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

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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.

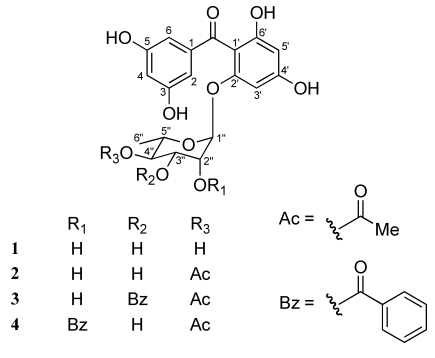
Key words *Hypericum pseudopetiolatum* var. *kiusianum*; benzophenone; rhamnoside; Clusiaceae

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 H_2O. EtOAc-soluble portions were subjected to a Sephadex LH-20 column (H_2O/MeOH), a Toyopearl HW-40F column (H_2O/MeOH), and a silica gel column ($CHCl_3$/MeOH) chromatographies to afford a mixture of benzophenone glycosides, which was purified by C_{18} HPLC (MeOH/H_2O) 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**), $C_{19}H_{20}O_{10}$, 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 ^{1}H-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 ^{13}C-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. ^{13}C-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 ^{1}H-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 $^1J_{C,H}$ (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 $C_{21}H_{22}O_{11}$. Although ^{1}H- and



	R_1	R_2	R_3
1	H	H	H
2	H	H	Ac
3	H	Bz	Ac
4	Bz	H	Ac

Chart 1. Petiolins F—I (**1**—**4**)

Table 1. ^{1}H- and ^{13}C-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, J=2.3)	106.4	6.59 (2H, d, J=2.2)
3, 5	158.5	—	158.6	—
4	106.1	6.51 (1H, t, J=2.3)	106.0	6.52 (1H, d, J=2.2)
1′	106.8	—	106.8	—
2′	159.3	—	158.9	—
3′	94.8	6.37 (1H, d, J=2.0)	94.4	6.33 (1H, d, J=2.1)
4′	163.9	—	163.8	—
5′	97.0	6.12 (1H, d, J=2.0)	97.0	6.13 (1H, d, J=2.1)
6′	163.1	—	163.1	—
C=O	198.2	—	198.0	—
1″	99.5	5.26 (1H, d, J=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, J=9.3, 3.5)	68.7	3.08 (1H, dd, J=9.8, 3.4)
4″	72.8	3.30 (1H, t, J=9.3)	73.9	4.79 (1H, t, J=9.8)
5″	69.9	3.42 (1H, dq, J=9.3, 6.3)	67.7	3.53 (1H, dq, J=9.8, 6.4)
6″	17.6	1.16 (3H, d, J=6.3)	17.3	1.03 (3H, d, J=6.4)
–OAc	—	—	170.4	—
			20.3	2.02 (3H, s)

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

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^{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-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 $C_{28}H_{26}O_{12}$ deduced from HR-ESI-MS. The ^{1}H- 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 $^1J_{C,H}$ value (174 Hz) of C-1″ indicated the connectivity of C-2′ and C-1″ by an α-glycoside linkage. The 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.

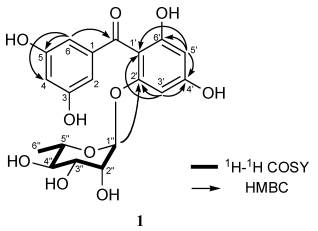

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Fig. 1. Selected 2D NMR Correlations for Petiolin F (**1**)

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

Position	**3**		**4**	
	^{13}C	^{1}H	^{13}C	^{1}H
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, br s)	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, d, *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)
	129.9×2	7.49 (2H, t, *J*=8.4)	130.0×2	7.54 (2H, t, *J*=7.8)
	128.7×2	7.94 (2H, dd, *J*=8.4, 1.3)	128.9×2	8.05 (2H, dd, *J*=7.8, 1.9)

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

Petiolin I (**4**) had the same molecular formula as that of **3**. The ^{1}H- 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 ^{1}H- and ^{13}C-NMR spectra, respectively. ESI-MS spectra were recorded on a JEOL JMS-T100LP.

Plant Material *Hypericum pseudopetiolatum* var. *kiusianum* 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. *kiusianum* (320 g) were extracted with MeOH (3 l×3), and the extracts were partitioned successively with *n*-hexane (300 ml×3), EtOAc (300 ml×3), and H_2O (300 ml). The EtOAc-soluble portions were subjected to a Sephadex LH-20 column (H_2O/MeOH, 10/0 to 0/10), a Toyopearl HW-40F column (H_2O/MeOH, 9/1 to 0/10), a silica gel column ($CHCl_3$/MeOH, 95/5 to 0/10), and C_{18} reversed-phase HPLC (Mighty sil RP-18, Kanto Chemical Co., Ltd., 10×250 mm; flow rate 3.0 ml/min; UV detection at 254 nm; eluent MeOH/H_2O, 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; $[\alpha]_D^{23}$ +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}; ^{1}H- and ^{13}C-NMR data (Table 1); ESI-MS *m/z*: 431 $(M+Na)^+$; HR-ESI-MS *m/z*: 431.0945 $(M+Na)^+$ (Calcd for $C_{19}H_{20}O_{10}Na$, 431.0954).

Petiolin G (**2**): Colorless amorphous solids; $[\alpha]_D^{23}$ −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}; ^{1}H- and ^{13}C-NMR data (Table 1); ESI-MS *m/z*: 473 $(M+Na)^+$; HR-ESI-MS *m/z*: 473.1048 $(M+Na)^+$ (Calcd for $C_{21}H_{22}O_{11}Na$, 473.1060).

Petiolin H (**3**): Colorless amorphous solids; $[\alpha]_D^{23}$ −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}; ^{1}H- and ^{13}C-NMR data (Table 2); ESI-MS *m/z*: 577 $(M+Na)^+$; HR-ESI-MS *m/z*: 577.1323 $(M+Na)^+$ (Calcd for $C_{28}H_{26}O_{12}Na$, 577.1322).

Petiolin I (**4**): Colorless amorphous solids; $[\alpha]_D^{23}$ −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}; ^{1}H- and ^{13}C-NMR data (Table 2); ESI-MS *m/z*: 577 $(M+Na)^+$; HR-ESI-MS *m/z*: 577.1331 $(M+Na)^+$ (Calcd for $C_{28}H_{26}O_{12}Na$, 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 °C for 16 h, individually. After evaporation of the solvent, the residue of each sample was subjected to a silica gel column (EtOAc/MeOH/H_2O, 20 : 3 : 2) to give methyl α-rhamnopyranoside {from **1**: 0.13 mg, $[\alpha]_D^{23}$ −67.9 (*c*=0.03, MeOH); from **2**: 0.22 mg, $[\alpha]_D^{23}$ −61.0 (*c*=0.08, MeOH); from **3**: 0.15 mg, $[\alpha]_D^{23}$ −71.4 (*c*=0.04, MeOH); from **4**: 0.17 mg, $[\alpha]_D^{23}$ −74.4 (*c*=0.04, MeOH)} and 2′,3,4′,5,6′-pentahydroxybenzophenone. 2′,3,4′,5,6′-Pentahydroxybenzophenone: ^{1}H-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 $C_{13}H_{10}O_6Na$, 285.0375). Authentic L-rhamnose was treated with 5% HCl/MeOH as described above to afford methyl α-L-rhamnopyranoside {$[\alpha]_D$ −64.8 (c=0.19, MeOH)}. *Rf* values of methyl α-L-rhamnopyranosides derived from **1**—**4** were consistent with that of authentic methyl α-L-rhamnopyranoside (*Rf* value: 0.66, silica gel TLC, EtOAc/MeOH/H_2O, 20 : 3 : 2).

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