Petiolins F—I, Benzophenone Rhamnosides from *Hypericum pseudopetiolatum* var. *kiusianum*

Naonobu TANAKA,^a Takaaki KUBOTA,^a Yoshiki KASHIWADA,^b Yoshihisa TAKAISHI,^b and Jun'ichi KOBAYASHI^{*,a}

^a Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060–0812, Japan; and ^b Graduate School of Pharmaceutical Sciences, University of Tokushima, Tokushima 770–8505, Japan.

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Four new benzophenone-*O*-rhamnosides, petiolins F—I (1–4), were isolated from aerial parts of *Hypericum pseudopetiolatum* var. *kiusianum*, and the structures were elucidated by spectroscopic data and chemical means.

Key words *Hypericum pseudopetiolatum* var. *kiusianum*; benzophenone; rhamnoside; Clusiaceae

The genus *Hypericum* (family Clusiaceae) are known to be a traditional medicine for the treatment of burns, bruises, swelling, inflammation, and anxiety as well as bacterial and viral infections.^{1–4} In our continuing search for new compounds from *Hypericum* spp.,^{5–7} four new benzophenone-*O*-rhamnosides, petiolins F—I (1–4), were isolated from aerial parts of *H. pseudopetiolatum* var. *kiusianum*. In this paper, we describe the isolation and structure elucidation of petiolins F—I (1–4).

The aerial parts of *H. pseudopetiolatum* var. *kiusianum* were extracted with MeOH, and the extracts were partitioned successively with *n*-hexane, EtOAc, and H₂O. EtOAc-soluble portions were subjected to a Sephadex LH-20 column (H₂O/MeOH), a Toyopearl HW-40F column (H₂O/MeOH), and a silica gel column (CHCl₃/MeOH) chromatographies to afford a mixture of benzophenone glycosides, which was purified by C₁₈ HPLC (MeOH/H₂O) to yield petiolins F (1, 0.0012%), G (2, 0.0038%), H (3, 0.0006%), and I (4, 0.0004%).

The molecular formula of petiolin F (1), C₁₉H₂₀O₁₀, was established by HR-electrospray ionization (ESI)-MS [*m/z* 431.0945 (M+Na)⁺, Δ −0.9 mmu]. IR absorptions at 3421 and 1629 cm^{−1} implied the presence of hydroxy and carbonyl functionalities. The ¹H-NMR spectrum showed proton signals of a 1,3,5-trisubstituted benzene ring [δ_{H} 6.58 (2H, d, *J*=2.3 Hz), 6.51 (1H, t, *J*=2.3 Hz)], a 1,2,3,5-tetrasubstituted benzene ring [δ_{H} 6.37 and 6.12 (1H each, d, *J*=2.0 Hz)], an anomeric proton [δ_{H} 5.26 (1H, d, *J*=1.5 Hz)], and a secondary methyl group [1.16 (3H, d, *J*=6.3 Hz)] (Table 1). The ¹³C-NMR spectrum revealed the presence of a carbonyl (δ_{C} 198.2) and 12 aromatic carbons, together with resonances for

a sugar moiety (Table 1). From these data, 1 was presumed to be a benzophenone glycoside. ¹³C-NMR chemical shifts of the sugar moiety were coincident with those of quercetin-3-*O*- α -rhamnoside.⁸ The aglycone of 1 was assigned as 2',3,4',5,6'-pentahydroxybenzophenone on the basis of heteronuclear multiple bond correlations (HMBC) (Fig. 1) and coupling patterns of aromatic protons in the ¹H-NMR (Table 1). The HMBC correlation for H-1'' to C-2' indicated that the rhamnosyl moiety was connected to C-2' through an oxygen atom, and its α -glycoside linkage was derived from the value for ¹*J*_{C,H} (172 Hz) of C-1'' obtained from the non-decoupled heteronuclear single quantum coherence (HSQC) spectrum.⁹ Methanolysis of petiolin F (1) yielded methyl α -rhamnopyranoside, which was assigned as L-form by comparison of its optical rotation with that of authentic methyl α -L-rhamnopyranoside. Thus, the structure of 1 was elucidated to be 2',3,4',5,6'-pentahydroxybenzophenone-2'-*O*- α -L-rhamnoside.

Petiolin G (2) showed the pseudomolecular ion peak at *m/z* 473 (M+Na)⁺ in the ESI-MS, and the HR-ESI-MS revealed the molecular formula to be C₂₁H₂₂O₁₁. Although ¹H- and

Table 1. ¹H- and ¹³C-NMR Data for Petiolins F (1) and G (2) in Acetone-*d*₆

Position	1		2	
	¹³ C	¹ H	¹³ C	¹ H
1	143.9	—	143.8	—
2, 6	106.7	6.58 (2H, d, <i>J</i> =2.3)	106.4	6.59 (2H, d, <i>J</i> =2.2)
3, 5	158.5	—	158.6	—
4	106.1	6.51 (1H, t, <i>J</i> =2.3)	106.0	6.52 (1H, d, <i>J</i> =2.2)
1'	106.8	—	106.8	—
2'	159.3	—	158.9	—
3'	94.8	6.37 (1H, d, <i>J</i> =2.0)	94.4	6.33 (1H, d, <i>J</i> =2.1)
4'	163.9	—	163.8	—
5'	97.0	6.12 (1H, d, <i>J</i> =2.0)	97.0	6.13 (1H, d, <i>J</i> =2.1)
6'	163.1	—	163.1	—
C=O	198.2	—	198.0	—
1''	99.5	5.26 (1H, d, <i>J</i> =1.5)	98.9	5.33 (1H, br s)
2''	70.4	3.39 (1H, br s)	70.3	3.51 (1H, br s)
3''	71.2	3.00 (1H, dd, <i>J</i> =9.3, 3.5)	68.7	3.08 (1H, dd, <i>J</i> =9.8, 3.4)
4''	72.8	3.30 (1H, t, <i>J</i> =9.3)	73.9	4.79 (1H, t, <i>J</i> =9.8)
5''	69.9	3.42 (1H, dq, <i>J</i> =9.3, 6.3)	67.7	3.53 (1H, dq, <i>J</i> =9.8, 6.4)
6''	17.6	1.16 (3H, d, <i>J</i> =6.3)	17.3	1.03 (3H, d, <i>J</i> =6.4)
–OAc	—	—	170.4	—
			20.3	2.02 (3H, s)

Coupling constants given (*J*, Hz) in parentheses.

Chart 1. Petiolins F–I (1–4)

* To whom correspondence should be addressed. e-mail: jkobay@pharm.hokudai.ac.jp

^{13}C -NMR resonances of **2** were similar to those of **1**, additional signals due to an acetoxy group [δ_{H} 2.02 (3H, s); δ_{C} 170.4 and 20.3] were observed in **2** (Table 1). From these data, **2** was estimated to be 2',3,4',5,6'-pentahydroxybenzophenone-2'-*O*- α -L-rhamnoside possessing an acetoxy group. The HMBC cross-peak of H-4'' to acetoxy carbonyl carbon and a low-field shift of H-4'' (δ_{H} 4.79 in **2**; δ_{H} 3.30 in **1**) indicated that the acetoxy group was connected to C-4''. The rhamnose moiety was assigned as L-form by the same procedure as described for **1**. Thus, the structure of **2** was assigned as 2',3,4',5,6'-pentahydroxybenzophenone-2'-*O*-(4''-acetoxy)- α -L-rhamnoside.

Petiolin H (**3**) had a molecular formula of $\text{C}_{28}\text{H}_{26}\text{O}_{12}$ deduced from HR-ESI-MS. The ^1H - and ^{13}C -NMR data (Table 2) revealed the presence of a 2',3,4',5,6'-pentahydroxybenzophenone moiety, an acetoxy group, a benzoyl group, and a rhamnosyl moiety. Connectivities of the acetoxy group and the benzoyl group to the rhamnosyl moiety were elucidated by HMBC correlations for H-3'' to the carbonyl carbon of the benzoyl group (δ_{C} 165.0), and H-4'' to acetoxy carbonyl carbon (δ_{C} 170.0), respectively. The HMBC cross-peak of H-1'' to C-2' and $^1J_{\text{C,H}}$ value (174 Hz) of C-1'' indicated the connectivity of C-2' and C-1'' by an α -glycoside linkage. The

Fig. 1. Selected 2D NMR Correlations for Petiolin F (**1**)

Table 2. ^1H - and ^{13}C -NMR Data for Petiolins H (**3**) and I (**4**) in Acetone- d_6

Position	3		4	
	^{13}C	^1H	^{13}C	^1H
1	143.5	—	143.8	—
2, 6	107.0	6.68 (2H, d, $J=2.3$)	106.5	6.66 (2H, d, $J=2.2$)
3, 5	158.6	—	158.9	—
4	106.4	6.46 (1H, t, $J=2.3$)	106.3	6.58 (1H, t, $J=2.2$)
1'	107.6	—	106.3	—
2'	158.8	—	158.6	—
3'	94.7	6.38 (1H, d, $J=2.0$)	94.8	6.34 (1H, d, $J=2.0$)
4'	163.9	—	164.4	—
5'	97.2	6.17 (1H, d, $J=2.0$)	97.6	6.17 (1H, d, $J=2.0$)
6'	163.4	—	163.8	—
C=O	197.8	—	197.9	—
1''	99.3	5.41 (1H, d, $J=1.8$)	96.2	5.57 (1H, d, $J=1.6$)
2''	68.0	3.99 (1H, brs)	72.8	5.07 (1H, dd, $J=3.6, 1.6$)
3''	72.9	4.59 (1H, dd, $J=10.1, 3.2$)	67.1	3.87 (1H, dd, $J=9.9, 3.6$)
4''	70.6	5.24 (1H, t, $J=10.1$)	74.1	4.96 (1H, t, $J=9.9$)
5''	68.0	3.72 (1H, dq, $J=10.1, 6.4$)	68.2	3.63 (1H, dq, $J=9.9, 6.4$)
6''	17.4	1.12 (3H, d, $J=6.4$)	17.6	1.11 (3H, dd, $J=6.4$)
-OAc	170.0	—	170.4	—
	20.2	1.96 (3H, s)	20.5	2.08 (3H, s)
-OBz	165.0	—	165.7	—
	130.7	—	130.4	—
	133.3	7.62 (1H, tt, $J=8.4, 1.3$)	133.7	7.66 (1H, tt, $J=7.8, 1.9$)
	129.9 $\times$ 2	7.49 (2H, t, $J=8.4$)	130.0 $\times$ 2	7.54 (2H, t, $J=7.8$)
	128.7 $\times$ 2	7.94 (2H, dd, $J=8.4, 1.3$)	128.9 $\times$ 2	8.05 (2H, dd, $J=7.8, 1.9$)

Coupling constants given (J , Hz) in parentheses.

rhamnose moiety was elucidated to be L-form in the same manner as described for **1**. Thus, the structure of **3** was elucidated to be 2',3,4',5,6'-pentahydroxybenzophenone-(4''-acetoxy-3''-benzoyl)-*O*- α -L-rhamnoside.

Petiolin I (**4**) had the same molecular formula as that of **3**. The ^1H - and ^{13}C -NMR spectral data of **4** (Table 2) revealed the presence of the same functional groups as found in **3**, while differences were observed for the proton resonances for the rhamnosyl moiety. The chemical shifts of H-2'' (δ_{H} 5.07) and H-4'' (δ_{H} 4.96) suggested that a benzoyl and an acetoxy groups were attached to C-2'' and C-4'', respectively. Positions of the acetoxy group and the benzoyl group were assigned as C-4'' and C-2'', respectively, by the HMBC cross-peaks of H-4'' to the acetoxy carbonyl carbon (δ_{C} 170.4) and H-2'' to the benzoyl carbonyl carbon (δ_{C} 165.7). The L-form of rhamnose moiety of **4** was assigned by the same procedure as described for **1**. Thus, the structure of **4** was elucidated to be 2',3,4',5,6'-pentahydroxybenzophenone-(4''-acetoxy-2''-benzoyl)-*O*- α -L-rhamnoside.

Experimental

General Optical rotations were recorded on a JASCO P-1030 digital polarimeter. IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometers, respectively. NMR spectra were measured with a JEOL ECA 500 spectrometer. The 2.05 and 205.7 ppm resonances of residual acetone were used as internal references for ^1H - and ^{13}C -NMR spectra, respectively. ESI-MS spectra were recorded on a JEOL JMS-T100LP.

Plant Material *Hypericum pseudopetiolatum* var. *kisianum* was collected in Kochi Prefecture, Japan in August 2005. Herbarium specimens were deposited in the botanical garden of the University of Tokushima (specimen number: UTP98013).

Extraction and Isolation The aerial parts of *H. pseudopetiolatum* var. *kisianum* (320 g) were extracted with MeOH (31 $\times$ 3), and the extracts were partitioned successively with *n*-hexane (300 ml $\times$ 3), EtOAc (300 ml $\times$ 3), and H₂O (300 ml). The EtOAc-soluble portions were subjected to a Sephadex LH-20 column (H₂O/MeOH, 10/0 to 0/10), a Toyopearl HW-40F column (H₂O/MeOH, 9/1 to 0/10), a silica gel column (CHCl₃/MeOH, 95/5 to 0/10), and C₁₈ reversed-phase HPLC (Mighty sil RP-18, Kanto Chemical Co., Ltd., 10 $\times$ 250 mm; flow rate 3.0 ml/min; UV detection at 254 nm; eluent MeOH/H₂O, 3 : 7) to afford petiolins F–I (**1**, 3.9 mg; **2**, 12.1 mg; **3**, 1.9 mg; **4**, 1.4 mg).

Petiolin F (1): Colorless amorphous solids; $[\alpha]_{\text{D}}^{23} +5.8$ ($c=0.87$ MeOH); UV (MeOH) λ_{max} 280 (ϵ 4650) and 307 (5730) nm; IR (KBr) ν_{max} 3421 and 1629 cm^{-1} ; ^1H - and ^{13}C -NMR data (Table 1); ESI-MS m/z : 431 ($\text{M}+\text{Na}^+$); HR-ESI-MS m/z : 431.0945 ($\text{M}+\text{Na}^+$) (Calcd for $\text{C}_{19}\text{H}_{20}\text{O}_{10}\text{Na}$, 431.0954).

Petiolin G (2): Colorless amorphous solids; $[\alpha]_{\text{D}}^{23} -4.9$ ($c=2.45$ MeOH); UV (MeOH) λ_{max} 277 (ϵ 6920) and 308 (8340) nm; IR (KBr) ν_{max} 3407, 1723, and 1627 cm^{-1} ; ^1H - and ^{13}C -NMR data (Table 1); ESI-MS m/z : 473 ($\text{M}+\text{Na}^+$); HR-ESI-MS m/z : 473.1048 ($\text{M}+\text{Na}^+$) (Calcd for $\text{C}_{21}\text{H}_{22}\text{O}_{11}\text{Na}$, 473.1060).

Petiolin H (3): Colorless amorphous solids; $[\alpha]_{\text{D}}^{23} -54.0$ ($c=0.38$ MeOH); UV (MeOH) λ_{max} 281 (ϵ 8660) and 306 (8800) nm; IR (KBr) ν_{max} 3417, 1723, and 1627 cm^{-1} ; ^1H - and ^{13}C -NMR data (Table 2); ESI-MS m/z : 577 ($\text{M}+\text{Na}^+$); HR-ESI-MS m/z : 577.1323 ($\text{M}+\text{Na}^+$) (Calcd for $\text{C}_{28}\text{H}_{26}\text{O}_{12}\text{Na}$, 577.1322).

Petiolin I (4): Colorless amorphous solids; $[\alpha]_{\text{D}}^{23} -19.2$ ($c=0.27$ MeOH); UV (MeOH) λ_{max} 275 (ϵ 8880) and 305 (8070) nm; IR (KBr) ν_{max} 3442, 1727, and 1619 cm^{-1} ; ^1H - and ^{13}C -NMR data (Table 2); ESI-MS m/z : 577 ($\text{M}+\text{Na}^+$); HR-ESI-MS m/z : 577.1331 ($\text{M}+\text{Na}^+$) (Calcd for $\text{C}_{28}\text{H}_{26}\text{O}_{12}\text{Na}$, 577.1322).

Methanolysis of Petiolins F–I (1–4) Petiolins F–I (**1–4**, 0.7, 0.7, 0.5, and 0.5 mg, respectively) were treated with 5% HCl/MeOH (50 ml) at 100 $^{\circ}\text{C}$ for 16 h, individually. After evaporation of the solvent, the residue of each sample was subjected to a silica gel column (EtOAc/MeOH/H₂O, 20 : 3 : 2) to give methyl α -rhamnopyranoside {from **1**: 0.13 mg, $[\alpha]_{\text{D}}^{23} -67.9$ ($c=0.03$, MeOH); from **2**: 0.22 mg, $[\alpha]_{\text{D}}^{23} -61.0$ ($c=0.08$, MeOH); from **3**: 0.15 mg, $[\alpha]_{\text{D}}^{23} -71.4$ ($c=0.04$, MeOH); from **4**: 0.17 mg, $[\alpha]_{\text{D}}^{23} -74.4$ ($c=0.04$, MeOH)} and 2',3,4',5,6'-pentahydroxybenzophenone. 2',3,4',5,6'-Pentahydroxybenzophenone: ^1H -NMR (acetone- d_6) δ_{H} : 6.59 (2H, d,

$J=1.6$ Hz), 6.47 (1H, t, $J=1.6$ Hz), 5.96 (2H, s); HR-ESI-MS m/z : 285.0382 ($M+Na$)⁺ (Calcd for C₁₃H₁₀O₆Na, 285.0375). Authentic L-rhamnose was treated with 5% HCl/MeOH as described above to afford methyl α -L-rhamnopyranoside {[α]_D −64.8 ($c=0.19$, MeOH)}. R_f values of methyl α -L-rhamnopyranosides derived from **1**–**4** were consistent with that of authentic methyl α -L-rhamnopyranoside (R_f value: 0.66, silica gel TLC, EtOAc/MeOH/H₂O, 20:3:2).

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