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Pinelloside, an antimicrobial cerebroside from *Pinellia ternata*

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Abstract

An antimicrobial cerebroside, pinelloside, was isolated from the air-dried tubers of *Pinellia ternata* (Thunb.) Breit. Its structure was determined as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,11*E*)-2-(2′*R*-hydroxyhexadecenoylamino)-4,11-octadecadiene-1,3-diol by chemical transformation and extensive spectroscopic analyses (IR, MS, ^{1}H and ^{13}C NMR, DEPT as well as 2D NMR techniques HMBC, HMQC, ^{1}H–^{1}H COSY and NOESY). The antimicrobial assay showed that this compound was inhibitory to the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*, with minimum inhibitory concentrations (MICs) of 20, 50, 30 and 10 μg/ml, respectively. The MICs of penicillin G against bacteria *B. subtilis*, *S. aureus*, *E. coli*, *P. fluorescens* and *H. pylori* were 0.80, 0.34, 0.56, 1.34 and 0.92, and those of ketoconazole against fungi *A. niger*, *C. albicans* and *T. rubrum* 0.90, 0.65 and 1.0 μg/ml, respectively.

Keywords: *Pinellia ternata*; Araceae; Antimicrobial; Cerebroside; Pinelloside; 4*E*,11*E*-sphingadienine

1. Introduction

The tuber of *Pinellia ternata* (Thunb.) Breit. (Araceae) is one of the main components in many decoctions in traditional Chinese medicine that has been applied since ancient times for anti-emetic, anti-tussive, sedative and anti-inflammatory purposes (Marki et al., 1987). Phytochemicals from this plant that have been previously characterized include alkaloids (Zhao, 1990), volatile oils (Wang et al., 1995) and polysaccharides (Tomoda et al., 1994; Gonda et al., 1994). In continuation of our ongoing project aiming at the characterization of new antimicrobial chemicals from nature (Liu et al., 2001, 2002; Meng et al., 2000), we found unexpectedly that the ethanol extract of *P. ternata* tubers exhibited pronounced antimicrobial activity in our preliminary bioassay. A subsequent bioassay-guided fractionation was therefore performed leading eventually to the isolation of a new antimicrobial cerebroside named pinelloside **1** (Fig. 1). Specific plant cerebrosides have been shown to be anti-ulcerogenic (Okuyama and Yamazaki, 1983), hepatoprotective (Jang et al., 1998) and enzyme (xanthine oxidase) inhibitory (Kong et al., 2001), but none have been described as antimicrobial. Here we report the structure determination and antimicrobial action of pinelloside **1**, a new member of this type of phytochemical.

2. Results and discussion

Compound **1** was obtained as a white amorphous solid. The positively charged HRESI-MS spectrum of **1** gave a protonated molecular ion $[M+H]^+$ at *m*/*z* 714.5520. This observation, combined with the ^{1}H and ^{13}C NMR spectral data of **1**, led us to postulate that its molecular formula is $C_{40}H_{75}NO_9$. In the ^{1}H NMR spectrum of **1**, a doublet ($J=7.5$ Hz) at δ 4.21, a hydroxymethyl giving signals at δ 3.77 (*br dd*, $J=12.0$, 6.0 Hz) and 3.59 (*dt*, $J=12.0$, 6.0 Hz) as well as four oxygenated methines resonating at δ 3.13 (*ddd*, $J=9.0$, 7.5, 4.0 Hz), 3.18 (*m*), 3.24 (*td*, $J=9.0$, 4.0 Hz) and 3.30 (*td*, $J=9.0$, 4.0 Hz) indicated the presence of a glucopyranosyl moiety. Furthermore, a six-proton triplet

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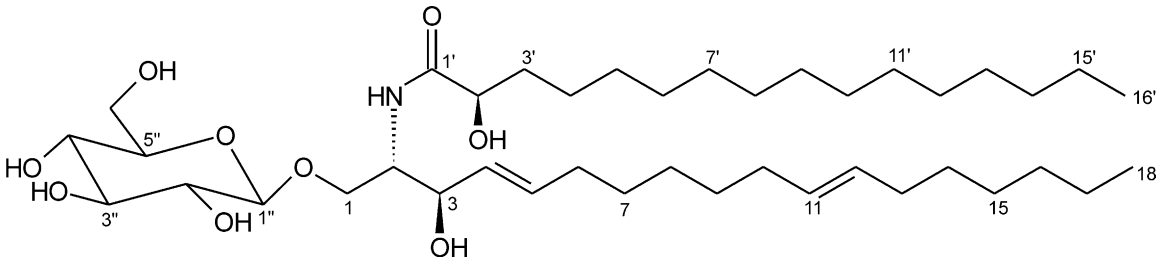

Fig. 1. Compound **1**.

($J=7.0$ Hz) at δ 0.87 and two pairs of olefinic proton signals at δ 5.56, 5.47, 5.42 and 5.34 (Table 1) demonstrated that compound **1** possessed two aliphatic chains containing two double bonds, suggesting that it was probably a cerebroside (Kong et al., 2001). This deduction was reinforced by analysis of the ^{13}C NMR spectrum (Table 1) as well as by methanolysis of **1**, which liberated the anticipated methyl β-D-glucose and two aliphatic molecules (**2** and **3**) (Fig. 2), all identified by EI and/or ESI mass spectroscopies. The presence of 1-*O*-glucopyranosyl, 2-amino and 3,2′-dihydroxy groups as well as the 4,5-double bond in the main chain was elucidated by analysis of the ^{1}H and ^{13}C NMR spectroscopic data of **1** which was assigned unambiguously by extensive 2D-NMR techniques (Table 1) (Fig. 3). The 11,12-double bond in the main chain of **1** was proven by ESI and EI mass spectra of **3**, giving the quasimolecular ion at 298 $[M+H]^+$ and an intense fragment ion at 227 $[M-C_5H_{10}]$ formed through a McLafferty rearrangement (Kong et al., 2001). It seems unusual that sphingoids isolated from vascular plants have an 11*E* double bond since this has only been observed with cerebrosides from the land annelid *Hirudo nipponica* (Noda et al., 1995). Regarding the stereochemistry, the formulated absolute configuration of compound **1** was based on the carbon chemical shifts at δ 69.2 (C-1), 53.7 (C-2), 71.8 (C-3), 175.2 (C-1′), 72.1 (C-2′), which happened to be fairly close to those previously reported for (2*S*, 3*R*, 2′*R*) sphingosine moieties (Kang et al., 1999; Chen et al., 2002; Liu et al., 1998). In conclusion, glycoside **1** is assigned as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,11*E*) - 2 - (2′*R* - hydroxyhexadecenoylamino) - 4,11-octadecadiene-1,3-diol, a hitherto undescribed cerebroside. We have named compound **1** pinelloside.

The antimicrobial activity of compound **1** against Gram-positive and -negative bacteria and against fungi was evaluated using the agar dilution method. The assay results indicated that compound **1** inhibited growth of bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and fungi *Aspergillus niger* and *Candida albicans*, with minimum inhibitory concentrations (MICs) of 20, 50, 30 and 10 μg/ml, respectively. However, it was not inhibitory to other test bacteria such as *Escherichia coli*, *Pseudomonas fluorescens* and *Helicobacter pylori* and the fungus *Trichophyton rubrum*. The MICs of the positive control penicillin G against *B. subtilis*, *S. aureus*, *E. coli*, *P. fluorescens* and *H. pylori* were 0.80, 0.34, 0.56, 1.34 and 0.92 while those of an antifungal reference ketoconazole against *A. niger*, *C. albicans* and *T. rubrum* were 0.90, 0.65 and 1.0 μg/ml, respectively.

Table 1
^{1}H and ^{13}C NMR spectral data of **1**[a] (DMSO-d_6:$CDCl_3$ = 2:1, v/v, 500 MHz)

Position	δ_C (DEPT)	δ_H, multiplicity (J in Hz)
1a	69.2 (CH_2)	4.01 *dd* (10.5, 5.5)
1b		3.67 *dd* (10.5, 4.0)
2	53.7 (CH)	3.91 *m*
3	71.8 (CH)	4.08 *br q* (5.5)
3-OH		4.92 *d* (5.5)
4	131.1 (CH)	5.41 *br dd* (16.0, 5.5)
5	129.8 (CH)	5.32 *br dt* (16.0, 6.5)
6	32.9 (CH_2)	1.98 *m*
7–9	30.0–29.5 (CH_2×3)	1.25–1.30 *m*
10, 13	32.6 (CH_2)	2.01 *m*
	32.2 (CH_2)	2.01 *m*
11	131.1 (CH)	5.45 *br dt* (15.0, 6.5)
12	132.3 (CH)	5.66 *br dt* (15.0, 6.5)
14–16	29.6–27.2 (CH_2×3)	1.25 *m*
17	23.0 (CH_2)	1.25 *m*
18	14.6 (CH_3)	0.87 *t* (7.0)
1′	175.2 (C)	
2′	72.1 (CH)	3.90 *m*
2′-OH		5.48 *d* (5.5)
3′	35.2 (CH_2)	1.95 *m*
4′	25.5 (CH_2)	1.25 *m*
5′–13′	30.0–29.5 (CH_2×9)	1.25 *m*
14′	32.2 (CH_2)	1.25 *m*
15′	23.0 (CH_2)	1.25 *m*
16′	14.6 (CH_3)	0.87 *t* (7.0)
1″	104.2 (CH)	4.21 *d* (7.5)
2″	74.0 (CH)	3.13 *ddd* (9.0, 7.5, 4.0)
2″-OH		5.02 *d* (4.0)
3″	77.2 (CH)	3.30 *td* (9.0, 4.0)
3″–OH		4.84 *d* (4.0)
4″	70.7 (CH)	3.24 *td* (9.0, 4.0)
4″-OH		4.86 *d* (4.0)
5″	77.3 (CH)	3.18 *m*
6″a	62.0 (CH_2)	3.77 *br dd* (12.0, 6.0)
6″b		3.59 *dt* (12.0, 6.0)
6″-OH		4.53 *t* (6.0)
NH		7.44 *d* (9.0)

[a] Assigned by COSY, HMQC, HMBC and NOESY experiments.

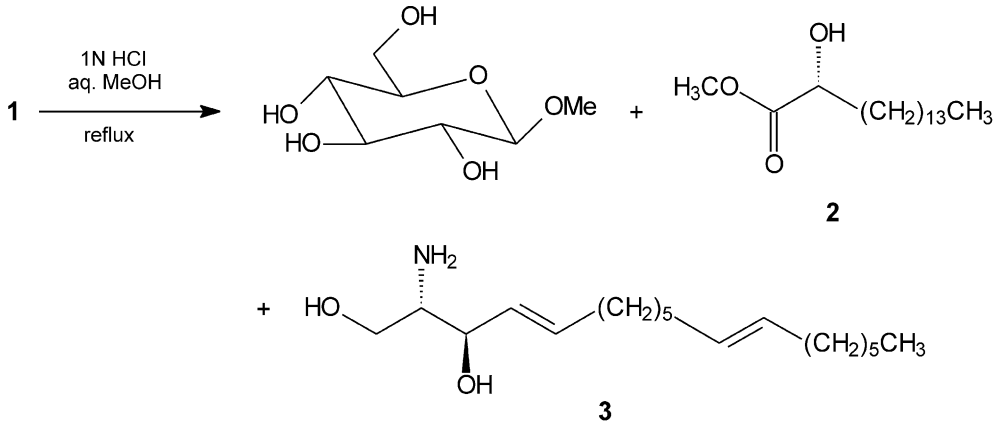

Fig. 2. Methanolysis of compound **1**.

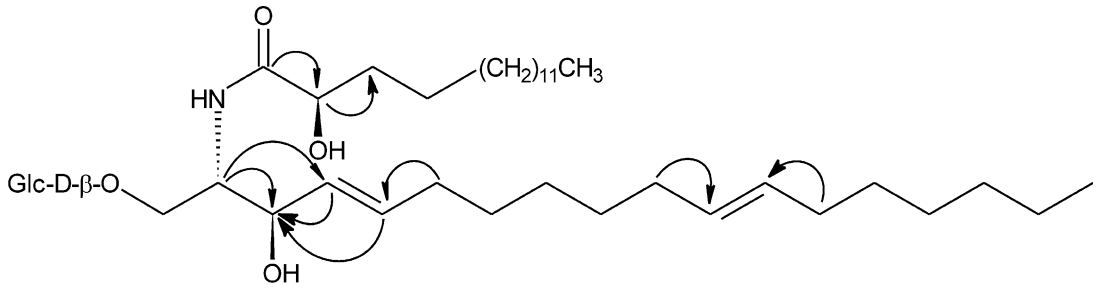

Fig. 3. Key correlations observed for analysis of the HMBC spectrum of compound **1**.

3. Experimental

3.1. General

Melting points were determined on a Boetius micro-melting point apparatus, and were uncorrected. All NMR spectra were measured on a Bruker DRX500 spectrometer in DMSO-d_6:$CDCl_3$ (2:1, v/v) with ^1H and ^{13}C nuclei observed at 500 and 125 MHz, respectively. The chemical shifts were expressed in ppm (δ) relative to an internal standard TMS; IR spectrum was recorded on a Nexus 870 FT-IR spectrometer. Silica GF_{254} for TLC and silica gel (200–300 mesh) for CC were produced by Qingdao Marine Chemical Company, China. Sephadex LH-20 was purchased from Pharmacia Biotec. AB, Uppsala, Sweden.

3.2. Plant material

Air-dried tubers of *Pinellia ternata*, purchased from Nanjing Materia Medica Company, were identified by Associate Professor L.X. Zhang, with a voucher specimen (JS-070801) deposited in the Herbarium of Nanjing University, Nanjing 210093, P. R. China.

3.3. Extraction and isolation

The coarsely chopped air-dried tubers of *Pinellia ternata* (5 kg) were extracted by refluxing with EtOH:H_2O (7:3, 2×5 l, 2 h each). Evaporation of the solvent from the extract under reduced pressure afforded an aqueous suspension which was extracted three times successively with petroleum and ethyl acetate to yield fractions A (9.2 g) and B (8.0 g), respectively. The antimicrobial fraction (B) was applied to a silica gel column (250 g) eluting with $CHCl_3$–CH_3OH (40:1, 300 ml; 20:1, 400 ml; 10:1, 500 ml; 1:1, 500 ml; CH_3OH only, 300 ml) gradient to give six crude fractions (F-1, 0.5 g; F-2, 1.0 g; F-3, 4.0 g; F-4, 0.5 g; F-5, 0.4 g, F-6, 1.1 g). Gel filtration of the antimicrobial fraction (F-4) over Sephadex LH-20 with MeOH afforded **1** (87 mg).

3.4. Antimicrobial bioassay

Evaluation of antimicrobial activities of fractions and pure phytochemicals was carried out using the method of agar dilution (Meng et al., 2001). The sample was dissolved in sterile water at a concentration of 0.1 mg/ml. Suitably quantified volumes of these test solutions were mixed with agar medium (20 ml) to prepare plates with a given concentration ($\leqslant$0.1 mg/ml) of the tested material. Test bacteria and fungi were subsequently incubated in these 'drug-containing' plates. The lowest concentration in which no growth of test microbes could be discerned was accordingly defined as the minimal inhibitory concentration (MIC). Penicillin G and ketoconazole were used as positive controls to the growth of bacteria and fungi, respectively.

3.5. Methanolysis of compound 1

A solution of **1** (10.2 mg) in a mixture of MeOH (2 ml), water (0.2 ml), and 12 N HCl (0.2 ml) was refluxed for 7 h (Qi et al., 2000). The reaction mixture was immediately cooled and dried by a stream of N_2, then subjected to gel filtration over Sephadex LH-20 with MeOH, which afforded the fatty acid methyl ester **2**, long chain base **3** and methyl glucopyranoside (Fig. 2).

3.5.1. 1-O-β-D-glucopyranosyl-(2S,3R,4E,11E)-2-(2′R-hydroxyhexadecenoylamino)-4,11-octadecadiene-1,3-diol (1)

White amorphous powder, $[\alpha]_D^{25}$: −6.0 (*c* 0.5, MeOH); m.p. 136–138 °C. IR (KBr): V_{max} = 3360, 2956, 2919, 2850, 1645, 1537, 1468, 1299, 1082, 1046, 962 and 721 cm^{-1}. Positive-charged HRESI-MS: m/z = 714.5520 $[M+H]^+$, cal. 714.5520 for $C_{40}H_{76}NO_9$, EI-MS: 497 (1), 272 (6), 227 (4), 138 (5), 69 (45), 44 (100). For ^1H and ^{13}C NMR spectral data, see Table 1.

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