Chemical constituents of *Pteris cretica* Linn. (Pteridaceae)

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1. Subject and Source

The group of fern from the genus *Pteris* (Pteridaceae) contains 250 cosmopolitan species (Mabberley, 1997). One species, *P. cretica* (Japanese name: ohbano-inomotoso) called Cretan brake fern is widely distributed in the temperate and warm area of Japan. Although some fern members of the family Pteridaceae are well known to contain carcinogenic C-14 illudane-type sesquiterpenoids (*e.g.* ptaquiloside, **1**), the rhizome and the aerial part as well as the young fronds of *P. cretica* are used in Chinese traditional medicine as antipyretic, antidote, and to treat burn (Gan, 1958; Jiang, 1977). For safety uses, investigation of the presence and distribution of the cytotoxic compounds in *Pteris* species is always necessary before uses in traditional medicines, since C_{14}- and C_{15}-illudane-type sesquiterpenoids are the chemical markers of Pteridaceae.

Plant material used in the present study was collected in May 2007 in Hiroshima City, Japan. A voucher specimen (07-PC-0513) has been deposited in the Department of Pharmacognosy, Graduate School Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, Japan.

2. Previous work

From the air-dried fronds of *P. cretica*, the presence of 19-hydroxycreticoside A (**2**), 2β,6β,15α-trihydroxy-*ent*-kaur-16-ene 2-*O*-β-D-allopyranoside (**3**), 2β,6β,15α-trihydroxy-*ent*-kaur-16-ene (**4**), 2β,6β,15α-trihydroxy-*ent*-kaur-16-ene 2-*O*-β-D-glucopyranoside (**5**), 2β,16α-dihydroxy-*ent*-kaurane (**6**), was reported (Hakamatsuka et

al., 1997). In addition, luteolin 8-C-rhamnoside 7-*O*-rhamnoside (**7**), luteolin

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7-*O*-glucopyranoside (**8**) and luteolin 7-*O*-gentiobioside (**9**) have been isolated from the aerial part of this fern (Imperato, 1994). In year 1985, Murakami and co-workers isolated 2β,6β,16α-trihydroxy-*ent*-kaurane 2-*O*-β-D-allopyranoside (**10**), 2β,6β,16α-trihydroxy-*ent*-kaurane 2-*O*-β-D-glucopyranoside (**11**), 2β,15α-16α-17-tetrahydroxy-*ent*-kaurane (**12**) 2β,15α,16α,17-tetrahydroxy-*ent*-kaurane 2-*O*-β-D-glucopyranoside (**13**), 2β,14α, 15α,16α,17-pentahydroxy-*ent*-kaurane (**14**), pterosins A, C, and S (**15**–**17**), and compound **6**, together with 2β,15α-dihydroxy-*ent*-kaur-16-ene (**18**), creticoside A (**19**) 2β,16α- dihydroxy-*ent*-kaurane (**20**) and creticoside B (**21**) from the rhizome of the plant, while the investigation by the same authors on the aerial part of the same plant demonstrated the presence of 2β,6β,16α-trihydroxy-*ent*-kaurane (**22**), pterosins B (**23**), and F (**24**) together with compounds **16** and **17** (Murakami et al., 1985). Herein we report the isolation of the chemical constituents and the characterization of a new kauranoid (**25**) of *P. cretica.*

3. Present study

The dried leaves of *P. cretica* (459 g) were extracted with MeOH (15 l) for a week. The concentrated MeOH extract (34 g) was suspended with H_2O and partitioned with EtOAc to give 7.6 g of EtOAc- and 25 g of H_2O-soluble fractions. The EtOAc-soluble fraction was chromatographed on silica gel column (particle size 0.063-0.200 mm, Merck) eluting with a solvent system containing CH_2Cl_2, MeOH and H_2O (17:6:1). Fractions having similar TLC behavior were combined and a number of eight fractions were obtained. Luteolin 7-*O*-β-D-glucopyranoside (**8**, 2.05 g) was precipitated from the fraction 2. ODS MPLC (solvent system 10% aqueous MeOH to 100% MeOH) of the remaining of the fraction 2 afforded compounds: **10** (5.1 mg), **16** (2.4 mg), **17** (1.7 mg), **19** (3.6 mg), and 2β,16α-dihydroxy-*ent*-kaurane 2-*O*-β-D-allopyranoside (**26**, 16 mg); (Katakawa et al., 2002). Fraction 3 was subjected to a column ODS MPLC (solvent system 10% aqueous MeOH to 100% MeOH) to give 21 fractions. Pure compounds identified as 2β,6β,16β-trihydroxy-*ent*-kaurane 2-*O*-β-D-allopyranoside, **8**, and 2**6** were isolated from fractions 3-5, 3-8 and 3-16, respectively. ODS HPLC of fraction 3-10 (solvent system:

CH_3CN 35%) afforded apigenin 7-*O*-glucopyranoside (**27**, 21.7 mg). Compounds **3** (1.2 mg), **19** (3.5 mg) and a new one identified as 2β,15β-dihydroxy-*ent*-kaur-16-ene 2-*O*-β-D-glucopyranoside (**25**, 12 mg) were obtained from ODS column chromatography (CC) (solvent system 10% aqueous MeOH to 100% MeOH) of fraction 5.

*Enzymatic hydrolysis of **25**:* Seven milligrams of crude hesperidinase were added to an aqueous solution of **25** (11 mg). The mixture was incubated at 37°C for 72 h. The solution was extracted with EtOAc to afford the aglycone **25a**, 4.2 mg, $[\alpha]_D^{20}$ –32.4 (0.19, MeOH). The remaining aqueous solution was evaporated and purified with silica gel CC (solvent system: CH_2Cl_2: MeOH: H_2O) to give D-glucose as identified by comparison with the chromatogram of the authentic sample in HPLC equipped with an optical rotation detector.

*Oxidation of **25a**:* Three milligrams of compound **25a** was dissolved in CH_2Cl_2 (2 ml) and 10 mg of pyridinium chlorochromate (PCC) was added (Lightner and Toan, 1987). The reaction mixture was stirred at room temperature for 6 hours and evaporated to give a brown residue. Compound **25b** (HRESIMS: *m/z* 323.1979 $[M+Na]^+$, $C_{20}H_{28}O_2Na$ requires 323.1981, 3.5 mg) was obtained from silica gel CC (solvent system hexane: EtOAc, 4:1) of the residue ($[\alpha]_D^{20}$ –56.1 (0.15, MeOH)).

2β,15β-Dihydroxy-*ent*-kaur-16-ene 2-*O*-β-D-glucopyranoside [**25**, amourphous powder $[\alpha]_D^{24}$ –9.4 (0.7, MeOH)] exhibited a molecular formula of $C_{26}H_{42}O_7Na$ as determined by the positive-ion HRESIMS (*m/z* 489.2820 $[M+Na]^+$, $C_{26}H_{42}O_7Na$ requires 489.2822). Its IR spectral data displayed strong bands due to polyhydroxyl (3395, 1078 and 1021 cm^{-1}) and an exomethylene (3081, 1654 and 906 cm^{-1}). Inspection of the ^1H NMR spectrum revealed the presence of a β-oriented anomeric proton (δ 5.05, d, *J*= 8 Hz, 1H), three quaternary methyl groups (δ 0.76, 0.85, 0.91, each singlet), an exomethylene (δ 5.45 and 5.14, each singlet), and a broad singlet and a multiplet oxygen bearing methine proton signals (δ 5.56 brs, H-15 and δ 4.48 m, H-2). The ^{13}C NMR spectrum displayed 26 signals, six of which were ascribable to a β-glucopyrannosyl unit and the remaining 20 to the aglycone of a kaurene-type diterpene (Murakami et al., 1997). The ^{13}C NMR data of **25** were very similar to those of **19** (Table 1), except for the downfield shift of the signals for C-13 (+0.9), C-15 (+1.3), C-17 (+2.9), and the upfield shift of that of C-16 (–5.1). These data suggested that the difference between **19** and **25** was the orientation of the

hydroxyl group at C-15. In order to confirm the planar structure of **25**, H-H COSY, HMQC, NOESY and HMBC experiments were carried out. Significant COSY correlations were observed between: H-1 and H-2, H-2 and H-3; H-5 and H-6, H-6 and H-7; H-9 and H-11, H-11 and H-12, H-12 and H-13, and H-13 and H-14. Moreover, the allocations of the functional groups were substantiated by careful interpretation of the HMBC spectral data. A strong long-range correlations between the proton at δ 5.56 (H-15) and the carbons at C-13 (δ 42.8), C-14 (δ 37.4), and C-17 (δ 110.7) (Fig. 2), and between the anomeric proton at δ 5.05 and the carbon (δ 72.2) concluded the attachment of the glucopyranosyl moiety to be at C-2 and the remaining hydroxymethine to be at C-15. The β-orientation of the C-2 and C-15 hydroxyl groups was concluded by the observation of the NOESY cross-peaks between H-2 (δ 4.48, m) and the equatorial-oriented H-1 2.11(brd, *J*= 12 Hz), and H-15 and H-14b. Enzymatic hydrolysis of **25** gave **25a** and D-glucose. Since the β-D-glucosylation-induced shift-trend rule (Kasai et al., 1977) could not be applied for **25**, when comparing its ^{13}C NMR data with those of **25a** (Table 1), the *ent*-kaurene nature of **25** was concluded by the observation of positive and negative Cotton effects at λ_{max} 241 nm (Δε +0.42) and λ_{max} 291 nm (Δε −2.81), respectively of compound **25b** obtained from the oxidation of **25a** with PCC. From the above data the structure of **25** was concluded to be as depicted.

4. Chemotaxonomic significance

The dried leaves, fronds aerial parts and rhizome of *Pteris cretica* contain pterosins and *ent*-kauranoids, which are the chemical markers of the family Pteridaceae. Pterosin B (**23**) derived from the carcinogenic compound **1**. Since **23** has been detected in all species of *Pteris* investigated, the presence of **1** in *Pteris* species cannot be omitted. Samples should thus be treated before their medicinal uses. The two flavonoids (**8** and **27**) were isolated from the leaves of *P. cretica* in high amount during the present study. Interestingly, our previous phytochemical investigation on one species of *Pteris* (*P. multifida*) demonstrated the presence of pterosins, caffeate and/or coumarate derivatives of quinic acid, flavonoids and sucrose in the plant (Harinantenaina et al., 2008). So far, *P. multifida* is the only *Pteris* species which has been proved to contain a large amount of quinic acid derivatives. The phytochemical investigation of six species of *Pteris* has been carried out and the results can be summarized in Table 2. The *ent*-kauranoids found in *Pteris* are C-2 and/or

C-6, and/or C-15, and/or C-16 and/or C-17 and/or C-18 hydroxylated. Mainly glucopyranosyl and/or allopyranosyl are the sugar moieties attached at C-2 and/or C-6 hydroxyl group of *ent*-kauranoids of *P. cretica*. Recently Ge and his group (Ge et al., 2008) have isolated a tetrahydroxylated (at C-2, C-14, C-15, and C-18) *ent*-kaurene from *P. multifida*. Although C-15 hydroxylated kaurene have been isolated from *P. cretica*, this is the first report on the isolation of an *ent*-kaurene with β-oriented C-15-hydroxyl group from *Pteris* species.

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Table 1. ^{1}H, ^{13}C NMR Spectral Data (400 and 150 MHz, respectively) for Compounds **19**, **25** and **25a** (in C_5D_5N)

Position	**25**		**19**	**25a**
	H	C	C	C
Aglycone				
1a	2.11(brd, *J*= 12 Hz)	47.9	48.0	50.2 (–2.3)[a]
1b	1.33 (t, *J*= 12 Hz)			
2	4.48 (m)	72.2	72.2	63.8 (+8.4)[a]
3a	1.80 (brt, *J*= 11 Hz)	49.6	50.0	52.0 (–2.4)[a]
3b	1.44 overlapped			
4	-	34.9	34.5	34.8
5	0.70 (dd, *J*= 11, 3 Hz)	55.4	56.0	55.3
6a	1.42 overlapped	19.2	19.6	19.3
6b	1.12 (m)			
7a,b	1.45 overlapped	34.5	36.7	34.7
8	-	48.0	48.0	47.7
9	1.10 (brd, *J*= 10 Hz)	54.0	54.4	53.9
10	-	41.3	41.3	41.3
11a	1.73 overlapped	18.5	18.3	18.5
11b	1.55 (brd, *J*= 10 Hz)			
12a	1.45 overlapped	33.0	33.1	32.9
12b	1.39 (m)			
13	2.69 (brs)	42.8	41.9	42.9
14a	1.78 (brd, *J*= 11 Hz)	37.4	36.9	37.5
14b	1.46 overlapped			
15	5.56 (brs)	84.2	82.9	84.3
16	-	155.8	160.9	155.7
17a	5.45 (brs)	110.7	107.8	110.6
17b	5.14 (brs)			
18	0.85 (s)	33.7	33.9	33.8
19	0.76 (s)	22.5	22.5	22.7
20	0.91 (s)	18.7	18.8	18.9
Glc				
1’	5.05 (d, *J*= 8 Hz)	102.8	102.6	
2’	4.04 (dd, *J*= 8, 8 Hz)	75.3	75.3	
3’	overlapped	78.7	78.3	
4’	overlapped	71.9	71.8	
5’	overlapped	78.4	78.6	
6’a	4.53 (dd, *J*= 12, 2 Hz)	63.0	63.0	
6’b	4.36 (dd, *J*= 12, 5 Hz)			

Glc: β-D-glucopyranosyl.
Assignments based on HSQC and HMBC.
[a]δ_{25}-δ_{25a}.

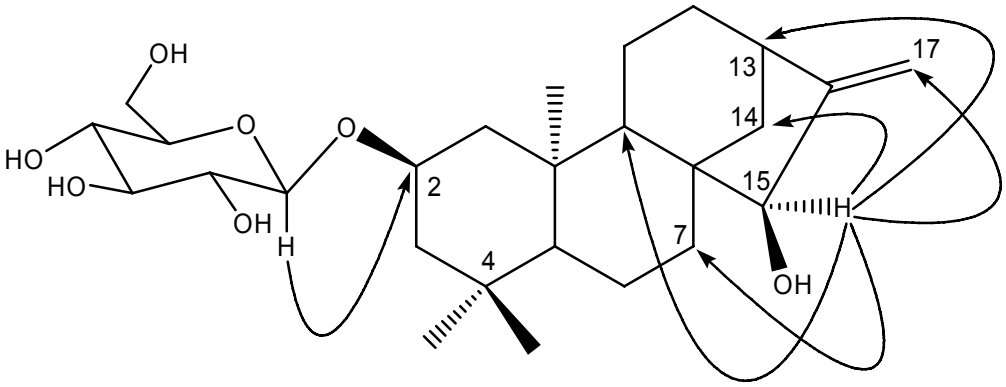


Fig. 1. Important HMBC correlations observed

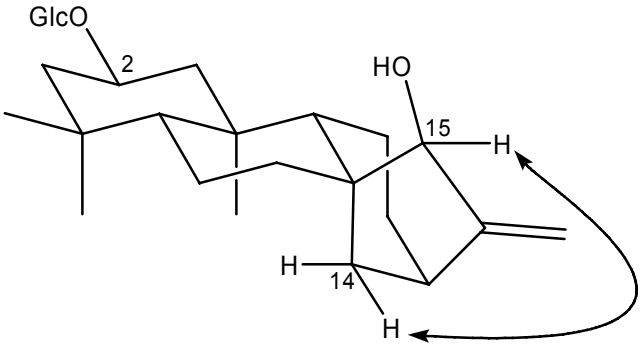
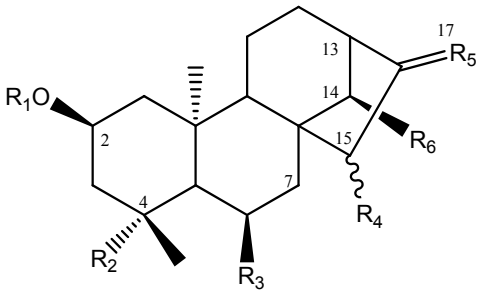


Fig. 2. Important NOESY correlation observed

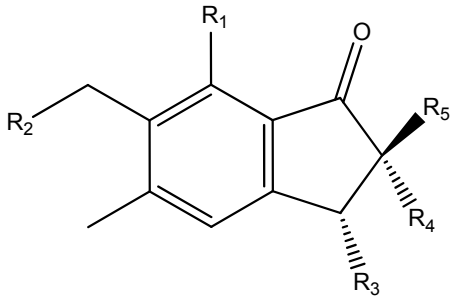
Table 2. Phytochemical investigation of six species of *Pteris*

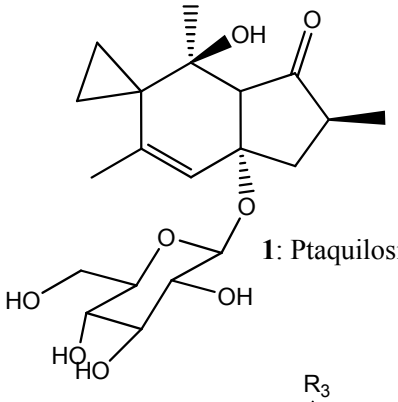
Species	**8**, **27**	Pterosins	Kauranoids
P. multifida Poir.[a]	+	+	+
P. multifida Poir.[b]	+	+	ND
P. cretica Linn.[a]	+	+	+
P. cretica Linn.[c]	+	+	+
P. tremula R. Br.[a]	–	+	+
P. dactylina Hook[a]	–	+	+
P. angustipinna Tagawa[a]	–	+	+
P. grevilleana Wall.[a]	–	+	–

[a]Previously reported investigation; [b]Our previous investigation (Harinantenaina et al., 2008); [c]Present investigation



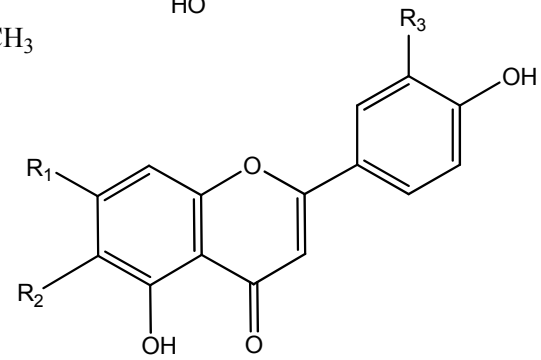
2 : R_1= β-D-glucopyranosyl, R_2= CH_2OH, R_3= H, R_4= α-OH, R_5= CH_2, R_6= H
3 : R_1= β-D-allopyranosyl, R_2= CH_3, R_3= OH, R_4= α-OH, R_5= CH_2, R_6= H
4 : R_1= H, R_2= CH_3, R_3=OH, R_4= α-OH, R_5= CH_2, R_6= H
5 : R_1= β-D-glucopyranosyl, R_2= CH_3, R_3=OH, R_4= α-OH, R_5= CH_2, R_6= H
6 : R_1= H, R_2= CH_3, R_3=H, R_4= H, R_5= α-OH, β-CH_3, R_6= H
10: R_1= β-D-allopyranosyl, R_2= CH_3, R_3=OH, R_4= H, R_5= α-OH, β-CH_3, R_6= H
11: R_1= β-D-glucopyranosyl, R_2= CH_3, R_3=OH, R_4= H, R_5= α-OH, β-CH_3, R_6= H
12: R_1= H, R_2= CH_3, R_3= H, R_4= α-OH, R_5= α-OH, β-CH_3, R_6= H
13: R_1= β-D-glucopyranosyl R_2= CH_3, R_3=H, R_4= α-OH, R_5= α-OH, β-CH_2OH, R_6= H
14: R_1= H, R_2= CH_3, R_3=H, R_4= α-OH, R_5= α-OH, β-CH_2OH, R_6= OH
18: R_1= H, R_2= CH_3, R_3=H, R_4= α-OH, R_5= CH_2, R_6= H
19: R_1= β-D-glucopyranosyl, R_2= CH_3, R_3=H, R_4= α-OH, R_5= CH_2, R_6= H
20: R_1= β-OH, R_2= CH_3, R_3=H, R_4= H, R_5= α-OH, β-CH_3, R_6= H
21: R_1= β-D-glucopyranosyl, R_2= CH_3, R_3=H, R_4= H, R_5= α-OH, β-CH_3, R_6= H
22: R_1= H, R_2= CH_3, R_3=OH, R_4= H, R_5= α-OH, β-CH_3, R_6= H
27: R_1= β-D-glucopyranosyl, R_2= CH_3, R_3=OH, R_4= α-OH, R_5= CH_2, R_6= H
25a: R_1= OH, R_2= CH_3, R_3=H, R_4= β-OH, R_5= CH_2, R_6= H





1: Ptaquiloside

15: R_1= CH_3, R_2= CH_2OH, R_3=H, R_4= CH_2OH, R_5= CH_3
16: R_1= CH_3, R_2= CH_2OH, R_3= OH, R_4= H, R_5= CH_3
17: R_1= CH_2OH, R_2= CH_2OH, R_3= OH, R_4= H, R_5= CH_3
23: R_1= CH_3, R_2= CH_2OH, R_3= H, R_4= CH_3, R_5= H
24: R_1= CH_3, R_2= CH_2Cl, R_3= H, R_4= CH_3, R_5= H



7: R_1= rhamnoyl, R_2= *O*-rhamnoside, R_3= OH
8: R_1= *O*-β-D-glucopyranosyl, R_2= H, R_3= H
9: R_1= H, R_2= *O*-gentiobioside, R_3= OH
27: R_1= β-D-glucopyranosyl, R_2= H, R_3= OH