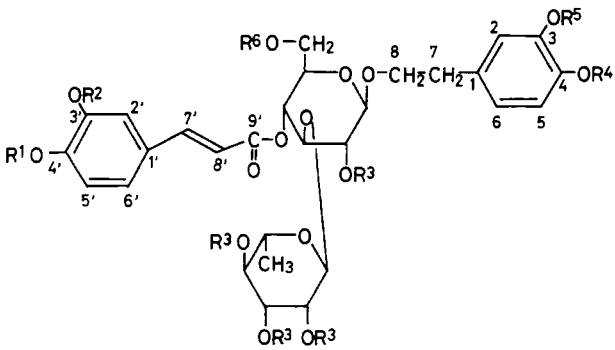
Faculty of Pharmaceutical Sciences, The University of Tokushima, Tokushima 770, Japan

Phtheirospermum japonicum Kanitz is distributed widely in East Asia. No reports on the constituents of the plant were found in the literature. In the course of the studies on the glycosidic constituents of the Scrophulariaceae, the constituents of the aerial parts of *P. japonicum* were examined. This paper describes the isolation and structure elucidation of a new phenolic glycoside, phtheirospermoside [**1**], and the identification of four phenolic glycosides and three iridoid glucosides.

The MeOH extract of the dried aerial parts of *P. japonicum* was separated by a combination of Si gel cc and reversed phase hplc. From the EtOAc-soluble fraction, phtheirospermoside [**1**] was isolated together with the known compounds, martynoside [**3**] (1), leucosceptoside A [**4**] (2), and acteoside [**5**] (3). From the *n*-BuOH-soluble fraction, three known iridoid glucosides, plantarenoside (4,5), aucubin (6), and geniposidic acid (7,8), and a known phenolic glycoside, forsythoside B [**6**] (9), were isolated in addition to **5**.

Phtheirospermoside [**1**] was isolated as an amorphous powder, $[\alpha]_D$ −93.1° (MeOH), whose molecular weight was confirmed by the observation of ion peaks at *m/z* 661 $[M+Na]^+$ and 677 $[M+K]^+$ in the fabms on addition of NaI and KI. Acetylation of **1** with Ac_2O and C_5H_5N gave the octaacetate **2**, in the ^{1}H-nmr spectrum of which signals due to three phenolic acetoxy groups and five alcoholic acetoxy groups were observed. The uv spectrum of **1** showed absorption maxima at 217, 245 (sh), 291, and 328 nm and are very similar to those of **4** and leucosceptoside B [**7**] (2). The ir spectrum of **1** showed the presence of a conjugated ester (1700 and 1630 cm^{-1}) and an aromatic ring (1610 and 1515 cm^{-1}). These spectral data suggest the presence of 3,4-dioxygenated β-phenethylalcohol and cinnamic acid moieties in the molecule.

The presence of a 3,4-dihydroxyphen-



1 R^1=Me, R^2=R^3=R^4=R^5=R^6=H
2 R^1=Me, R^2=R^3=R^4=R^5=R^6=Ac
3 R^1=R^3=R^5=R^6=H, R^2=R^4=Me
4 R^1=R^3=R^4=R^5=R^6=H, R^2=Me
5 R^1=R^2=R^3=R^4=R^5=R^6=H
6 R^1=R^2=R^3=R^4=R^5=H, R^6=β-D-apiosyl
7 R^1=R^3=R^5=H, R^2=R^4=Me, R^6=β-D-apiosyl

ethyl alcohol moiety in the structure was suggested from the fact that signals at δ 6.56 (1H, dd, J=8 and 2 Hz), 6.68 (1H, d, J=8 Hz), 6.70 (1H), and 2.79 (2H, t, J=7.5 Hz) were observed in the ^{1}H-nmr spectrum and was supported from the fact that the ^{13}C-nmr signals (Table 1) corresponding to this moiety were completely identical to those of **4** and **5**. The ^{1}H- and ^{13}C-nmr signals due to the unsaturated ester moiety were similar but slightly different when compared to those of **4**. Comparisons of ^{1}H-nmr data of **1**, **4**, and methyl isoferulate (Table 2) suggested that isoferulate is present as the ester component of **1**. Upon irradiation at δ 3.88 (3H, s, OMe), an nOe (9.3%) was observed for the signal at δ 6.93 (1H, d, J=8 Hz), supporting that the 4′-hydroxy group is methylated. Hydrolysis of **1** with methanolic NaOH gave methyl isoferulate. Hydrolysis of **1** with 2% H_2SO_4 gave glucose and rhamnose. The ^{13}C-nmr spectrum of **1** was completely identical in the sugar region with those of **4** and **5**, indicating that the linkage pattern of the two sugars and the esterification site were identical with those in **4** and **5**. On the basis of the findings mentioned above, phtheirospermoside was elucidated to have the structure 3,4-dihydroxy-β-phenethyl-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-isoferuloyl-β-D-glucopyranoside [**1**].

TABLE 1. ^{13}C-nmr Chemical Shifts (δ)[a] of Phtheirospermoside [**1**].

3,4-Dihydroxyphenethyl alcohol	1	131.4	Glucose	1	104.1
	2	116.3		2	75.9
	3	144.5		3	81.6
	4	146.5		4	70.3
	5	117.1		5	76.1
	6	121.2		6	62.3
	7	36.5	Rhamnose	1	102.8
	8	72.0		2	72.1[b]
Isoferulate moiety	1′	128.7		3	72.2[b]
	2′	112.5		4	73.8
	3′	151.5		5	70.7
	4′	147.9		6	18.4
	5′	122.9	OMe		56.4
	6′	114.9			
	7′	147.5			
	8′	115.7			
	9′	168.0			

[a]The spectrum was taken in CD_3OD.
[b]May be interchanged.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—

TABLE 2. Comparison of ^{1}H-nmr Data of Isoferulate Portion of Phtheirospermoside [**1**] and Leucosceptoside A [**4**] to those of Methyl Isoferulate (measured in CD_3OD).

Proton	Compound		
	1	**4**	Methyl isoferulate
2′-H	7.08	7.19	7.06
5′-H	6.93	6.81	6.91
6′-H	7.07	7.08	7.03
OMe	3.88	3.88	3.76
			3.88

Uv spectra were measured with a Hitachi 330 spectrometer. Ir spectra were taken with a Hitachi 215 spectrometer. ^{1}H- (200 MHz) and ^{13}C- (50.1 MHz) nmr spectra were measured with a JEOL JNM FX-200 spectrometer. TMS was used as the internal standard, and chemical shifts were given in δ (ppm) values. Mass spectra were determined on a JEOL JMS D-300 spectrometer. Optical rotations were measured with a JASCO model ORD/UV-5. Kieselgel 60 PF_{254} (Merck) was used for cc, and precoated Si gel plates (0.25 mm, Merck) were used for tlc. A reversed-phase column (M&S pack C-18B, 20 i.d. × 250 mm) was used for preparative hplc.

PLANT MATERIAL.—The plant material used was collected in early October 1985, in Kisawa Village, Tokushima Prefecture, Japan, and identified as *P. japonicum* by Dr. K. Murakami of this Faculty. A voucher speciman (Y. Takeda No. 3) was deposited in the herbarium of Faculty of Pharmaceutical Sciences, The University of Tokushima, Tokushima, Japan.

ISOLATION PROCEDURES.—Dried aerial parts of *P. japonicum* (210 g) were extracted with MeOH (3.5 liters) at room temperature for 10 days. The extract was concentrated in vacuo. The residue was partitioned between 90% MeOH (300 ml) and *n*-hexane (300 ml × 3). The 90% MeOH layer was concentrated in vacuo and the residue was partitioned between H_2O (300 ml) and EtOAc (300 ml × 3). The EtOAc extract was dried and evaporated in vacuo to give a residue (3.5 g). The aqueous layer was extracted with *n*-BuOH (300 ml × 3). The *n*-BuOH extract was evaporated in vacuo to give a residue (7 g).

The residue from the EtOAc-soluble fraction was subjected to a Si gel (90 g) column with $CHCl_3$/MeOH as eluent. $CHCl_3$-MeOH (9:1, 250 ml), $CHCl_3$-MeOH (85:15, 500 ml), and $CHCl_3$-MeOH (8:2, 1 liter) were eluted successively, collecting 9-ml fractions. Fractions 37–46 gave a residue (138 mg) that was separated by preparative hplc (MeOH-H_2O 1:1, 7 ml/min, detection 230 nm) to give martynoside [**3**] (45 mg) as an amorphous powder. Fractions 56–67 gave a residue (179 mg) that was separated by preparative hplc (MeOH-H_2O, 4:6, 6.5 ml/min, detection 240 nm) to give leucosceptoside A [**4**] (18.9 mg) from the faster eluate and phtheirospermoside [**1**] (30.6 mg) from the slower eluate, both as amorphous powders. Frctions 111–205 were combined and evaporated to give acteoside [**5**] (342 mg) as an amorphous powder.

The residue from the *n*-BuOH-soluble fraction was subjected to Si gel (80 g) cc with $CHCl_3$/MeOH as eluent. $CHCl_3$-MeOH (85:15, 500 ml), $CHCl_3$-MeOH (8:2, 1 liter), and $CHCl_3$-MeOH (7:3, 500 ml) were eluted successively, collecting 8-ml fractions. Fractions 55–70 gave a residue (310 mg), an aliquot (210 mg) of which was purified by preparative hplc (MeOH-H_2O, 1:1, 7 ml/min, detection 230 nm) to give plantarenoside (98 mg) as an amorphous powder. Fractions 101–145 gave acteoside [**5**] (3 g). Fractions 146–165 gave a residue (363 mg) that was subjected to a polyamide C-200 (6 g, Wako) column with H_2O as eluent, collecting 25-ml fractions. On evaporation, fraction 1 gave a residue (147 mg) that was separated on a Si gel (45 g) column with $CHCl_3$/MeOH/H_2O as eluent. $CHCl_3$-MeOH-H_2O (82.5:17.5:1, 500 ml) and $CHCl_3$-MeOH-H_2O (7:3:0.1, 1 liter) were eluted successively, collecting 6-ml fractions. Fractions 75–90 gave aucubin (9 mg), and fractions 111–190 gave geniposidic acid (21 mg) as amorphous powders. Fractions 181–200 of the first column gave a residue (177 mg), an aliquot (118 mg) of which was purified by preparative hplc (H_2O-MeOH, 1:1, 7 ml/min, detection 230 nm) to give forsythoside B [**6**] (43 mg) as an amorphous powder.

PHTHEIROSPERMOSIDE [**1**].—An amorphous powder, $[\alpha]^{23}_D$ −93.1° (*c*=0.2, MeOH); uv λ max (MeOH) nm (ε) 217 (20508), 245 (sh) (11570), 291 (15480), 328 (17670); ir ν max (KBr) cm^{-1} 3650–3100, 1700, 1630, 1610, 1515, 1270, 815; ^{1}H nmr (CD_3OD) δ 1.09 (3H, d, *J*=6 Hz), 2.79 (2H, t, *J*=7.5 Hz), 3.88 (3H, s, OMe), 4.37 (1H, d, *J*=8 Hz), 4.93 (1H, t, *J*=9 Hz), 5.19 (1H, br s), 6.32 (1H, d, *J*=16 Hz), 6.56 (1H, dd, *J*=8, 2 Hz), 6.68 (1H, d, *J*=8 Hz), 6.70 (1H), 6.93 (1H, d, *J*=8 Hz), 7.07 (1H, dd, *J*=8 and 1.5 Hz), 7.08 (1H), 7.62 (1H, d, *J*=16 Hz); ^{13}C nmr see Table 1; fabms *m/z* 661 $[M+Na]^+$ (+NaI) and 677 $[M+K]^+$ (+KI).

KNOWN COMPOUNDS ISOLATED.—Martynoside [**3**], $[\alpha]^{26}_D$ −86.7° (*c*=0.39, MeOH). ^{1}H and ^{13}C nmr were essentially as reported (2). Leucosceptoside A [**4**], $[\alpha]^{26}_D$ −85.3° (*c*=0.25, MeOH); ^{1}H and ^{13}C nmr were essentially as reported (2). Acteoside [**5**], $[\alpha]^{26}_D$ −98.7° (*c*=0.30, MeOH); ^{1}H- and ^{13}C-nmr spectra were identical with those of an authentic sample. Forsythoside B [**6**], $[\alpha]^{26}_D$ −67.2° (*c*=1.37, MeOH), was identified by comparisons of ^{1}H- and ^{13}C-nmr spectra with those of **5** and leucosceptoside B [**7**] (2). Plantarenoside, $[\alpha]^{26}_D$ −120° (*c*=1.05, MeOH); ^{1}H and ^{13}C nmr were essentially as reported (4). Aucubin was identified with an authentic sample by cochromatography on tlc and ^{1}H nmr. Geniposidic acid was identified by ^{1}H-nmr spectra and by conversion to the pentaacetate, the ^{1}H nmr of which was the same as that of authentic specimen.

PHTHEIROSPERMOSIDE OCTAACETATE [**2**].—Acetylation of **1** (5 mg) with a mixture of Ac_2O (0.5 ml) and C_5H_5N (0.5 ml) in the usual way gave the octaacetate **2** (8.5 mg). Ir ν max ($CHCl_3$) cm^{-1} 1760, 1640, 1520, 1380, 1270–1220,

1050; ^{1}H nmr ($CDCl_3$) δ 1.04 (3H, d, J=6.5 Hz), 1.86, 1.93, 2.01, 2.06, 2.08, 2.25, 2.27, 2.30 (each 3H, s, 8×OAc), 2.87 (1H, t, J=6 Hz), 3.86 (3H, s, OMe), 4.40 (1H, d, J=8 Hz), 6.25 (1H, d, J=16 Hz), 6.95 (1H, d, J=8.5 Hz), 7.03 (1H, d, J=8.5 Hz), 7.07 (1H), 7.23 (1H), 7.34 (1H, dd, J=8.5, 2 Hz), 7.62 (1H, d, J=16 Hz); fabms m/z 997 $[M+Na]^+$ (+ NaI) and 1013 $[M+K]^+$ (+KI).

ALKALINE HYDROLYSIS OF PHTHEIROSPERMOSIDE [**1**].—Phtheirospermoside [**1**] (18.7 mg) was dissolved in 0.2 N methanolic NaOH and stirred for 2 h at room temperature under an N_2 atmosphere. The solution was neutralized with Amberlite IR-120B. The ion exchange resin was removed by filtration, and the filtrate was evaporated in vacuo. The residue was partitioned between EtOAc (20 ml) and H_2O (20 ml). The EtOAc layer was dried and evaporated in vacuo to give a residue (1.2 mg). ^{1}H nmr (CD_3OD) δ 3.75 and 3.87 (each 3H, s, 2×OMe), 6.29 (1H, d, J=16 Hz), 6.91 (1H, d, J=8.5 Hz), 7.03 (1H, dd, J=8.5, 2 Hz), 7.06 (1H), 7.56 (1H, d, J=16 Hz); eims m/z 208.0738 $[M]^+$. Calcd for $C_{11}H_{12}O_4$: 208.0736. This compound was identified with an authentic sample of methyl isoferulate by co-chromatography and comparison of ^{1}H nmr.

IDENTIFICATION OF SUGAR PORTION.—Phtheirospermoside [**1**] (2.3 mg) was dissolved in 2% H_2SO_4 aqueous solution (5 ml), and the solution was refluxed for 3 h. The cooled reaction mixture was neutralized with Amberlite IRA-410 (OH form). After the ion exchange resin was removed by filtration, the filtrate was concentrated in vacuo. The residue was subjected to gc (column 1.5% OV-1, 2m; carrier gas N_2, 50 ml/min; column temperature 170°; detection temperature 190°) after trimethylsilylation. Glucose (t_R 12.9 and 19.1 min) and rhamnose (t_R 3.9 and 5.1 min) were identified by comparisons with authentic samples.

ACKNOWLEDGMENTS

The author thanks Dr. K. Murakami of this Faculty for identification of plant material and the staff of Analytical Centre of this Faculty for measurements of nmr and mass spectra.

LITERATURE CITED

1. H. Sasaki, H. Taguchi, T. Endo, I. Yoshioka, K. Higashiyama, and H. Otomasu, *Chem. Pharm. Bull.*, **26**, 2111 (1978).
2. T. Miyase, A. Koizumi, A. Ueno, T. Noro, M. Kuroyanagi, S. Fukushima, Y. Akiyama, and T. Takemoto, *Chem. Pharm. Bull.*, **30**, 2732 (1982).
3. L. Birkofer, C. Kaiser, and U. Thomas, *Z. Naturforsch.*, **23b**, 1051 (1968).
4. Y. Ozaki, S. Johne, and M. Hesse, *Helv. Chim. Acta*, **62**, 2708 (1979).
5. A. Bianco, P. Casiola, M. Guiso, C. Iavarone, and C. Trogolo, *Gazz. Chim. Ital.*, **111**, 201 (1981).
6. P. Karrer and H. Schmid, *Helv. Chim. Acta*, **29**, 525 (1946).
7. Y. Takeda, H. Nishimura, and H. Inouye, *Phytochemistry*, **14**, 2647 (1975).
8. R. Guarnaccia, K.M. Madyastha, E. Tegtmeyer, and C.J. Coscia, *Tetrahedron Lett.*, 5152 (1972).
9. K. Endo, K. Takahashi, T. Abe, and H. Hikino, *Heterocycles*, **19**, 261 (1982).

Received 30 July 1987