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Iotrochotamides I and II: New ceramides from the Indonesian sponge *Iotrochota purpurea*

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Our search for biologically active marine natural products led to the isolation of two new ceramides, iotrochotamide I (1) and iotrochotamide II (2), together with three known 6-bromoindole alkaloids (6-bromo-1*H*-indole-3-carbaldehyde (3), 6-bromo-1*H*-indole-3-carboxylic acid methyl ester (4), and 6-bromo-1*H*-indole-3-carboxylic acid ethyl ester (5)) from the sponge *Iotrochota purpurea* collected in Indonesia. The structure elucidation of these compounds was secured by spectroscopic methods (¹H, ¹³C, DEPT, COSY, HMQC and HMBC), accurate mass measurements (ESI, EI and GS-MS) as well as comparison with known compounds.

Keywords: Iotrochota purpurea; iotrochotamide; ceramides; bromoindole alkaloids

1. Introduction

Sponges have long been recognised as a rich source of structurally novel lipids including unique sterols, fatty acids, phospholipids and triglycerides (Naglc, McClatchey, & Gerwick, 1992). Sphingolipids, e.g. ceramides, cerebrosides, glycosphingolipids (GSL), sphingomylein and sphingosine derivatives or analogs are important constituents of cell membranes of eukaryotic cells and are assumed to play important roles in antigen antibody reactions and the transmission of biological information (Gao, Yang, Wang, & Liu, 2001; Qu, Zhang, & Liu, 2004). Recently, some sphingolipids have been reported to exhibit anti-ulcergenic, anti-hepatotoxic, anti-tumour, immunostimulatory, antiviral, antifungal, cytotoxic and Ca⁺-ATPase activities (Gao et al., 2001; Muralidhar, Kumar, Krishna, Rao, & Rao, 2005; Qu et al., 2004). Because of their promising biological activities, they have aroused increasing research interest. Previous studies on the sponges belonging to *Iotrochota* sp. have revealed bioactive metabolites including 6-bromoindole alkaloids (Carletti, Banaigs, & Amade, 2000; Guella, Mancini, Duhet, Richer de Froges, & Pietra, 1989),

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sphingolipids, a toxin, ecdysteroids, and tyrosine derivatives (Blunt, Copp, Munro, Northcete, & Prinsep, 2003; Muralidhar, Krishna, Kumar, Rao, & Rao, 2003). In continuation of our investigation of the chemical constituents from Indonesian marine sponges, we describe herein the isolation and structural elucidation of two new ceramides, iotrochotamide I (1) and iotrochotamide II (2), together with three known 6-bromoindole alkaloids (6-bromoin-1*H*-indole-3-carbaldehyde (3), 6-bromo-1*H*-indole-3-carboxylic acid methyl ester (4), and 6-bromo-1*H*-indole-3-carboxylic acid ethyl ester (5)). 6-Bromo-1*H*indole-3-carboxylic acid methyl ester (4) and 6-bromo-1*H*-indole-3-carboxylic acid ethyl ester (5) are known sponge metabolites previous reported for the first time by Segraves & Crews (Segraves & Crews, 2005) from *Semenospongia* sp. and Guella et al. (Guella et al., 1989) from *Iotrochota birotulata*, respectively.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer-241 MC polarimeter. ¹H NMR and ¹³C NMR spectra were measured on a Bruker DRX 500 spectrometer (¹H: 500 MHz and ¹³C: 125 MHz). The UV spectra were carried out in methanol using a Perkin–Elmer Lambda 25 UV/VIS spectrophotometer. Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT TSQ 7000 mass spectrometer. Positive-ion electrospray ionisation mass spectra (ESI-MS) were performed on a Thermofinnigan LCQ DECA mass spectrometer coupled to an Agilent 1100 high performance liquid chromatography (HPLC) system equipped with a photodiode-array detector. Vacuum liquid chromatography (VLC) was carried out on silica gel 60(0.040–0.063 mm, Merck). Column chromatographic separations were performed over silica gel 60 (0.040–0.063 mm, Merck) and Lichroprep RP-18 (Merck). TLC analysis was carried out on aluminum sheets precoated with silica gel 60 F_{254S} (Merck, Darmstadt, Germany). TLC visualisation was by UV absorption at λ 254 and 336 nm followed by spraying with anisaldehyde/H₂SO₄ reagent and heating at 110°C for 2 min. All solvents were distilled prior to use. Spectral grade solvents were utilised for chromatographic analysis.

2.2. Animal material

The sponges were collected by diving at a depth of 8 m from Pulau Baranglompo Island (south) in Indonesia in July 1997. The animals were deep frozen after collection and then freeze-dried prior to extraction. Taxonomic identification was carried out by Dr. Rob W. M. van Soest (Zoological Museum, University of Amsterdam).

2.3. Extraction and isolation

The freeze-dried sponge (150 g) was extracted with methanol six times at room temperature at 48 h intervals. The combined alcoholic extracts were concentrated under reduced pressure. The concentrated extract was diluted with water and sequentially extracted with ethyl acetate and *n*-butanol. The ethyl acetate fraction was concentrated under vacuum to yield brown coloured gummy residue (1.8 g), which was subjected to VLC on silica gel using eluants with increasing polarity starting from *n*-hexane through ethyl acetate to methanol. Six fractions were collected and monitored through

silica gel TLC. Fraction 4 on further column chromatography over silica gel column using *n*-hexane-ethyl acetate gradient elution yielded compounds 1 (15 mg) and 2 (8 mg) (Figure 1). Fraction 7 was subjected to RP-18 column using H_2O -MeOH (1:1) to give compounds 3 (5 mg), 4 (4 mg) and 5 (5 mg).

Iotrochotamide I (1). Colourless amorphous powder 25 $[\alpha]_D^{25}$ -8.3° (*c* 0.1, CHCl₃). EI–MS: m/z (rel. int.%) 663 M⁺ (30.7), 365 (17.9), 281 (3.5), 97 (37.9), 57 (84.9), 43 (100). IR (KBr) γ_{max} : 3550, 3360, 1650, 2930, 2850, 1395 cm⁻¹. For ¹H and ¹³C NMR data, see Table 1.

Iotrochotamide II (2). Colourless amorphous powder $[\alpha]_D^{25} -10.5^\circ$ (*c* 0.1, CHCl₃). ESI-LC/MS: *m*/*z* 720.3 M + H⁺. IR (KBr) γ_{max} : 3560, 3380, 1662, 2927, 2856, 1386 cm⁻¹. For ¹H and ¹³C NMR data, see Table 1.

6-Bromo-1*H***-indole-3-carbaldehyde (3).** Colourless needles; $UV\lambda_{max}$ (MeOH) nm: 222.0, 247.3, 265.0 and 305. EI-MS: m/z (rel. int.%) 225/223 [M]⁺ (100), 224/222 [M-H]⁺ (82.8), 196/194 [M-CHO]⁺ (12), 144 [M-Br]⁺ (20.6), 115 [M-(CHO + Br)]⁺ (30.5). ¹H NMR (CD₃OD, 500 MHz): 9.91 (1H, s, H-8), 8.13 (1H, s, H-2), 8.10 (1H, d, 8.5 Hz, H-4), 7.67 (1H, d, 1.6 Hz, H-7), 7.35 (1H, dd, 8.5, 1.6 Hz, H-5).

6-Bromo-1*H***-indole-3-carboxylic acid methyl ester (4).** Yellow solid; UV λ_{max} (MeOH) nm: 219.3, 250.4 and 279.9. EI-MS: m/z (rel. int.%) 253/255 [M]⁺ (92), 224/222 [M–CH₃O]⁺ (100), 196/194 [M–CH₃OCO]⁺ (11), 144 [M–(CH₃O + Br)]⁺ (10), 115 [M–(CH₃OCO + Br)]⁺ (29). ¹H NMR (CDCl₃, 500 MHz): 8.56 (1H, br s, N*H*), 8.06 (1H, d, 8.5 Hz, H-4), 7.89 (1H, d, 3.1 Hz, H-2), 7.58 (1H, d, 1.6 Hz, H-7), 7.39 (1H, dd, 8.5, 1.6 Hz, H-5), 3.95 (3H, s, OCH₃).

6-Bromo-1*H***-indole-3-carboxylic acid ethyl ester (5).** Greenish white needles; UV λ_{max} (MeOH) nm: 219.6, 250.5 and 280.0. EI-MS: m/z (rel. int.%) 269/267 [M]⁺ (91), 293/241 [M-C₂H₅]⁺ (22), 224/222 [M-C₂H₅O]⁺ (100), 196/194 (12), 144 (10), 115 (33.8). ¹H NMR (CDCl₃, 500 MHz): 8.55 (1H, br s, N*H*), 8.05 (1H, d, 8.5 Hz, H-4), 7.89 (1H, d, 2.9 Hz, H-2), 7.59 (1H, d, 1.6 Hz, H-7), 7.37 (1H, dd, 8.5, 1.6 Hz, H-5), 4.39 (2H, q, 6.9 Hz, -OCH₂-),1.42 (3H, t, 6.9 Hz, -CH₃).

Methanolysis (Muralidhar et al., 2003). Compound 1 and 2 (6 mg) were separately treated with 3 mL of 1N HCl in MeOH at 90°C for 15 h. Cold water (10 mL) was poured to the reaction mixture. The reaction mixture in each case was extracted with *n*-hexane $(15 \times 3 \text{ mL})$ to give a corresponding fatty acid methyl ester. The combined organic layer



Figure 1. Structures of componds 1 and 2.

	i				i		
	Compo	und 1			Compo	ound 2	
No.	$\delta_{\rm H}$ (m, J in Hz)	δ _C (m)	¹ H ⁻¹ H COSY	No.	$\delta_{\rm H}$ (m, J in Hz)	δ _C (m)	¹ H ⁻¹ H CC
1	A 3.95 (dd, 3.7, 11.0)	62.5 t	2	1A	3.95 (dd, 3.7, 11.2)	62.4 t	2
	B 3.70 (dd, 3.2, 11.0)			1B	3.69 (dd, 3.5, 11.2)		
2	3.92 (m)	54.5 d	1, 3, N H	7	3.90 (m)	54.5 d	1, 3, NH
3	4.30 (t, 5.0)	74.6 d	2, 4	С	4.30 (t, 5.0)	74.5 d	2, 4
4	5.52 (dd, 6.3, 15.4)	128.7 d	5	4	5.53 (dd, 6.6, 15.4)	128.7 d	5
5	5.76 (dt, 6.6, 15.4)	134.4 d	9	5	5.77 (dt, 6.9, 15.4)	134.2 d	9
9	2.07 (q, 6.9)	32.2 t	7	9	2.06 (q, 7,0)	32.3 t	7
7	1.37 (m)	31.9 (t)	8	7	1.36 (m)	31.9 (t)	8
8-16	1.20-1.35 (m)	29.1 t-29.9 t		8-21	1.20–1.35 (m)	29.1 t-29.9 t	
17	1.15 (q, 6.6)	39.0 t	18	22	1.15 (m)	39.0 t	18
18	1.52 (septet, 6.9)	27.9 d	19, 20	23	1.53 (septet, 6.6)	27.9 d	19, 20
19, 20	0.86 (d, 6.6)	22.6 q	18	24, 25	0.86 (d, 6.6)	22.6 q	18
2NH	6.25 (d, 7.3)	4	2	2NH	6.25 (d, 7.3)	4	7
1′		$174.0 { m s}$		1′	n. V	$174.0\mathrm{s}$	
2'	2.23 (t, 8.2)	36.8 t	3′	2′	2.22 (t, 8.2)	36.8 t	3,
3'	1.64 (m)	25.7 t	4	3,	1.63 (p, 7.9)	25.7 t	4
4'-20'	1.20–1.35 (m)	29.1 t–29.9 t		4'-20'	1.20–1.35 (m)	29.1 t - 29.9 t	
21′	1.23 (m)	22.7 t	22'	21'	1.23 (m)	22.7 t	22'
22′	0.88 (t, 6.5)	14.0 q	21′	22′	0.88 (t, 6.5)	14.0 q	21′

¹H⁻¹H COSY

Table 1. NMR data of compound 1 and 2 in CDCl₃ (500 and 125 MHz).

was concentrated under reduced pressure to yield colourless residue. The residue was analysed by GC-, EI-MS and ¹H NMR.

n-Docosanoic acid methyl ester (2 mg) from compound 1 and 2: ¹H NMR (CDCl₃, 500 MHz) $\delta_{\rm H}$: 0.85 (3H, t, J = 6.3 Hz), 1.25–1.32 (br s, $-{\rm CH}_2$ -groups), 1.65 (2H, m, H-3), 2.30 (2H, t, J = 7.8 Hz, H-2), 3.67 (3H, s, OCH₃). GC- and EI-MS m/z: 354 [M]⁺. The MeOH/H₂O phase was evaporated and gave a long chain base. The long chain bases were subjected to EI-MS and ¹H NMR.

(*E*)-2-Amino-19-methylicos-4-ene-1,3-diol (2 mg) from compound 1: ¹H NMR (CDCl₃, 500 MHz) $\delta_{\rm H}$: 0.85 (6H, d, J = 6.3 Hz), 1.20–1.32 (br s –CH₂–groups), 1.56 (1H, m), 2.09 (2H, m), 3.72 (1H, dd, J = 3.3, 11 Hz, H-1B), 3.97 (1H, dd, J = 3.5, 11.0 Hz, H-1A), 3.94 (1H, m, H-2), 4.31 (1H, t, J = 5.2 Hz, H-3), 5.50 (1H, dd, J = 6.5, 15.4 Hz, H-4), 5.75 (1H, dd, J = 6.7, 15.4 Hz, H-5). EI-MS m/z: 341 [M]⁺.

(*E*)-2-Amino-23-methyltetracos-4-ene-1,3-diol (0.5 mg) from compound 2: EI-MS m/z: 397 [M]⁺.

3. Results and discussion

Compound 1 was obtained as colourless amorphous powder, with an $[\alpha]_{D}^{25}$ of -8.3 (c 0.1, CHCl₃). EI-MS showed the characteristic molecular ion peak at m/z 663 [M]⁺, which, in conjunction with 1D and 2D NMR, was compatible with the molecular formula $C_{43}H_{85}NO_3$. The IR spectrum showed absorption bands at 3550 cm⁻¹ (hydroxyl); 3360, 1650 cm^{-1} (amide); and 2930, 2850 and 1395 cm^{-1} (aliphatic). The structural elucidation and complete ¹H and ¹³C assignments were achieved by 2D NMR techniques (¹H-¹H COSY and HMQC) and chemical methods. A large methylene envelope at $\delta_{\rm H}$ 1.20–1.35 in ¹H NMR and $\delta_{\rm C}$ 29.1–29.9 ppm in ¹³C NMR suggested the existence of two long aliphatic chains (Qu et al., 2004). The presence of a sphingosine moiety was indicated by signals for non-equivalent methylene proton signals at $\delta_{\rm H}$ 3.70 (1H, dd, J = 3.2, 11.0 Hz, H-1B) and 3.95 (1H, dd, J = 3.7, 11.0 Hz, H-1A), and two methine proton signals at $\delta_{\rm H}$ 3.92 (1H, m, H-2) and 4.30 (1H, t, J = 5.0 Hz, H-3). A characteristic amide NH signal was seen at $\delta_{\rm H}$ 6.25 (1H, d, J = 7.3 Hz), exchangeable with D₂O, which was also confirmed by the downfield signals at $\delta_{\rm C}$ 174.0 (C-1') and 54.5 (C-2) in ¹³C NMR. In addition, the ¹H NMR revealed the presence of two olefinic proton signals at $\delta_{\rm H}$ 5.52 (1H, dd, J = 6.3, 15.4 Hz, H-4) and 5.76 (1H, dt, J = 6.6, 15.4 Hz, H-5), which were also confirmed by the signals at $\delta_{\rm C}$ 128.7 (C-4) and 134.4 (C-5) in its ¹³C NMR spectrum (Table 1). The geometry of the double bond at C-4 was deduced to be E from the ${}^{1}H^{-1}H$ coupling constant (J = 15.4 Hz) between H-4 and H-5 (Hattori, Adachi, & Shizuri 1998). The presence of a triplet signal at $\delta_{\rm H}$ 2.23 (2H, t, J=8.2 Hz) due to the methylene protons connected to amide carbonyl indicated that the *N*-acyl moiety in **1** was a non-hydroxy fatty acid (Gao et al., 2001). In ¹H–¹H COSY (Figure 2), two spin systems were observed, the first spin system assigned to the sphingosine base. The H-1A and H-1B correlated with H-2, assumed to be geminal with the nitrogen of the secondary amide group correlated with H-3. H-3 correlated with the olefinic methine proton H-4 and this further correlated with H-5. H-5 also correlated with H₂-6 ($\delta_{\rm H}$ 2.07). H_2 -6 gave a cross peak with H_2 -7 (δ_H 1.37). H_2 -7 showed a cross peak with multiple proton signal at $\delta_{\rm H}$ 1.20–1.35, which showed correlation with a methine proton at $\delta_{\rm H}$ 1.52 (septet, J = 6.9 Hz), which also correlated with two terminal methyls at $\delta_{\rm H}$ 0.86 (6H, d, J = 6.6 Hz). Thus the connectivity -O-CH₂-CH(NH-)CH(O-)-CH=CH-CH₂-CH₂-(CH₂)_n-CH

(CH₃)₂ was established. The second spin system corresponding to the saturated fatty acyl moiety was evident from ${}^{1}H^{-1}H$ COSY spectrum. H₂-2' correlated with H₂-3', and this further correlated with multiple proton signal at $\delta_{\rm H}$ 1.20–1.35, which showed correlation with methyl proton signal at $\delta_{\rm H}$ 0.88 (t, J = 6.5 Hz). The length and branching pattern of the long chain base and fatty acid were determined on the basis of the results of its methanolysis followed by EI-, GC-MS and ¹H NMR analyses of the methanolysis products. The MeOH layer provided a C₂₁ sphingosine with an isopropyl terminus at $\delta_{\rm H}$ 0.86 (6H, d, J = 6.6 Hz) identified by ¹H NMR spectral features and further confirmed by the presence of fragment ion peaks at m/z 341 [M]⁺ and m/z 43 [M-C₁₈H₃₆NO₂]⁺ due to loss of the terminal isopropyl group in EI-MS. The *n*-hexane layer afforded a fatty acid methyl ester that showed a molecular ion peak at m/z 354 [M]⁺ upon GC- and EI-MS spectral analyses, suggesting the methyl ester of *n*-docosanoic acid. EI-MS spectrum of 1 confirmed the *n*-docosanoyl moiety by the presence of significant fragment ion peaks at m/z 365 (a) and 281 (b) (Figure 3) (Yaoita, Kohata, Kakuda, Machida, & Kikuchi, 2002). The relative stereochemistry of 1 at C-2 and C-3 was determined to be D-erythro (2S, 3R), since ¹H NMR spectral data of **1** [H-1A at $\delta_{\rm H}$ 3.95 (dd, J = 3.7, 11.0 Hz, H-1B at $\delta_{\rm H}$ 3.70 (dd, J = 3.2, 11.0 Hz), H-2 at $\delta_{\rm H}$ 3.92 (m) and H-3 at $\delta_{\rm H}$ 4.30 (t, $J = 5.0 \,\rm{Hz}$)] were in a good agreement with that of N-docosanoyl-D-erythro-(2S,3R)-16-methyl-heptadecasphing-4(E)-enine isolated from marine sponge Haliclona koremella [H-1A at $\delta_{\rm H}$ 3.94 (dd, J=3.8, 11.0 Hz), H-1B at $\delta_{\rm H}$ 3.68 (dd, J = 3.0, 11.0 Hz), H-2 at δ_{H} 3.89 (m) and H-3 at δ_{H} 4.30 (dd, J = 3.4, 6.6 Hz)] (Hattori et al., 1998). The stereochemistry of 1 at C-2 and C-3 was further confirmed to be 2S, 3R configuration by comparing the optical rotation values of 1 $([\alpha]_D^{25} - 8.3^{\circ})$ and N-docosanoyl-D-erythro-(2S,3R)-16-methyl-heptadecasphing-4(E)-enine $([\alpha]_D - 6.0^\circ)$. On the basis of this evidence, the structure of 1 was determined to be (2S,3R,4E)-N-docosanoyl-2-amino-19- methyl-icosa-4-ene-1,3-diol, and was named iotrochotamide I.

Compound 2 was obtained as a colourless amorphous powder. ESI-LC/MS displayed a pseudomolecular ion peak at m/z 720.3 [M+H]⁺, thus differing from compound 1 by 56 mass units, suggested the presence of four additional methylene groups. The ¹H and ¹³C



Figure 2. Some key ¹H-¹H COSY correlations of compound 1.



Figure 3. Characteristic fragmentation pattern of compound 1.

NMR spectra of **2** closely resembled those of **1** except for the integration of the aliphatic methylene protons at $\delta_{\rm H}$ 1.20–1.35. The lengths of LCB and fatty acid were determined by methanolysis followed GC-, EI-MS and ¹H NMR analyses of the methanolysis products. GC- and EI-MS spectra of FAM showed a molecular ion peak at m/z 354 [M]⁺, indicating that the FAM was the same as that of **1** *n*-docosanoic acid methyl ester, and the LCB had to be (*E*)-2-amino-23-methyl-tetracos-4-ene-1,3-diol. This was supported by EI-MS of LCB, which gave a molecular ion peak at m/z 397 [M]⁺. The optical rotation value of **2** ($[\alpha]_{\rm D}^{25} - 10.5^{\circ}$) in comparison with **1** ($[\alpha]_{\rm D} - 8.3^{\circ}$) showed that **2** has the same absolute configuration as that of **1** for C-2 and C-3 parts. Based on this evidence, the structure of **2** was unambiguously determined to be (2S,3R,4E)-*N*-docosanoyl-2-amino-23-methyl-tetracos-4-ene-1,3-diol, and the name iotrochotamide II was given to this new natural product. This is the first isolation of ceramide from the sponge *Iotrochota purpurea*.

The structures of compounds (3) (Rasmussen, Jensen, Anthoni, Christophersen, & Nielsen, 1993), (4) Segraves & Crews. (2005), and (5) Guella et al. (1989) were determined by interpretation of 1D and 2D NMR spectral data and confirmed by comparison with the reported values.

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