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Papuamides E and F, Cytotoxic Depsipeptides from the Marine Sponge *Melophlus* sp

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Abstract

Two known papuamides C (1) and D (2) together with two new depsipeptides, papuamides E (3) and F (4), were isolated from an undescribed sponge of the genus *Melophlus* collected in the Solomon Islands. The planar structures of the compounds were elucidated on the basis of spectroscopic studies. Papuamides C–F (1–4) showed cytotoxicity against brine shrimp with LD₅₀ values between 92 and 106 µg/mL.

Keywords

Papuamide; *Melophlus*; Depsipeptide; Cytotoxic

1 Introduction

Marine sponges that are active against human immunodeficiency virus (HIV) have served as a rich source of cyclic depsipeptides incorporating rare amino acid residues. The marine sponges *Theonella swinhoei* and *Theonella mirabilis* (family Theonellidae) from Papua New Guinea have yielded papuamides A–C, cyclic depsipeptides with structurally unique features including unprecedented acid moieties.¹ Callipeltin A, isolated from the New Caledonian marine sponge *Callipelta* sp.², neamphamide A, obtained from *Neamphius huxlei*³ and mirabamides A–D from *Siliquariaspongia mirabilis*⁴ are also well recognized for their potent HIV-inhibitory activity. Mirabamide C and novel congeners mirabamides E–H with anti-HIV activity were also recently reported from *Stelletta clavosa* (family Ancorinidae).⁵

As part of our continuing search for bioactive metabolites from marine invertebrates, in the present study, we isolated two new depsipeptides (3–4) along with the previously reported papuamides C and D¹ from the butanol extract of a marine sponge *Melophlus* sp. Marine sponges of the genus *Melophlus* (family Ancorinidae) have been reported to yield compounds belonging to tetramic acids and saponins.^{6–9} This is the first report of

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Supplementary data

¹H NMR, HSQC, HMBC and HSQC-TOCSY spectra for papuamides E and F.

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depsipeptides from *Melophlus* sp. In this paper, we describe the isolation, structure elucidation and bioactivity of these new depsipeptides.

2 Results and discussion

The sponge was extracted three times at room temperature using MeOH followed by CH₂Cl₂. The extracts were combined, evaporated *in vacuo* and partitioned between H₂O and CH₂Cl₂. The aqueous layer was further partitioned with n-BuOH and the active n-BuOH extract was subjected to bioassay guided fractionation using C18 bonded silica. Further purification by reverse-phase C18 HPLC afforded compounds (**1–4**).

The major metabolites, compounds **1** and **2**, showed protonated molecular ions [M+H]⁺ at *m/z* 1399.73678 and 1385.7203 in the HRMS (ESI), respectively. A marinlit (2010) search for the corresponding masses revealed a match to the known papuamides C and D.¹⁰ The NMR spectral data of **1** and **2** were identical to the literature values of papuamides C and D, respectively.

Compounds **3** and **4** were isolated in smaller quantities compared with **1** and **2**. Comparison of the ¹H NMR spectra of **3** and papuamide C (**1**) indicated strong similarities over most of the molecule. Detailed 2D NMR data analysis of **3** together with comparison to **1** enabled us to identify 11 amino acid residues present in **3** to be identical to the corresponding residues in **1**: two glycine (Gly) residues, threonine (Thr), aminobutenoic acid (Aba), 3,4-dimethylglutamine (3,4-DiMeGln), 3-hydroxyleucine (3-OHLeu), 3-methoxyalanine (3-OMeAla), alanine (Ala), *N*-methylthreonine (NMeThr), β-methoxytyrosine (β-OMeTyr) and homoproline (Hpr) (Table 1).

A molecular formula of C₆₆H₁₀₂N₁₂O₂₀ for **3** was suggested by a pseudomolecular ion at 1369.7250 by HRMS (ESI) ([M+H]⁺; calcd. for C₆₆H₁₀₃N₁₂O₂₀ 1369.7250; = 0.0 ppm) which differs from papuamide C (**1**) by loss of an oxygen atom. The quaternary oxygenated carbon signal at δ_C 78.8 ppm in **1** has been replaced in **3** by a methine at δ_H 2.41 ppm, δ_C 48.7 ppm as suggested by the HMBC correlation of the proton Me-2_{Htda} (δ_H 1.05 ppm) to the carbon C-2_{Htda} (δ_C 48.7 ppm) and the absence of any HMBC correlations in **3** to the quaternary carbon (C-2_{Dhtda} in **1**). The upfield shift of H-3_{Dhtda} (from δ_H 4.88 ppm to δ_H 4.72 ppm) and Me-2_{Dhtda} (from δ_C 22.4 ppm to δ_C 14.2 ppm) are further consistent with the replacement of the 2,3-dihydroxy-2,6,8-trimethyl-4,6-decadienoic acid moiety (Dhtda) of papuamide C (**1**) with a 3-hydroxy-2,6,8-trimethyl-4,6-decadienoic acid moiety (Htda) in **3**, accounting for the difference in molecular formula between the two compounds. The chemical shifts for the Htda moiety in **3** were consistent with the proton and carbon chemical shifts assigned for the Htda moiety in mirabamide H⁵. However, a large (~15 Hz) coupling was not evident even if the actual coupling could not be resolved for H4 and H5 of

Htlda of compound **3**. Furthermore, the agreement of the chemical shifts of all the NMR signals of Htlda moiety in compound **3** to the Htlda moiety in mirabamide **1** suggests a *Z* geometry to the C4–C5 olefin and *E* geometry to the C6–C7 double bond.

The HRESI(+)MS of papuamide **F** (**4**) indicated a molecular formula of $C_{65}H_{100}N_{12}O_{20}$ which differed from that of papuamide **E** (**3**) by loss of CH_2 . The *N*-methyl (δ_C 31.1 ppm, δ_H 3.15 ppm) signal in **3** was absent in the HSQC spectrum of **4**. Compound **3** differed from compound **4** in that the threonine residue of **3** was *N*-methylated while **4** was not. This relationship was similar to that of papuamides **C** and **D** in that papuamide **C** was *N*-methylated while papuamide **D** was not. Thus, **4** and **3** are analogues of **1** and **2**, respectively, in which the Dhtlda moiety has been replaced with Htlda. Moreover, the C4–C5 olefin of the Htlda was assigned a *Z* geometry on the basis of coupling of approximately 11 Hz between H4 and H5 as in the papuamides¹ and mirabamides⁵. The agreement of the chemical shifts of all NMR signals in Htlda near the C6–C7 double bond suggests the same stereochemistry (*E* geometry) as in **1** and **2**.

The optical rotation signs reported for all papuamides were same to that of the obtained for the compounds **3** and **4** indicating that all the amino acid residues in compounds **3** and **4** possessed identical configurations to papuamides **A–D**¹. The compounds, **3** and **4**, were obtained as optically active white powders that are concluded as new analogues of papuamides **A–D** and proposed as papuamides **E** and **F**, respectively.

Brine shrimp assay¹¹ revealed these analogues (**1–4**) to be cytotoxic with LD₅₀ values of 92, 92, 104 and 106 $\mu\text{g/mL}$, respectively. However, papuamides **C–F** (**1–4**) were found to be inactive against methicillin resistant *Staphylococcus aureus* (ATCC 10537), vancomycin resistant *Enterococcus faecium* (ATCC 12952), wild type *Candida albicans* (ATCC 32354) and amphotericin resistant *Candida albicans* (ATCC 90873).

3 Experimental section

3.1 General experimental procedures

Optical rotations were recorded on a Bellingham Stanley ADP220 polarimeter. NMR spectra were recorded on a Varian spectrometer operating at 400 MHz. Chemical shifts are referenced to residual MeOH (δ_C 49.0; δ_H 3.31) in CD_3OD . High resolution ESI-MS analyses were obtained using Thermo Scientific LTQ Orbitrap Discovery LC-MS in positive electrospray ionisation mode. Reverse phase flash chromatography was performed on Bakerbond C18 40 μm prep LC packing. Semi-preparative HPLC was performed using a Waters 515 HPLC system with a Alltech 10 μm C18 (250 x 10 mm) column.

3.2 Animal material (collection and taxonomy)

The marine sponge *Melophlus* sp. (family Ancorinidae) was collected by hand using scuba at a depth of 10 m from Karumolum Pt, Russell Island in the Solomon Islands (S8° 85.76' and E159° 6.98') on 21st June 2006. The marine sponge was identified by Prof. John Hooper of Queensland Museum, Australia. Voucher specimens of the sponge, SOL06-1-018, are preserved at University of Utah and The University of the South Pacific.

3.3 Extraction, isolation and purification

The frozen sponge was cut into small pieces, extracted using MeOH (3 x 1000 mL) and followed by CH_2Cl_2 (3 x 1000 mL). The extracts were combined and evaporated to dryness under vacuum. The crude (4.5 g) was partitioned with CH_2Cl_2 –H₂O (3:1). The aqueous layer was further partitioned with *n*-BuOH. The resulting biologically active *n*-BuOH extract was subjected to RP-silica chromatography pre-equilibrated with aqueous MeOH

(20% H₂O). The column was eluted with a stepwise gradient of 20–100% MeOH_(aq). The active (100% MeOH) elute was rechromatographed on RP-silica using stepwise gradient 20–100% MeOH_(aq) to yield 12 fractions. The eighth fraction 80% MeOH_(aq) was subjected on isocratic RP-HPLC using 83% MeOH_(aq) at a flow rate of 4.0 mL/min and monitoring at a wavelength of 254 nm to yield 5 fractions. The fourth fraction was further purified on RP-HPLC using isocratic elution with 42% MeCN_(aq) to yield pure compounds **1** (4.2 mg, *t_R* = 25.3 min), **2** (4.1 mg, *t_R* = 18.5 min), **3** (1.8 mg, *t_R* = 31.0 min) and **4** (2.0 mg, *t_R* = 22.1 min).

3.3.1 Papuamide E (3)—white powder; $[\alpha]_D^{25} +68.5$ (c 0.07, MeOH); ¹H NMR (400 MHz, CD₃OD), see Table 1; HRMS (ESI): *m/z* 1383.7407 [M + H]⁺ (calcd for C₆₆H₁₀₃N₁₂O₂₀, 1383.7406), HRMS (ESI): *m/z* 1405.7230 [M + Na]⁺ (calcd for C₆₆H₁₀₂N₁₂O₂₀Na, 1405.7226).

3.3.2 Papuamide F (4)—white powder; $[\alpha]_D^{25} +107.1$ (c 0.03, MeOH); ¹H NMR (400 MHz, CD₃OD), see Table 1; HRMS (ESI): *m/z* 1369.7250 [M + H]⁺ (calcd for C₆₅H₁₀₁N₁₂O₂₀, 1369.7250), HRMS (ESI): *m/z* 1391.7067 [M + Na]⁺ (calcd for C₆₅H₁₀₀N₁₂O₂₀Na, 1391.7069).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1NMR Spectroscopic Data (400 MHz, CD₃OD), for Papuamides E (3) and F (4)

	Papuamide E (3)		Papuamide F (4)	
	δ_C	δ_H^a	δ_C	δ_H^a
Homoproline (Hpr)				
1	n.o.	–	n.o.	–
2	52.7	5.32 m	52.7	5.18 d (9.6)
3	27.3	2.20 m, 1.77 m	27.5	2.25 m
4	21.1	1.26 m	21.7	1.75 s
5	26.9	1.76 m	25.8	1.72 ovl
6	44.3	4.15 m	44.9	4.06 m
β-Methoxytyrosine (β-OMeTyr)				
1	n.o.	–	n.o.	–
2	54.0	5.15 d (5.2)	54.0	5.22 m
3	85.5	4.25 d (9.6)	84.9	4.33 m
3-OMe	56.1	3.10 s	56.9	3.15 s
1'	129.3	–	129.1	–
2', 6'	130.6	7.21 d (8.4)	130.3	7.20 d (8.4)
3', 5'	116.0	6.77 d (8.4)	116.0	6.76 d (8.4)
4'	159.2	–	158.7	–
N-Methylthreonine (NMeThr/Thr-2)				
1	n.o.	–	n.o.	–
2	59.3	3.37 m	60.1	3.93 m
3	64.1	3.89 m	66.8	3.94 m
4	20.0	0.52 d (6.4)	19.7	0.76 d (6.0)
N-Me	31.1	3.15 s		
Alanine (Ala)				
1	n.o.	–	175.0	–
2	51.1	4.68 m	51.1	4.32 m
3	15.8	1.46 d (7.2)	17.4	1.47 d (7.6)
Glycine (Gly-1)				
1	n.o.	–	n.o.	–
2	43.8	3.85 s	43.9	4.13 m, 3.85 m
3-Methoxyalanine (3-OMeAla)				
1	n.o.	–	n.o.	–
2	56.0	4.18 m	56.0	4.35 m
3	70.9	3.44 m, 3.46 m	71.9	3.83 m, 3.66 m
3-OMe	59.5	3.44 s	59.2	3.38 s
3-Hydroxyleucine (3-OHLeu)				
1	n.o.	–	n.o.	–
2	54.0	4.75 m	54.9	4.87 m

	Papuamide E (3)		Papuamide F (4)	
	δ_C	δ_H^a	δ_C	δ_H^a
3	77.4	5.40 m	77.6	5.40 m
4	29.6	2.04 m	29.4	2.03 m
5	17.6	0.92 m	16.8	0.91 ovl
5'	19.8	0.90 m	19.6	0.90 ovl
3,4 - Dimethylglutamine (3,4-DiMeGln)				
1	n.o.	–	n.o.	–
2	58.7	4.21 m	58.5	4.32 m
3	39.7	2.15 m	38.0	2.20 m
4	42.9	2.64 m	42.4	2.59 m
5	180.5	–	n.o.	–
3-Me	13.6	0.95 ovl	14.0	0.98 d (6.8)
4-Me	15.5	1.19 d (7.2)	15.5	1.16 d (7.2)
Aminobutenoic acid (Aba)				
1	n.o.	–	n.o.	–
2	n.o.	–	n.o.	–
3	132.0	6.55 m	131.1	6.57 q (7.2)
4	13.0	1.77 d (4.3)	12.9	1.78 d (4.0)
Threonine (Thr-1)				
1	n.o.	–	n.o.	–
2	59.6	4.60 d (3.2)	59.7	4.60 d (3.2)
3	68.9	4.40 m	68.9	4.40 dd (6.4, 3.6)
4	19.8	1.24 d (6.4)	19.8	1.24 d (6.4)
Glycine (Gly-2)				
1	n.o.	–	n.o.	–
2	44.0	4.14 m	45.0	3.39 m, 3.38 m
2,3-Dihydroxy-2,6,8-trimethyl-4,6-decadienoic acid (Dhtda)				
1	178.5	–	178.4	–
2	48.7	2.41 m	48.7	2.42 m
3	71.5	4.72 m	71.3	4.69 t (9.6)
4	129.8	5.29 ovl	129.8	5.29 m
5	137.9	6.09 m	137.8	6.08 dd (11.6, 2.8)
6	132.0	–	132.0	–
7	139.6	5.26 m	139.7	5.26 m
8	35.4	2.36 m	35.4	2.37 m
9	31.3	1.35 m, 1.30 m	31.1	1.39 m, 1.29 m
10	12.3	0.89 ovl	12.3	0.88 ovl
2-Me	14.2	1.05 d (6.4)	14.1	1.05 d (6.8)
6-Me	16.8	1.80 s	16.8	1.80 s
8-Me	20.8	0.98 d (6.8)	20.8	0.98 ovl

Ovl: overlapped; n.o.: not observed.

^a Coupling constants are in parentheses and given in hertz. Although an HMBC was recorded, more assignments thus appear not possible.