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PETIOLINS D AND E, PHLOROGLUCINOL DERIVATIVES FROM *HYPERICUM PSEUDOPETIOLATUM* VAR. *KIUSIANUM*

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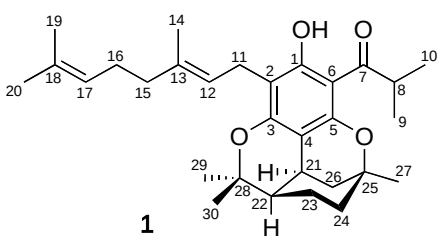
Abstract - A new phloroglucinol derivative possessing citran skeleton, petiolin D (**1**), and a new chromone glucoside, petiolin E (**2**), were isolated from aerial parts of *Hypericum pseudopetiolatum* var. *kiusianum.* The structures of **1** and **2** were elucidated by spectroscopic data, and a single-crystal X-ray diffraction analysis of **1** revealed that **1** was a racemic mixture.

INTRODUCTION

The plants, belonging to 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.[1,2,3,4] In our continuing search for bioactive compounds from *Hypericum* spp,[5,6,7,8,9,10] we previously isolated new phloroglucinol derivatives, petiolins A – C, from the aerial parts of *Hypericum pseudopetiolatum* var. *kiusianum*.[11] Further investigation of extracts from this plant resulted in the isolation of a new phloroglucinol derivative possessing citran skeleton, petiolin D (**1**), and a new chromone glucoside, petiolin E (**2**), were isolated from aerial parts of *H. pseudopetiolatum* var. *kiusianum*. In this paper, we describe the isolation and structure elucidation of **1** and **2**.



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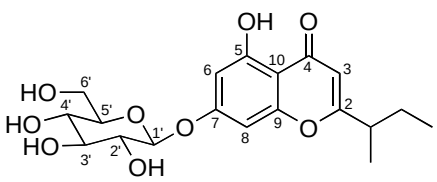

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RESULTS AND DISCUSSION

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. *n*-Hexane-soluble portions were subjected to a silica gel column (*n*-hexane/EtOAc) and then a Sephadex LH-20 column (EtOH) chromatographies to afford a mixture of phloroglucinol derivatives, which was purified by a C_{18} column (MeOH/H_2O) and C_{18} HPLC (MeOH/H_2O) to yield petiolin D (**1**, 0.0008%). EtOAc-soluble portions were applied to a Sephadex LH-20 column (MeOH/H_2O), a C_{18} column (MeOH/H_2O), a silica gel column ($CHCl_3$/MeOH) chromatographies, and C_{18} HPLC (MeOH/H_2O) to give petiolin E (**2**, 0.0008%).

The molecular formula of petiolin D (**1**), $C_{30}H_{42}O_4$, was established by HRESIMS [*m/z* 489.2973 $(M+Na)^+$, Δ -0.8 mmu]. ^1H and ^{13}C NMR data (Table 1) of **1** revealed the presence of one hydrogen-bonded hydroxyl (δ_H 14.07), one fully substituted benzene ring (δ_C 163.1, 160.4, 156.0, 110.8, 106.0, and 104.9), two trisubstituted olefins [δ_H 5.20 and 5.08 (each 1H, t, J = 7.0 Hz); δ_C 134.2, 131.0, 124.5, and 122.9], one 2-methylpropanoyl group [δ_H 3.79 (1H, sept, J = 6.5 Hz), 1.19 and 1.18 (each 3H, d, J = 6.5 Hz); δ_C 209.8, 38.9, 19.6, and 19.1], two sp^3 quaternary carbons attached to an oxygen atom [δ_C 84.5 and 75.9], two methines [δ_H 2.82 (1H, brs) and 2.20 (1H, m); δ_C 45.9 and 27.6], three methylenes [δ_H 2.18 (1H, ddd, J = 13.2, 4.4, 3.0 Hz), 1.86 (1H, dd, J = 13.2, 1.5 Hz), 1.82, 1.47, 1.32, 0.90 (each 1H, m); δ_C 37.4, 34.8, and 21.9], and three tertiary methyl groups [δ_H 1.55, 1.43, and 1.06 (each 3H, s); δ_C 29.6, 28.7, and 24.2]. The presence of a geranyl group was implied by ^1H–^1H COSY correlations of H_2-11 to H-12 and H_2-15 to H-17, HMBC correlations of H_3-14 to C-12, C-13, and C-15, H_3-19 to C-17, C-18, and C-20, and NOESY correlations of H_2-11 to H_3-14 and H-12 to H_2-15. The ^1H–^1H COSY spectrum suggested the connectivity of C-24 to C-26. HMBC correlations of H_3-27 to C-24, C-25, and C-26 indicated that an oxygenated sp^3 quaternary carbon (C-25) was attached to C-24, C-26, and C-27. Connectivities of C-28 to C-22, C-29, and C-30 were deduced from HMBC correlations of H_3-30 to C-22, C-28, and C-29. ^{13}C NMR chemical shifts of a benzene ring suggested the 1,3,5-trihydroxy substitution. HMBC correlations of H_2-11 to C-1, C-2, and C-3, and H-21 to C-4 indicated that C-11 and C-21 were attached to C-2 and C-4, respectively (Figure 1). The molecular formula and the unsaturation degree of **1** implied that C-3 and C-5 were connected to C-25 or C-28 through an ether linkage, respectively.

Petiolin D (**1**) was crystallized from methanol/water as colorless platelets. A single-crystal X-ray diffraction analysis of **1** revealed the structure and relative stereochemistry. This crystal consisted of a pair of enantiomers, suggesting that **1** was a racemate. The ORTEP drawing of one enantiomer of **1** was shown in Figure. 2. Thus, the structure of petiolin D was elucidated to be **1**, a new phloroglucinol derivative possessing citran skeleton.

Table 1. ^{1}H and ^{13}C NMR Data for Petiolin D (**1**) in $CDCl_3$.

position	^{13}C	^{1}H[a]
1	163.1	-
2	110.8	-
3	160.4	-
4	106.0	-
5	156.0	-
6	104.9	-
7	209.8	-
8	38.9	3.79 (1H, sept, J = 6.5 Hz)
9	19.1	1.19 (3H, d, J = 6.5 Hz)
10	19.6	1.18 (3H, d, J = 6.5 Hz)
11	21.2	3.26, 3.22 (each 1H, dd, J = 14.3, 7.0 Hz)
12	122.9	5.20 (1H, t, J = 7.0 Hz)
13	134.2	-
14	16.0	1.77 (3H, s)
15	39.7	1.96 (2H, m)
16	26.6	2.05 (2H, m)
17	124.5	5.08 (1H, t, J = 7.0 Hz)
18	131.0	-
19	17.5	1.58 (3H, s)
20	25.5	1.65 (3H, s)
21	27.6	2.82 (1H, brs)
22	45.9	2.20 (1H, m)
23	21.9	1.32, 0.90 (each 1H, m)
24	37.4	1.82, 1.47 (each 1H, m)
25	75.9	-
26	34.8	2.18 (1H, ddd, J = 13.2, 4.4, 3.0 Hz) 1.86 (1H, dd, J = 13.2, 1.5 Hz)
27	28.7	1.43 (3H, s)
28	84.5	-
29	29.6	1.55 (3H, s)
30	24.2	1.06 (3H, s)
OH-1	-	14.07 (1H, s)

[a]Coupling constants given (J, Hz) in parentheses.

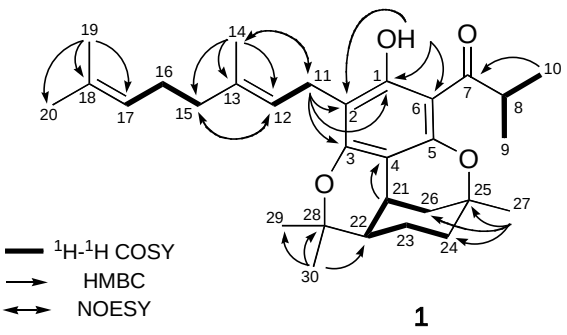

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Figure 1. Selected 2D NMR correlations for p etiolin D (**1**)

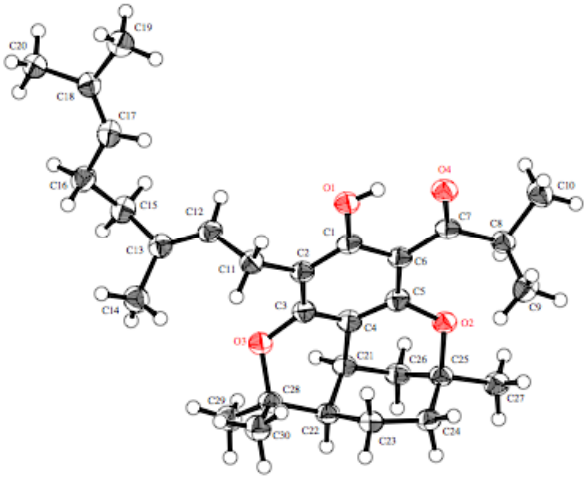

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Figure 2. ORTEP drawing of petiolin D (**1**)

Petiolin E (**2**) showed the pseudomolecular ion peak at *m/z* 431 $(M+Cl)^-$ in the ESIMS, and the HRESIMS analysis revealed the molecular formula to be $C_{19}H_{24}{}^{35}ClO_9$ [*m/z* 431.11126 $(M+{}^{35}Cl)^-$, Δ +1.2 mmu]. IR absorptions (1662, 1621, and 1579 cm^{-1}) suggested the presence of chromone functionality.[12] The ^1H NMR spectrum showed signals of a pair of *meta*-coupled aromatic protons [δ_H 6.69 and 6.48 (each 1H, d, *J* = 1.8 Hz)], an olefin proton [δ_H 6.11 (1H, s)], an isobutyl group [δ_H 2.67 (1H, tq, *J* = 7.0, 7.0 Hz), 1.76 and 1.64 (each 1H, dq, *J* = 7.0, 7.0 Hz), 1.30 and 0.94 (each 3H, d, *J* = 7.0 Hz)], and an anomeric proton [δ_H 5.03 (1H, d, *J* = 6.9 Hz)]. The ^{13}C NMR spectrum exhibited signals due to a conjugated carbonyl carbon, six aromatic carbons, a trisubstituted olefin, an isobutyl group, and a sugar moiety (Table 2). From these data, **2** was elucidated to be a isobutylchromone glycoside. The aglycone of **2** was deduced to be 5,7-dihydroxy-2-isobutylchromone from the analysis of the ^1H-^1H COSY and HMBC spectra (Figure 3). ^{13}C NMR chemical shifts of the sugar moiety in **2** were coincident with those of quercetin-3-*O*-β-D-glucoside.[13] The HMBC correlation of H-1' to C-7 indicated that the glucosyl moiety was connected to a hydroxyl group at C-7, and its β-glycoside linkage was derived from the *J*-value (6.9 Hz) of the anomeric proton signal.

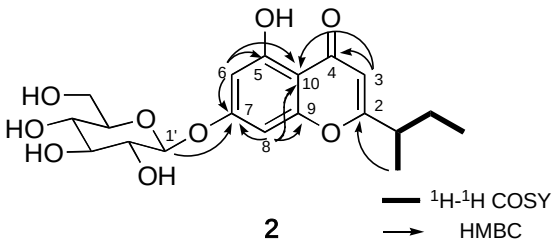

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Figure 3. Selected 2D NMR correlations for petiolin E (**2**)

Table 2 ^{1}H and ^{13}C NMR Data for Petiolin E (**2**) in CD_3OD.

position	^{13}C	^{1}H[a]
2	176.3	-
3	106.9	6.11 (1H, s)
4	184.1	-
5	162.8	-
6	100.9	6.48 (1H, d, *J* = 1.8 Hz)
7	164.6	-
8	95.7	6.69 (1H, d, *J* = 1.8 Hz)
9	159.3	-
10	107.8	-
1'	101.4	5.03 (1H, d, *J* = 6.9 Hz)
2'	74.5	3.30 - 3.51 (1H, m)
3'	77.6	3.30 - 3.51 (1H, m)
4'	71.0	3.30 - 3.51 (1H, m)
5'	78.1	3.30 - 3.51 (1H, m)
6'	62.2	3.90 (1H, brd, *J* = 12.0 Hz)
		3.70 (1H, dd, *J* = 12.0, 5.6 Hz)
2-iBu	41.6	2.67 (1H, tq, *J* = 7.0, 7.0 Hz)
	28.4	1.76, 1.64 (each 1H, dq, *J* = 7.0, 7.0 Hz]
	18.0	1.30 (3H, d, *J* = 7.0 Hz)
	11.7	0.94 (3H, t, *J* = 7.0 Hz)

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

The sugar moiety was assigned as D-glucopyranose by chiral HPLC analysis of *O*-benzoyl derivatives of the methanolysis products of **2**.[14] Thus, the structure of **2** was elucidated to be 5,7-dihydroxy-2-isobutylchromone-7-*O*-β-D-glucopyranoside.

Petiolin D (**1**) is a new phloroglucinol derivative consisting of a citran skeleton, a geranyl group, and a 2-methylpropanoyl group, while petiolin E (**2**) is a new chromone-7-*O*-β-D-glucopyranoside having an isobutyl group at C-2. Though various phloroglucinol derivatives consisted of a citran skeleton were found in natural sources,[15,16] a derivative having a citran skeleton with a geranyl group attached to its benzene ring like petiolin D (**1**) has not been reported so far. Petiolins D (**1**) and E (**2**) showed no cytotoxicity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells (both IC_{50} >10 μg/mL), while **1** and **2** exhibited a weak antifungal activity against *Aspergillus niger* (both MIC, 33.3 μg/mL).

EXPERIMENTAL

General Experimental Procedures

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 by a JEOL ECA 500 spectrometer. The 7.27 and 76.9 ppm resonances of residual

$CHCl_3$ were used as internal references for ^1H and ^{13}C NMR spectra, respectively. ESIMS 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 (3L x 3), and the extracts were partitioned successively with *n*-hexane (300 mL x 3), EtOAc (300 mL x 3), and H_2O (300 mL). The *n*-hexane-soluble portions were subjected to a silica gel column (*n*-hexane / EtOAc), a Sephadex LH-20 column (EtOH), a C_{18} column (MeOH/H_2O, 85: 15) chromatographies, and then C_{18} reversed-phase HPLC (Mightysil RP-18, Kanto Chemical Co., Ltd, 10 x 250 mm; flow rate 3.0 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 95:5) to afford petiolin D (**1**, 2.5 mg, 0.0008%). The EtOAc-soluble portions were applied to a Sephadex LH-20 column (H_2O → MeOH), a C_{18} column (MeOH/H_2O), a silica gel column ($CHCl_3$/MeOH), and then C_{18} reversed-phase HPLC (Mighty sil RP-18, Kanto Chemical Co. Ltd, 10 x 250 mm; flow rate 3.0 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 1:1, 0.1 % TFA) to give petiolin E (**2**, 2.4 mg, 0.0008%).

Petiolin D (1): Colorless crystal; mp 110 – 112 ^oC; UV (MeOH) λ_{max} 240 (ε 15100), 299 (14200), 364 (2470) nm; IR (KBr) ν_{max} 3411 and 1613 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 489 $(M+Na)^+$; HRESIMS: *m/z* 489.2973 $(M+Na)^+$ (calcd for $C_{30}H_{42}O_4Na$, 489.2981).

Petiolin E (2): Colorless amorphous; $[\alpha]^{23}_D$ -34.4 (*c* 0.48 MeOH); UV (MeOH) λ_{max} 257 (ε 9200), 286 (3695), and 320 (2175) nm; IR (KBr) ν_{max} 3408, 1662, 1621, and 1579 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESIMS *m/z* 431 $(M+^{35}Cl)^-$; HRESIMS: *m/z* 431.1113 $(M+^{35}Cl)^-$ (calcd for $C_{19}H_{24}{}^{35}ClO_9$, 431.1101).

X-Ray Analysis of Petiolin D (1)

Petiolin D (**1**) was crystallized as colorless platelets from MeOH/water. The crystal having approximate dimensions of 0.20x0.20x0.02 mm was mounted in a roop. All measurements were made on a Rigaku RAXIS PAPID imaging plate area detector with graphite monochromated Cu-Kα radiation (1.54187Å) at -180^{o}C. Crystal data: Formula $C_{30}H_{42}O_4$, Formula weight 466.66, Space group *P*-1(#2), *a*=9.56002(17)Å,

b=9.74718(18), c=28.0157(5), α=88.8543(7)°, β=86.5058(7), γ=85.7846(7), V=2598.38(8)Å^3, Z=4, D_{calcd}=1.193 g/cm^3, 39766 reflections measured, 9338 reflections unique, $2\theta_{max}$=136.5°, R_{int}=0.062, R_1= Σ ||Fo|-|Fc|| / Σ |Fo| = 0.0572 for 6648 reflections with I>2σ(I), wR_2=[Σ (w(Fo^2-Fc^2)^2 / Σ w(Fo^2)^2)$^{1/2}$ = 0.1581 for all reflections, goodness of fit 1.043. The structure was solved by direct methods (SIR2002)[17] and expanded using Fourier thechniques.[18] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. All calculations were performed using CrystalStructure[19] except for refinement, which was performed using SHELXL-97.[20] Crystallographic data for petiolin D (**1**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 692371).

Stereochemical Assignment of the Sugar Moiety in Petiolin E (2).

Petiolin E (**2**, 0.3 mg) was treated with 3% HCl/MeOH (300 μL) at 110 °C for 1 h. After the solvent was removed by nitrogen stream, to the residue was added EtOAc (100 μL), and the EtOAc solution was extracted with H_2O (100 μL x 3). The aqueous fraction evaporated in vacuo was treated pyridine (100 μL), triethylamine (15 μL), and benzoyl chloride (15 μL), at rt for 21 h. After addition of MeOH (100 μL), the reaction mixture was extracted with *n*-hexane (100 μL x 3). The *n*-hexane-soluble fraction was evaporated in vacuo to afford *O*-benzoyl/methyl derivative of the sugar units of **2**. Authentic D- and L-glucose were treated with benzoyl chloride as described above to afford *O*-benzoyl/methyl derivatives of D- and L-glucose, respectively. The *O*-benzoyl/methyl derivatives were subjected to chiral HPLC analyses using Chiralpak OP(+) (Daicel Chemical Industry, Ltd., 4.6 x 250 mm; MeOH; flow rate 0.5 mL/min; UV detection at 254 nm). The retention time of *O*-benzoyl/methyl derivative of methanolysis product of **2** was found to be 13.7 min, while the retention times of *O*-benzoyl/methyl derivatives of authentic D- and L-glucose were found to be 13.7 and 14.5 min, respectively.

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