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(–)-Pteroside N and pterosinone, new BACE1 and cholinesterase inhibitors from *Pteridium aquilinum*

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ABSTRACT

Bioassay-guided fractionation of the ethanolic extract from the whole plants of *Pteridium aquilinum* has resulted in the isolation of a new pterosin glycoside, (–)-pteroside N (**1**), and a new *seco*-illudoid sesquiterpene, pterosinone (**2**). Their structures were identified by analysis of the spectroscopic data including extensive 2D NMR. All of the isolates were evaluated for the anti-Alzheimer disease (anti-AD) activity through enzyme inhibition of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1). (–)-Pteroside N (**1**) showed moderate BACE1 inhibitory activity (IC_{50} value: 30.6 μM), but exhibited potent inhibitory activity against AChE and BChE (IC_{50} values: 4.47 and 7.39 μM, respectively). On the other hand, pterosinone (**2**) showed mild AChE and BChE inhibitory activity (IC_{50} value: 87.7 and 72.9 μM), but exhibited potent inhibitory activity against BACE1 (IC_{50} value: 19.4 μM). The results of the present study demonstrate that sesquiterpenoids from *P. aquilinum* might be beneficial in the treatment of AD.

1. Introduction

Bracken fern (*Pteridium aquilinum* (L.) Kuhn., family Dennstaedtiaceae) is one of the most ubiquitous ferns that is widely distributed vascular plant species in Southeast Asia, South and Central America, and Oceania, has been widely consumed traditionally for foods in Asia, including Korea and Japan (Yu et al., 2015; Gil da Costa et al., 2012). Also, bracken rhizomes and the whole plant has long been used as traditional folk medicine to treatment ailments of neonatal jaundice, hypertension, injury, leucorrhoea, lower back pain, rheumatic arthritis, and eczema (Chen et al., 2008). Bracken contains many nutrients, such as carbohydrates, carotenoids, fat, protein, vitamins, and trace mineral (Wang and Wu, 2013; Xu et al., 2009). A previous study on the chemical constituents of this plant led to the isolation of pterosin sesquiterpenoids (Mohammad et al., 2016; Kovganko et al., 2004), illudane and illudalane sesquiterpenes (Castillo et al., 1998), flavonoids (Imperato, 1995,1997), oligosaccharides (Wang and Wu, 2013; Xu et al., 2009), steroids, and tannins (Pamukcu et al., 1980). Bracken was reported to possess several pharmacological activities, such as immunomodulatory activity (Song et al., 2017; Latorre et al., 2009), antioxidant (Wang and Wu, 2013), smooth muscle relaxant activity (Sheridan et al., 1999), and antitumor activity (Chen et al., 2013a,b). As part of an ongoing research program for the discovery of plant-derived anti-AD potential, the hot water extract of the whole plants of bracken was found to inhibit BACE1, AChE, and BChE. Bioactivity-guided purification of the EtOAc- and buthanol-soluble fraction of the whole plants of bracken led to the isolation of a new pterosin glycoside, (–)-pteroside N (**1**), and a new *seco*-illudoid sesquiterpene, pterosinone (**2**) (Fig. 1). Their chemical structures were determined by the analysis of 1D and 2D NMR spectra such as HSQC, HMBC, and HRMS. We report herein the isolation and structure determination of these pterosin glycoside and *seco*-illudoid sesquiterpene, and isolated compounds were evaluated for their potential to inhibitory potential against BACE1, AChE, and BChE.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, and its molecular formula was determined as $C_{20}H_{28}O_8$ by HRESIMS (*m/z* 419.16747 $[M + Na]^+$; calcd 419.16764), which required seven degrees of unsaturation in the molecule. The UV absorbance maxima at 211, 263 and 305 nm were consistent with 1-indanone derivatives (Mohammad et al., 2016). The ^1H and ^{13}C NMR data of **1** (Table 1) suggested the presence of a characteristic pentasubstituted benzene

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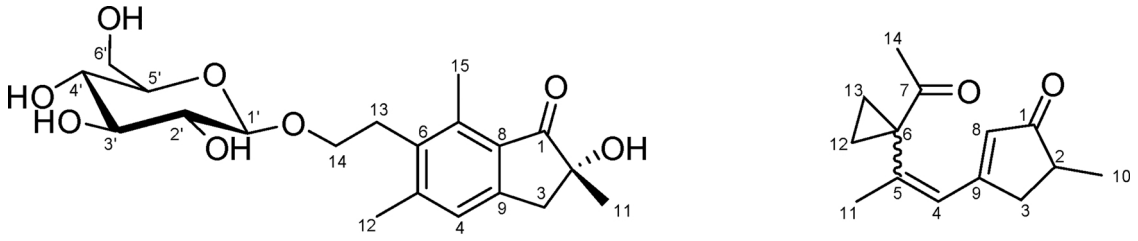

1 **2**

Fig. 1. Structure of compounds **1** and **2** isolated from *P. aquilinum*.

Table 1
^{1}H and ^{13}C NMR data of Compound **1**[a].

Position	δ_H mult. (J = Hz)	δ_C mult.
1		210.0 s
2		78.3 s
3		42.9 s
4	7.12 s	127.1 d
5		147.3 s
6		136.9 s
7		139.8 s
8		130.9 s
9		151.4 s
11	1.33 s	25.6 q
12	2.46 s	21.5 q
13	3.11 d (7.7)	30.3 t
14	3.91 dd (16.8, 7.7) 3.65 m	69.3 t
15	2.65 s	14.2 q
1'	4.31 d (7.7)	104.6 d
2'	3.19 t (9.1)	75.3 d
3'	3.35 t (9.1)	78.3 d
4'		71.7 d
5'		78.2 d
6'	3.85 d (11.9) 3.66 d (11.9)	62.8 t

[a] Measured at 700 and 175 MHz; obtained CD_3OD with TMS as an internal standard. Data assignment was based on HSQC and HMBC experiments.

moiety [δ_H 7.12 (1H, s, H-4); δ_C 151.4, 147.3, 139.8, 136.9, 130.9, and 127.1], three quaternary methyl groups [δ_H 2.65 (3H, s, H-15), 2.46 (3H, s, H-12), and 1.33 (3H, s, H-11); δ_C 25.6, 21.5, and 14.2], three methylene groups [including an oxygenated ones, δ_H 3.91 (1H, dd, J = 16.8, 7.7, H_a-14) and 3.65 (1H, m, H_b-14); δ_C 69.3, δ_H 3.11 (2H, d, J = 7.7 Hz, H-13); δ_C 30.3, and 3.08 (1H, d, J = 16.8 Hz, H_a-3), 3.01 (1H, d, J = 16.8 Hz, H_b-3); δ_C 42.9]. In addition, ^{1}H and ^{13}C NMR data revealed a set of β-glucopyranosyl group [δ_H 4.31 (1H, d, J = 7.7 Hz, H-1′); δ_C 104.6 (C-1′), 75.3 (C-2′), 78.3 (C-3′), 71.7 (C-4′), 78.2 (C-5′), 62.8 (C-6′)] (Table 1). The ^{13}C NMR and DEPT spectra of **1** (Table 1) showed 14 distinct carbon signals excluding the carbon signals corresponding to the glucose moiety. These carbon signals also supported the presence of a pentasubstituted aromatic moiety, including one conjugated ketone signal at δ_C 210.0, which is typical of C-1 of a pterosin-type sesquiterpenoid skeleton (Mohammad et al., 2016; Fukuoka et al., 1983). Identification of the sugar moiety was proved by acid hydrolysis and NMR data of compound **1** where glucose could be detected by TLC analysis. The β-configuration for the glucose was determined from its coupling constant of anomeric proton ($J_{1,2}$ > 7.0 Hz) (Liu et al., 2010; Hong et al., 2013). The HMBC correlation of H-1′ (δ_H 4.31) with C-14 (δ_C 69.3) demonstrated that the β-glucopyranose unit was connected to C-14 (Fig. 2). The ^{13}C NMR spectra of **1** were almost superimposable to those of pterosin N except for the presence of one glucose moiety (Kuroyanagi et al., 1974; Wu et al., 2014). The specific rotation of **1** and its aglycone were $[\alpha]_D^{29}$ −49.4 (c = 0.05, MeOH) and $[\alpha]_D^{29}$ −29.4 (c = 0.2, MeOH) respectively, which was equivalent to that of (−)-pterosin N (Wu et al., 2014). Therefore, the structure of **1** was determined as (−)-pteroside N.

Compound **2** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{14}H_{18}O_2$ by HRESIMS for the $[M+H]^+$ ion at m/z 219.1375 (calcd for $C_{14}H_{18}O_2$ $[M+H]^+$, 219.1380), possessing six degrees of unsaturation in the molecule. Inspection of the ^{1}H and ^{13}C NMR spectra (Table 2), obtained with the aid of 2D (^{1}H-^{1}H COSY, HSQC, and HMBC) NMR spectra, revealed that this compound exists mixture of two geometric stereoisomers [**2a** (major isomer) and **2b** (minor isomer)], in a ratio of 2.5 : 1 in $CDCl_3$ (see Supplementary data, Figs. S9 and S10).

The ^{1}H and ^{13}C NMR spectra in combination with the HSQC spectrum showed the presence of four olefinic proton signals [**2a**, δ_H 6.29 (1H, brs, H-4), and 6.01 brs (1H, brs, H-8); **2b**, δ_H 6.28 (1H, brs, H-4) and 6.08 brs (1H, brs, H-8)], two tertiary methyl groups [**2a**, δ_H 1.18 (3H, d, J = 7.0 Hz, H-10); **2b**, δ_H 1.22 (3H, d, J = 7.7 Hz, H-10)], two methyl ketone groups [**2a**, δ_H 2.16 (3H, s, H-14); **2b**, δ_H 2.15 (3H, s, H-14)], two quaternary methyl signals coupled with one olefinic proton (H-4) [**2a**, δ_H 2.08 (3H, d, J = 1.4 Hz, H-11); **2b**, δ_H 2.12 (3H, d, J = 0.7 Hz, H-11)], two methine signals [**2a**, δ_H 2.43 (1H, dt, J = 10.3, 7.0, 2.8 Hz, H-2); **2b**, δ_H 2.48 (1H, td, J = 7.0, 2.8 Hz, H-2)], two methylene signals [**2a**, δ_H 2.95 (1H, ddd, J = 18.2, 6.3, 1.4 Hz, H_a-3) and 2.30 (1H, td, J = 18.2, 1.4 Hz, H_b-3); **2b**, δ_H 3.08 (1H, ddd, J = 18.2, 7.0, 1.4 Hz, H_a-3) and 2.42 (1H, overlapped, H_b-3)]. Additional four methylene proton signals [**2a**, δ_H 2.58 (2H, brd, J = 4.2 Hz, H-13) and 1.03 (2H, brd, J = 3.5 Hz, H-12), δ_C 21.4 (t) and 21.3 (t); **2b**, δ_H 1.45 (2H, q, J = 3.5 Hz, H-13) and 1.06 (2H, q, J = 3.5 Hz, H-12), δ_C 17.95 (t) and 17.93 (t)] appeared in a symmetrical pair of quartets (A_2B_2 type) which were assigned to two methylene groups of cyclopropane ring conjugated with other unsaturated function (Hayashi et al., 1973). The ^{13}C NMR spectrum of **2a** and **2b** displayed 14 carbon signals including three methyls, three methylenes, three methines (including two olefinic signals), and five quaternary carbons (including two ketone), respectively. The ^{1}H and ^{13}C NMR spectra of **2** (Table 2) were similar to those of hypacrone except for a methyl group instead of *gem*-dimethyl group at C-2. Hypacrone is a *seco*-illudoid sesquiterpene isolated from fresh shoots of fern, *Hypolepis punctata*, also possessing a reactive cyclopropane ring and giving pterosin Z on hydrolysis (Hayashi et al., 1973, 1975). Thus, compound **2** can also be expected to be produced by hydrolysis of pterosin B (Chen et al., 2013a,b; Potter and Baird, 2000). Complete assignment of the ^{1}H and ^{13}C NMR chemical shifts were obtained using DEPT, ^{1}H–^{1}H COSY, HSQC, and HMBC NMR correlations (Fig. 2). Therefore, **2** was identified as 3-[2-(1-acetylcyclopropyl)-1-propen-1-yl]-5-methyl-2-cyclopenten-1-one, named pterosinone. However, the geometric configuration of **2** remains to be clarified. The **2** was novel, according to the SciFinder database.

To evaluate the anti-AD potential, the inhibitory activity of compounds **1** and **2** against BACE1, AChE, and BChE was evaluated by respective *in vitro* inhibition assays (Table 3). Compounds **1** and **2** showed concentration-dependent inhibitory activity against BACE1 with a value of IC_{50} values (concentration required to decrease activity