New Ceramide from *Alocasia macrorrhiza*

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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 (2S,3S,4R)-2N-[(2'R)-2'-hydroxy-hexacosanoyl]-tetradecane-1,3,4-triol based on extensive 1D, 2D NMR, El-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 (2S,3S,4R)-2N-[(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 KNY polarimeter. El-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. ¹H-NMR (500 MHz) and ¹³C-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).

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-H₂O (10:1) as eluent to yield 1 (34.5 mg).

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

White amorphous powders; mp 112-114 °C; [α]D 25 +13.1° (c 1.00, pyridine); IR νKBr 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 C₄₀H₇₁NO₅Na: 678.6012); ¹H (500 MHz,
New Ceramide from Alocasia macrorrhiza

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 Na₂SO₄. Evaporation of the n-hexane yielded a fatty acid methyl ester la as a white amorphous powder. The H₂O layer was neutralized with conc-NH₄OH and extracted with ether. The ether layer was dried over Na₂SO₄, filtered and then concentrated to yield a long chain base.

2-Hydroxy-hexacosanoic acid methyl ester (la)
White amorphous powder; mp 60-62 °C - 1.5 °C (c 0.5, CHCl₃); El-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 (85.7), 83 (45.2), 57 (100) and 55 (82.3); ¹H-NMR (500 MHz, CDCl₃) δ: 4.18 (1H, br s, H-2), 3.79 (3H, s, CH₃-O), 0.89 (3H, t, J= 8.7 Hz); ¹³C-NMR (125 MHz, CDCl₃) δ: 175.8 (C-1'), 70.4 (C-2'), 52.4 (CH₃-O), 34.4 (C-3'), 21.4-30.8 (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 Ac₂O (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 ~1x20 cm) using hexane-ethyl acetate (5 : 1, 100 mL) as eluent, yielding derivative 1b (1.4 mg) as white crystal; mp 105-108 °C; [α]D ²⁰ +26.5 ° (c 0.1, MeOH); ¹H-NMR (500 MHz, CDCl₃) 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 C₄₀H₆₄NO₆ (Observed m/z: 678.6013 [M+Na]+; Calcd. for C₄₀H₆₄NO₆Na: 678.6012), suggesting one degree of unsaturation. The ¹H- and ¹³C-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 ¹H-¹H COSY, HMOC and HMBC spectra. The ¹H-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 ¹³C-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), 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 ¹H-¹H 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.

Table I. ¹H- and ¹³C-NMR spectral data of 1

<table>
<thead>
<tr>
<th>C</th>
<th>δc</th>
<th>δh (J, Hz)</th>
<th>¹H-¹H COSY</th>
<th>HMBC (H to C)</th>
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<tr>
<td>NH</td>
<td>-</td>
<td>7.50 (d, 9.1)</td>
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<tr>
<td>1a</td>
<td>60.4 (t)</td>
<td>3.61 (dd, 6.1, 12.5)</td>
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<tr>
<td>1b</td>
<td>3.72 (dd, 6.0, 12.8)</td>
<td>4.00 (m)</td>
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<td>C-1', C-3, C-4</td>
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<tr>
<td>2</td>
<td>51.3 (d)</td>
<td>4.00 (m)</td>
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<td>C-1', C-3, C-4</td>
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<tr>
<td>3</td>
<td>74.5 (d)</td>
<td>3.42 (dd, 3.2, 4.5)</td>
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<td>4</td>
<td>71.0 (d)</td>
<td>3.40 (m)</td>
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<td>31.8 (t)</td>
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<td>5b</td>
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<td>6-13</td>
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<td>14</td>
<td>13.2 (q)</td>
<td>0.89 (l, 8.7)</td>
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</table>

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

DMSO-d₆ and ¹³C-NMR (125 MHz, DMSO-d₆): see Table I.