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New Ceramide from *Alocasia macrorrhiza*

Nguyen Quyet Tien, PhamHoang Ngoc, Pham Hong Minh, Phan Van Kiem[1], Chau Van Minh[1], and Young Ho Kim[2]

Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghiado, Caugiay, Hanoi, Vietnam, [1]Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghiado, Caugiay, Hanoi, Vietnam, and [2]College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea

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A new ceramide alomacrorrhiza A was isolated from the ethanolic extract of the plant *Alocasia macrorrhiza* (L.) Schott. Its chemical structure was elucidated as (2*S*,3*S*,4*R*)-2*N*-[(2'*R*)-2'-hydroxy-hexacosanoyl]-tetradecane-1,3,4-triol based on extensive 1D, 2D NMR, EI-MS, FAB-MS, HR-FAB-MS spectroscopic data and chemical degradation studies.

Key words: Araceae, *Alocasia macrorrhiza*, Ceramide, Alomacrorrhiza A

INTRODUCTION

Alocasia macrorrhiza (L.) Schott (Araceae) is widely distributed in Vietnam, and used as a folk medicine to treat inflammation, eczema and abscess (Chi, 1997 and Loi, 2001). Alocasin, an anti-fungal protein and trypsin inhibitor has been isolated from the giant taro *A. macrorrhiza* (Wang and Ng, 2003; Bradbury *et al.*, 1990; Hammer *et al.*, 1989). We report herein the isolation and structure elucidation of a new ceramide (2*S*,3*S*,4*R*)-2*N*-[(2'*R*)-2'-hydroxy-hexacosanoyl]-tetradecane-1,3,4-triol (**1**) from the ethanolic extract of *A. macrorrhiza*.

MATERIALS AND METHODS

General experimental procedures

Melting points were determined using an Electrothermal IA-9200. IR spectrum was obtained on a Hitachi 270-30 type spectrometer with KBr discs. Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter. EI-MS spectrum was obtained using a Hewlett Packard 5989 B MS spectrometer. FAB-MS and HR-FAB-MS spectra were obtained using a JEOL JMS-DX 300 spectrometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer and TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck).

Correspondence to: Young Ho Kim, College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
Tel: 82-42-821-5933, Fax: 82-42-823-6566
E-mail: yhk@cnu.ac.kr

Plant material

Roots of *A. macrorrhiza* was collected in Hoabinh province, Vietnam in December 1999 and identified by Prof. Nguyen Tien Ban, Institute of Ecology, Biological Resources, VAST of Vietnam. A voucher specimen (VN-63) is deposited at the herbarium of the Institute of Chemistry, VAST, Vietnam.

Isolation

The dried and powdered roots of *A. macrorrhiza* (2.0 kg) were extracted three times with hot EtOH repeatedly to give ethanolic extract (210.0 g), which was suspended in water and extracted using *n*-hexane, chloroform, ethyl acetate and *n*-butanol, respectively. The ethyl acetate extract (11.5 g) was subjected to chromatography on a silica gel column, using chloroform-methanol (9:1) as eluent to yield six fractions (Fr. A-F). Fraction C (1.2 g) was followed by CC on a YMC RP-8 using a MeOH-H_2O (10:1) as eluent to yield **1** (34.5 mg).

(2*S*,3*S*,4*R*)-2*N*-[(2'*R*)-2'-Hydroxy-hexacosanoyl]-tetradecane-1,3,4-triol (1)

White amorphous powders; mp 112-114 °C; $[\alpha]_D^{25}$ +13.1° (*c* 1.00, pyridine); IR ν_{max}^{KBr} cm^{-1}: 3434, 3368, 2953, 2916, 2851, 1645, 1620, 1546, 1467; positive FAB-MS *m/z*: 678.6 $[M+Na]^+$; HR-FAB-MS *m/z*: 678.6013 $[M+Na]^+$ (Calcd. for $C_{40}H_{81}NO_5Na$: 678.6012); ^{1}H- (500 MHz,

DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Table I.

Acid hydrolysis of 1

Ceramide **1** (20 mg) was refluxed with 0.9 N HCl in 82% aqueous MeOH (15 ml) at 68°C for 18 h. The resulting solution was extracted with *n*-hexane, and combined organic phase was dried over Na_2SO_4. Evaporation of the *n*-hexane yielded a fatty acid methyl ester **1a** as a white amorphous powder. The H_2O layer was neutralized with conc-NH_4OH and extracted with ether. The ether layer was dried over Na_2SO_4, filtered and then concentrated to yield a long chain base.

2-Hydroxy-hexacosanoic acid methyl ester (1a)

White amorphous powder; mp 60-62 °C;$[\alpha]_D^{25}$ -1.5° (*c* 0.5, $CHCl_3$); EI-MS (70 eV) *m/z* (%): 426 $[M]^+$ (9.3), 412 (22.7), 398 (61.0), 367 (8.0), 159 (15.4), 145 (23.9), 126 (13), 111 (13), 97 (65.7), 83 (45.2), 57 (100) and 55 (82.3); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 4.18 (1H, br s, H-2), 3.79 (3H, s, $C\underline{H}_3$-O), 0.89 (3H, t, J = 8.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 175.8 (C-1'), 70.4 (C-2'), 52.4 ($\underline{C}H_3$-O), 34.4 (C-3'), 21.4-30.9 (C-4' to C-25') and 14.1 (C-26').

Acetylation of 1

Compound **1** (4 mg) was added to dry pyridine (0.25 mL) and Ac_2O (0.5 mL) and left overnight. After usual workup, the reaction mixture was chromatographed on a silica gel column (Merck, 70-230 mesh, 10 g, column Φ1×20 cm) using hexane-ethyl acetate (5 : 1, 100 mL) as eluent, yielding derivative **1b** (1.4 mg) as white crystal; mp 105-108 °C; $[\alpha]_D^{25}$ +26.5° (*c* 0.1, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.57 (d, J = 9.1 Hz, NH), 4.33-4.95 (m, 5H, carbinol protons), 2.18 (3H, s, OAc), 2.08 (3H, s, OAc), 2.05 (3H, s, OAc), 2.02 (3H, s, OAc) and 0.88 (6H, t, 8.7 Hz).

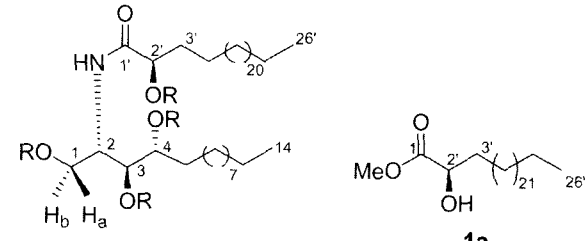
RESULTS AND DISCUSSION

Repeated column chromatography on silica gel and YMC RP-8 of the ethyl acetate extract of *A. macrorrhiza* roots yielded a new ceramide **1**. Compound **1** was found as white amorphous powders. The IR spectrum of **1** exhibited hydroxyl absorption at 3434 cm^{-1} and amide functionality at 1645 and 1546 cm^{-1}. Its HR-FAB-MS spectra provided the molecular formula $C_{40}H_{81}NO_5$ (Observed *m/z*: 678.6013 $[M+Na]^+$; Calcd. for $C_{40}H_{81}NO_5Na$: 678.6012), suggesting one degree of unsaturation. The ^{1}H- and ^{13}C-NMR spectra were typical of a ceramide possessing a long chain base and 2-hydroxy fatty acid (Table I). Assignments of all protons and carbons of **1** were made by ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The ^{1}H-NMR spectrum of **1** (in DMSO) showed a doublet at δ 7.50 (d, J = 9.1 Hz) due to an NH proton, a broad singlet at δ 1.26 (methylene protons) and carbinol protons appearing between δ 3.40 and 3.86 suggesting it to be a ceramide. The ^{13}C-NMR spectrum of **1** (in DMSO) showed carbonyl carbon signals at δ 173.2 (s), carbinol carbons at δ 60.4 (t), 71.0 (d), 71.0 (d) and 74.5 (d), methine carbon at δ 51.3 (d), methylene carbons at δ 34.0-21.5 and two methyl carbons at δ 13.2 (q). In the ^{1}H-^{1}H COSY spectrum (Table I), the NH doublet at δ 7.50 showed a cross peak at δ 4.00 attributed to the H-2 proton. The latter proton showed coupling with two doublet doublets at δ 3.72, 3.61 and one doublet doublet at δ 3.42, assigned to protons H-1 and H-3, respectively. The H-3 proton also showed coupling with the multiplet at δ 3.40 assigned to H-4. The

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

C	δ_C	δ_H (J, Hz)	^{1}H-^{1}H COSY	HMBC (H to C)
long chain base				
NH	-	7.50 (d, 9.1)	H-2	C-1', C-2', C-1, C-2, C-3
1a	60.4 (t)	3.61 (dd, 6.1, 12.5)	H-1b, H-2	C-2, C-3
1b		3.72 (dd, 6.0, 12.8)	H-1a, H-2	C-2, C-3
2	51.3 (d)	4.00 (m)	H-1a, H-1b, NH, H-3	C-1', C-3, C-4
3	74.5 (d)	3.42 (dd, 3.2, 4.5)	H-2, H-4	C-1, C-2, C-4
4	71.0 (d)	3.40 (m)	H-3, H-5a, H-5b	C-2, C-3
5a	31.8 (t)	1.55 (m)	H-4, H-6	
5b		1.28 (m)	H-4, H-6	
6- 13	21.5- 30.8 (t)	1.26 (br s)		
14	13.2 (q)	0.89 (t, 8.7)	H-13	
N-acyl moiety				
1'	173.2 (s)	-		
2'	71.0 (d)	3.86 (dd, 8.0, 3.7)	H-3'a, H-3'b	C-1', C-3'
3'a	34.0 (t)	2.00 (m)	H-2', H-4'	C-1', C-2'
3'b		2.20 (m)	H-2', H-4'	C-1', C-2'
4'- 25'	21.5- 30.8 (t)	1.26 (br s)		
26'	13.2 (q)	0.89 (t, 8.7)	H-25'	

[a]Chemical shift as ppm. Assignments were assigned on the basis of DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra.



1 R=H
1b R=Ac

1a

Fig. 1. Structures of Compounds **1**, **1a**, and **1b**

other carbinol proton appearing at δ 3.86 showed only cross peaks to δ 2.00 (m) and 2.20 (m). The above ^{1}H-^{1}H correlation studies suggested the placement of three hydroxyl groups in long chain base and one hydroxyl group in N-acetyl moiety. Moreover, the H-C long-range correlations between NH proton and carbon C-1' (δ 173.2)/C-2' (δ 71.0)/C-1 (60.4)/C-2 (51.3)/C-3 (74.5), and between proton H-2 (δ 4.00) and carbon C-3 (δ 74.5)/C-4 (71.0) were observed in the HMBC spectrum. This confirmed the location of carbonyl group at C-1', three methine carbinol groups at C-2', C-3, and C-4, and one methylene carbinol at C-1. The stereochemistry of ceramide **1** was determined as 2*S*, 3*S*, 4*R*, 2'*R* by comparison of the ^{1}H- and ^{13}C-NMR data of **1** (measured in Pyridin-d_5, data not shown) with that of (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]-1,3,4-hexadecanetriol (Huang *et al.*, 1995) as well as with that of the other (2*S*,3*S*,4*R*,2'*R*)-cerebrosides (Ryu *et al.*, 2003) and (2*S*,3*S*,4*R*,2'*R*)-phytosphingosine moieties (Kang *et al.*, 2001). Compound **1** on acetylation with Py-Ac_2O (see experimental part) gave peracetyl derivative **1b** that showed four acetyl groups at δ 2.18, 2.08, 2.05, and 2.02 in the ^{1}H-NMR spectrum. Furthermore, **1** was methanolyzed with methanolic hydrochloric acid, 2-hydroxy-hexacosanoic acid methyl ester (**1a**) was obtained together with long-chain base (see experimental part). This evidence confirmed that the fatty acid must be 2-hydroxy-hexacosanoic acid. The length of the long chain base was deduced from the result of the MS spectral data of fatty acid methyl ester and the HR-FAB-MS spectrum of **1**. Based on above data, **1** was elucidated as (2*S*,3*S*,4*R*)-2*N*-[(2'*R*)-2'-hydroxy-hexacosanoyl]-tetradecane-1,3,4-triol, which was named alomacrorrhiza A.

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