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—3 In our continuing search for new compounds from *Hypericum* spp.; 3—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 by 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: −0.0004%]. IR absorptions at 3421 and 1629 cm−1 indicated the presence of hydroxy and carbonyl functionalities. The 1H-NMR spectrum showed proton signals of a 1,3,5-trisubstituted benzene ring [δH 6.58 (2H, d, J=2.3 Hz), 6.51 (1H, t, J=2.3 Hz)], a 1,2,3,5-tetrasubstituted benzene ring [δH 6.37 and 6.12 (1H each, d, J=2.0 Hz)], an anomic proton [δH 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 (δC 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 J1,2’H (172 Hz) of C-1” obtained from the non-decoupled heteronuclear single quantum coherence (HSQC) spectrum. 9 Methanalysis of petiolin F (1) yielded methyl α-hexamethylenemalonate, which was assigned as 1-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.

Pettolin 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 13C-NMR data for petiolins F (1) and G (2) in Acetone-d6 are shown in Table 1.

![Chart 1. Petiolins F—I (1—4)](image)
The rhamnose moiety was assigned as L-form by the same procedure as described for I. Thus, the structure of 2 was ascribed to be 2',3',4',5,6'-pentahydroxybenzophenone-2'-O-α-rhamnopyranoside (Fig. 1; Table 1). From these data, 2 was estimated to be 2',3',4',5,6'-pentahydroxybenzophenone-2'-O-α-rhamnose possessing an acetoxy group. The HMBC cross-peak of H-4" to acetoxy carbonyl carbon and a low-field shift of H-4" (∆δH 4.79 in 2; ∆δH 3.30 in I) 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 I. Thus, the structure of 2 was ascribed to be 2',3',4',5,6'-pentahydroxybenzophenone-2'-O-α-rhamnose.

Petiolin H (3) had a molecular formula of C28H26O12 deduced from HR-ESI-MS. The 1H- and 13C-NMR data (Table 1) 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 (∆δC 165.0), and H-4" to acetoxy carbonyl carbon (∆δC 170.0), respectively. The HMBC cross-peak of H-1" to C-2" and 1JCH 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 I. Thus, the structure of 3 was elucidated to be 2',3',4',5,6'-pentahydroxybenzophenone-4'-acetoxyl-3'-benzoyl)-O-α-l-rhamnose.

Petiolin I (4) had the same molecular formula as that of 3. The 1H- and 13C-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" (∆δH 5.07) and H-4" (∆δH 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 2"- and 3"-pentahydroxybenzophenone-(4'-acetoxyl-3'-benzoyl)-O-α-l-rhamnose.

Experimental

General: Optical rotations were recorded on a JASCO P-1030 digital polarimeter. IR and UV spectra were recorded on a 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 13C-NMR spectra, respectively. ESI-MS spectra were recorded on a JEOL JMS-T100LP.

Plant Material: Hypericum pseudopetiolatum var. kiussianum 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. kiussianum (320 g) were extracted with MeOH (31×3), and the extracts were partitioned successively with n-hexane (300 ml×3), EtOAc (300 ml×3), and H2O (300 ml). The EtOAc-soluble portions were subjected to a Sephadex LH-20 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., 10×250 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, 1.12 mg, 3, 1.9 mg, 4, 1.4 mg).

Petiolin F (1): Colorless amorphous solids; [α]23D +5.8 (c = 0.87 MeOH); UV (MeOH) λmax 280 (ε 4650) and 307 (5730) nm; IR (KBr) νmax 3421 and 1629 cm⁻¹; 1H- and 13C-NMR data (Table 1); ESI-MS m/z 431 (M+Na)⁺; HR-ESI-MS m/z 431.0945 (M+Na)⁺ (Calcd for C19H20O10Na, 431.0954).

Petiolin G (2): Colorless amorphous solids; [α]23D -4.9 (c = 2.45 MeOH); UV (MeOH) λmax 277 (ε 6920) and 308 (8340) nm; IR (KBr) νmax 3407, 1723, and 1627 cm⁻¹; 1H- and 13C-NMR data (Table 1); ESI-MS m/z 473 (M+Na)⁺; HR-ESI-MS m/z 473.1048 (M+Na)⁺ (Calcd for C22H25O11Na, 473.1060).

Petiolin H (3): Colorless amorphous solids; [α]23D -54.0 (c = 0.38 MeOH); UV (MeOH) λmax 281 (ε 8660) and 306 (8800) nm; IR (KBr) νmax 3417, 1723, and 1627 cm⁻¹; 1H- and 13C-NMR data (Table 2); ESI-MS m/z 577 (M+Na)⁺; HR-ESI-MS m/z 577.1323 (M+Na)⁺ (Calcd for C22H25O11Na, 577.1322).

Petiolin I (4): Colorless amorphous solids; [α]23D +19.2 (c = 0.27 MeOH); UV (MeOH) λmax 275 (ε 8880) and 305 (8070) nm; IR (KBr) νmax 3442, 1727, and 1619 cm⁻¹; 1H- and 13C-NMR data (Table 2); ESI-MS m/z 577 (M+Na)⁺; HR-ESI-MS m/z 577.1331 (M+Na)⁺ (Calcd for C22H25O11Na, 577.1322).

Meethanolysis of Petiolins F-I (1-4): Petiolins F-I (1-4, 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/H2O, 20:3:2) to give methyl α-rhamnopyranoside (from I, 0.13 mg, [α]23D -67.9 (c = 0.03, MeOH); from 2: 0.02 mg, [α]23D -61.0 (c = 0.08, MeOH); from 3, 0.15 mg, [α]23D -71.4 (c = 0.04, MeOH); from 4: 0.17 mg, [α]23D -74.4 (c = 0.04, MeOH) and 2',3',4',5,6'-pentahydroxybenzophenone. 2',3',4',5,6'-Pentahydroxybenzophenone: 1H-NMR (acetone-d6) δH 6.52 (2H, d, d, H = 3.5 Hz). The coupling constants are given in parentheses.
$J = 1.6 \text{ Hz}$), 6.47 (1H, $J = 1.6 \text{ Hz}$), 5.96 (2H, s); HR-ESI-MS $m/z$: 285.0382 (M+Na)$^+$ (Calcd for C$_{13}$H$_{10}$O$_6$Na, 285.0375). Authentic $\alpha$-rhamnose was treated with 5% HCl/MEOH as described above to afford methyl $\alpha$-$\alpha$-rhamnopyranoside ($[\alpha]_D -64.8 \ (c = 0.19, \text{MeOH})$). $R_f$ values of methyl $\alpha$-$\alpha$-rhamnopyranosides derived from 1—4 were consistent with that of authentic methyl $\alpha$-$\alpha$-rhamnopyranoside ($R_f$ value: 0.66, silica gel TLC, EtOAc/MeOH/H$_2$O, 20 : 3 : 2).

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References