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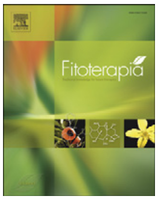

Two new peroxy fatty acids with antibacterial activity from *Ophioglossum thermale* Kom

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ABSTRACT

Two new peroxy fatty acids, thermalic acids A (**1**) and B (**2**), together with eight known compounds, (3β)-methyl-3-hydroxy-urs-11-en-28 oate (**3**), luteolin (**4**), quercetin (**5**), 3-methoxyquercetin (**6**), ophioglonol (**7**), ophioglonol 4′-O-α-D-glucopyranoside (**8**), pedunculosumoside B (**9**), syringol (**10**), were isolated from the herba of *Ophioglossum thermale* Kom. The structures of **1** and **2** were identified by HRESIMS, EIMS, 1D and 2D NMR, and electronic circular dichroism (ECD) spectra. Both two acids exhibited potential antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. This is the first report of peroxy fatty acids isolated from herbaceous plants of Ophioglossaceae.

1. Introduction

The genus *Ophioglossum* is a small terrestrial plant that is distributed worldwide [1]. Phytochemical researches revealed that the herba of *Ophioglossum* contains massive fatty acids and their esters [2,3], amino acid [4], flavonoids [5–11], and polysaccharoses [12,13] which are known to have various biological activities. *Ophioglossum thermale* Kom. is a Chinese herb of heat-clearing and detoxifying, and widely used as a traditional antidote for poisonous snake bite. However, there is no more report on its constituents except rarely common constituents [2]. The present paper describes the isolation and identification of two new peroxy fatty acids, thermalic acids A (**1**) and B (**2**), together with eight known compounds, (3β)-methyl-3-hydroxy-urs-11-en-28 oate (**3**) [14], luteolin (**4**) [15], quercetin (**5**) [16], 3-methoxyquercetin (**6**) [2], ophioglonol (**7**) [5], ophioglonol 4′-O-α-D-glucopyranoside (**8**) [5], Pedunculosumoside B (**9**) [6], and syringol (**10**) [17] (Fig. 1) from the methanol extract of the herba of *O. thermale*. The antibacterial activity assay shows that both new compounds exhibited potential antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*.

2. Results and discussion

Two new peroxy acids, thermalic acids A (**1**) and B (**2**), were isolated from the methanol extract of the whole plant of *O. thermale* by successive open column chromatography (CC) over silica gel, MCI gel, Sephadex LH-20, and ODS, as well as medium pressure liquid chromatography (MPLC) and preparative HPLC (prep-HPLC).

Thermalic acid A (**1**) was obtained as a colorless gummy oil and its molecular formula was deduced to be $C_{18}H_{28}O_5$ by HRESIMS at *m/z* 325.2011 $[M + H]^+$ (Calcd. for $C_{18}H_{29}O_5$ 325.2010). The ^{1}H NMR spectrum of **1** showed four olefinic protons at δ_H 5.38 (^{4}H, m), while two oxymethine protons resonated at δ_H 5.09 (^{1}H, t, $J = 3.2$ Hz) and 5.04 (^{1}H, t, $J = 3.1$ Hz). A terminal methyl group was observed as triplet at δ_H 0.88 (^{3}H, t, $J = 4.0$ Hz). A broad hump between δ_H 1.30–2.80 showed the presence of nine methylene groups. The ^{13}C NMR and DEPT spectra showed eighteen signals comprising one methyl, nine methylene, six methane, and two quaternary carbons. It showed the presence of a ketone at δ_C 204.1 (s) and a carboxyl at δ_C 180.5 (s), respectively. The two downfield oxymethine carbons at δ_C 89.6 (d) and 91.6 (d), comparing with NMR the data of urticic acid [δ_C 91.2 (d) and 89.1 (d); detailed comparison see Table S1] from literature [18] and combing the HMBC correlations from δ_H 5.04 (^{1}H, t, $J = 3.1$ Hz) and δ_H 5.09 (^{1}H, t, $J = 3.2$ Hz) to δ_C 204.1 (s), is present in five-membered 4-oxo-1,2-dioxolane moiety (Fig. 2). The four olefinic carbons at δ_C 130.1 (d), 129.7 (d), 128.2 (d), and 128.1 (d) and a methylene at δ_C 25.5 (t) were observed, suggesting a chain of $CH{=}CH{-}CH_2{-}CH{=}CH$ [19]. The presence of *n*-butyric acid moiety was evident by mass fragments in EIMS spectrum at *m/z* 87, 73, and 59 (Fig. 3). Further peak at *m/z* 128 supported that the 4-oxo-1,2-dioxolane moiety was attached to *n*-butyric acid unit. The fragments at *m/z* 180, 123, 97, 83, and 57, and the ^{1}H–^{1}H COSY correlations from H-9 [δ_H 1.98 (^{2}H, m)] to H-10 [δ_H 5.38 (^{1}H, m), H-11 [δ_H 5.38 (^{1}H, m)] to H-12 [δ_H 2.79 (^{2}H, dd, $J = 14.8$, 6.8 Hz)], H-14 [δ_H 5.38 (^{1}H, m)] to H-15 [δ_H 2.00 (^{2}H, m)], and H-15

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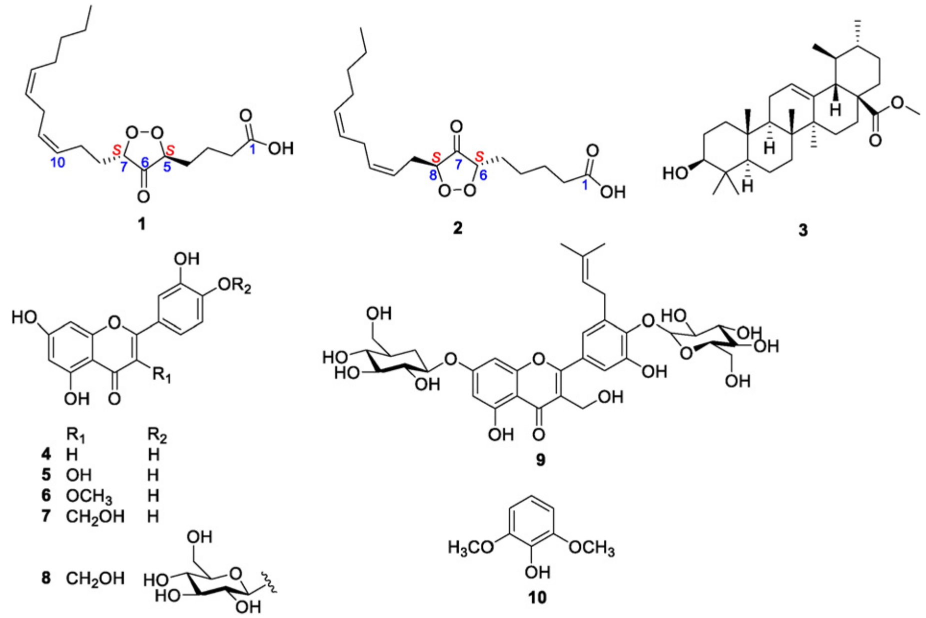

Fig. 1. Structures of compounds **1**–**10**.

[δ_H 2.00 (^{2}H, m)] to H-16 [δ_H 1.33 (^{2}H, m), combing with the HMBC correlations from H-18 [δ_H 0.88 (^{3}H, t, 4.0)] to C-17 (δ_C 22.7 t) and C-16 (δ_C 31.9 t), confirmed the presence of a 3,6-undecanediene unit. The key ^{1}H–^{1}H COSY correlations from H-7 [δ_H 5.04 (^{1}H, t, $J = 3.1$ Hz)] to H-8 [δ_H 2.02 (^{2}H, m)] and HMBC correlations from H-8 [δ_H 2.02 (^{2}H, m)] to C-6 (δ_C 204.1 s) and C-7 (δ_C 89.6 d) implied that the 3,6-undecanediene moiety was attached to C-7 of 4-oxo-1,2-dioxolane. Thus, the planar structure of **1** was established as 5,7-dioxolan-6-oxo-10,13-octadecadienoic acid. The detailed ^{1}H and ^{13}C NMR data of **1** were shown in Table 1 on the basis of ^{1}H–^{1}H COSY, HSQC, and HMBC correlations.

The *Z*/*E* configurations of C(10)═C(11) and C(13)═C(14) were determined based on the chemical shifts of C-9, C-12, and C-15, and the NOE correlations. It was reported that the chemical shift of CH_2 attaching to *E* double bond is about δ_C 33, whereas attaching to *Z* double bond is about δ_C 27 [20]. In our experiment, the chemical shifts of C-9 which is adjacent to the C(10)═C(11) bond and C-15 which is adjacent to the C(13)═C(14) bond appear at δ_C 29.1 and 29.3, respectively. Combing the existence of NOE correlations from H-12 δ_H [2.79 (^{2}H, dd, J=14.8, 6.8 Hz)] to H-9 δ_H [1.98 (^{2}H, m)] and H-15 δ_H [2.00 (^{2}H, m)] (Fig. 4), the configurations of C(10)═C(11) and C(13)═C(14) were assigned as *Z*, *Z* which also is supported by the similar ^{13}C NMR data of (Z,Z)-CH═CH–CH_2–CH═CH fragment between **1** and linoleic acid [19]. The relative and absolute configurations at C-5 and C-7 were assigned by NOESY experiment and calculation of electronic circular dichroism (ECD) data, respectively. The absence of NOE correlation from H-5 [δ_H 5.09 (^{1}H, t, $J = 3.2$ Hz)] to H-7 [δ_H 5.04 (^{1}H, t, $J = 3.1$ Hz)] suggested a *trans*-configuration between C-5 and C-7 [21,22]. The ECD data of two possible structures were calculated and compared with experimental spectrum. Two geometries were previously optimized by Density Functional Theory (DFT) method at the B3LYP/6-31G(d,p) level. Excitation energies and rotational strengths were calculated using time-dependent Density Functional Theory (TDDFT) at the B3LYP/6-31G(d,p) level [23]. The ECD spectrum was simulated from electronic excitation energies and velocity rotational strengths [24]. The results showed that the theoretical ECD data for 5*S*,7*S*-isomer was in good agreement with the experimental spectrum (Fig. 5**A**). Hence, the structure of **1** was established as (5*S*,7*S*)-(10*Z*,13*Z*)-5,7-dioxolan-6-oxo-10,13-octadecadienoic acid and named thermalic acid A.

Thermalic acid B (**2**) was obtained as a colorless gummy oil and its molecular formula was deduced to be $C_{18}H_{28}O_5$ by HRESIMS at *m*/*z* 325.2004 [M + H]$^+$ (Calcd. for $C_{18}H_{29}O_5$ 325.2010). The NMR spectra of **2** (Table 1) exhibited characteristic NMR features of an isomer of **1** bearing a 4-oxo-1,2-dioxolane, a carboxyl, and a chain of two double bond. Comparison of the NMR data of **2** with **1** indicated that the 4-oxo-1,2-dioxolane of **2** was located at C-6, C-7, and C-8, implying the

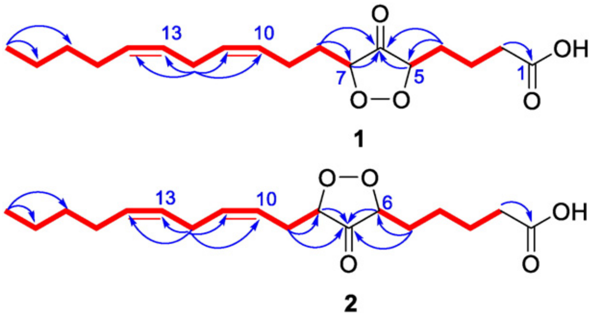

Fig. 2. Key ^{1}H–^{1}H COSY (▬) and HMBC (→) correlations for **1** and **2**.

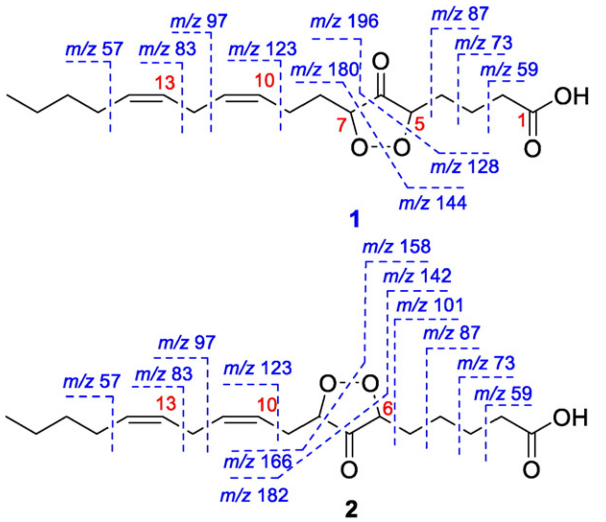

Fig. 3. Structures and mass fragmentations of **1** and **2**.