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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 LD_{50} values between 92 and 106 μg/mL.

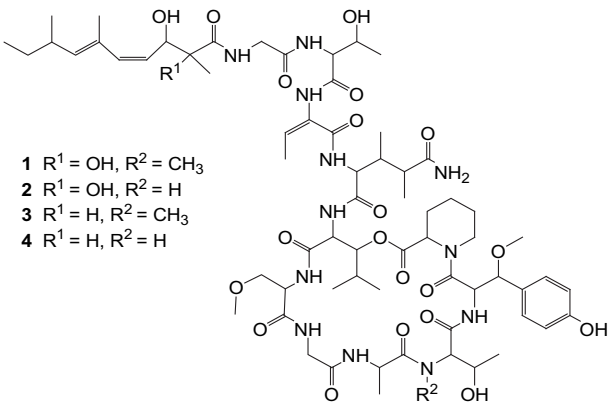
1. Introduction

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 recognised 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 D[1] 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 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 CH_2Cl_2. The extracts were combined, evaporated *in vacuo* and partitioned between H_2O and CH_2Cl_2. 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**).



1 R^1 = OH, R^2 = CH_3
2 R^1 = OH, R^2 = H
3 R^1 = H, R^2 = CH_3
4 R^1 = H, R^2 = H

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 (2011) search for the corresponding masses revealed a match to the known papuamides C and

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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 ^{1}H 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 $C_{66}H_{102}N_{12}O_{20}$ for **3** was suggested by a pseudomolecular ion at 1369.7250 by HRMS (ESI) ($[M+H]^+$; calcd for $C_{66}H_{103}N_{12}O_{20}$ 1369.7250; Δ=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 H[5]. However, a large (~15 Hz) coupling was not evident even if the actual coupling could not be resolved for H4 and H5 of Htda of compound **3**. Furthermore, the agreement of the chemical shifts of all the NMR signals of Htda moiety in compound **3** to the Htda moiety in mirabamide H 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 Dhtda moiety has been replaced with Htda. Moreover, the C4–C5 olefin of the Htda was assigned a *Z* geometry on the basis of coupling of approximately 11 Hz between H4 and H5 as in the papuamides[1] and mirabamides.[5] The agreement of the chemical shifts of all NMR signals in Htda 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 assay[11] revealed these analogues (**1**–**4**) to be cytotoxic with LD_{50} values of 92, 92, 104 and 106 μg/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 *C. albicans* (ATCC 90873).

Table 1
NMR spectroscopic data (400 MHz, CD_3OD), 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	5.32m	52.7	5.18d (9.6)
3	27.3	2.20m, 1.77m	27.5	2.25m
4	21.1	1.26m	21.7	1.75s
5	26.9	1.76m	25.8	1.72ovl
6	44.3	4.15m	44.9	4.06m
β-Methoxytyrosine (β-OMeTyr)				
1	n.o.	—	n.o.	—
2	54.0	5.15d (5.2)	54.0	5.22m
3	85.5	4.25d (9.6)	84.9	4.33m
3-OMe	56.1	3.10s	56.9	3.15s
1′	129.3	—	129.1	—
2′, 6′	130.6	7.21d (8.4)	130.3	7.20d (8.4)
3′, 5′	116.0	6.77d (8.4)	116.0	6.76d (8.4)
4′	159.2	—	158.7	
N-Methylthreonine (NMeThr/Thr-**2**)				
1	n.o.	—	n.o.	—
2	59.3	3.37m	60.1	3.93m
3	64.1	3.89m	66.8	3.94m
4	20.0	0.52d (6.4)	19.7	0.76d (6.0)
N-Me	31.1	3.15s		
Alanine (Ala)				
1	n.o.	—	175.0	—
2	51.1	4.68m	51.1	4.32m
3	15.8	1.46d (7.2)	17.4	1.47d (7.6)
Glycine (Gly-**1**)				
1	n.o.	—	n.o.	—
2	43.8	3.85s	43.9	4.13m, 3.85m
3-Methoxyalanine (3-OMeAla)				
1	n.o.	—	n.o.	—
2	56.0	4.18m	56.0	4.35m
3	70.9	3.44m, 3.46m	71.9	3.83m, 3.66m
3-OMe	59.5	3.44s	59.2	3.38s
3-Hydroxyleucine (3-OHLeu)				
1	n.o.	—	n.o.	—
2	54.0	4.75m	54.9	4.87m
3	77.4	5.40m	77.6	5.40m
4	29.6	2.04m	29.4	2.03m
5	17.6	0.92m	16.8	0.91ovl
5′	19.8	0.90m	19.6	0.90ovl
3,4-Dimethylglutamine (3,4-DiMeGln)				
1	n.o.	—	n.o.	—
2	58.7	4.21m	58.5	4.32m
3	39.7	2.15m	38.0	2.20m
4	42.9	2.64m	42.4	2.59m
5	180.5	—	n.o.	—
3-Me	13.6	0.95ovl	14.0	0.98d (6.8)
4-Me	15.5	1.19d (7.2)	15.5	1.16d (7.2)
Aminobutenoic acid (Aba)				
1	n.o.	—	n.o.	—
2	n.o.	—	n.o.	—
3	132.0	6.55m	131.1	6.57q (7.2)
4	13.0	1.77d (4.3)	12.9	1.78d (4.0)
Threonine (Thr-**1**)				
1	n.o.	—	n.o.	—
2	59.6	4.60d (3.2)	59.7	4.60d (3.2)
3	68.9	4.40m	68.9	4.40dd (6.4, 3.6)
4	19.8	1.24d (6.4)	19.8	1.24d (6.4)
Glycine (Gly-**2**)				
1	n.o.	—	n.o.	—
2	44.0	4.14m	45.0	3.39m, 3.38m
2,3-Dihydroxy-2,6,8-trimethyl-4,6-decadienoic acid (Dhtda)				
1	178.5	—	178.4	—
2	48.7	2.41m	48.7	2.42m
3	71.5	4.72m	71.3	4.69t (9.6)
4	129.8	5.29ovl	129.8	5.29m
5	137.9	6.09m	137.8	6.08dd (11.6, 2.8)
6	132.0	—	132.0	—
7	139.6	5.26m	139.7	5.26m
8	35.4	2.36m	35.4	2.37m
9	31.3	1.35m, 1.30m	31.1	1.39m, 1.29m
10	12.3	0.89ovl	12.3	0.88ovl
2-Me	14.2	1.05d (6.4)	14.1	1.05d (6.8)

Table 1 (*continued*)

	Papuamide E (**3**)		Papuamide F (**4**)	
	δ_C	δ_H[a]	δ_C	δ_H[a]
6-Me	16.8	1.80s	16.8	1.80s
8-Me	20.8	0.98d (6.8)	20.8	0.98ovl

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.

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 Orbitarp 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 (250×10 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 (3×1000 mL) and followed by CH_2Cl_2 (3×1000 mL). The extracts were combined and evaporated to dryness under vacuum. The crude (4.5 g) was partitioned with CH_2Cl_2–H_2O (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% H_2O). 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); ^{1}H NMR (400 MHz, CD_3OD), see Table 1; HRMS (ESI): *m*/*z* 1383.7407 $[M+H]^+$ (calcd for $C_{66}H_{103}N_{12}O_{20}$, 1383.7406), HRMS (ESI): *m*/*z* 1405.7230 $[M+Na]^+$ (calcd for $C_{66}H_{102}N_{12}O_{20}Na$, 1405.7226).

3.3.2. Papuamide F **(4).** White powder; $[\alpha]_D^{25}$ +107.1 (c 0.03, MeOH); ^{1}H NMR (400 MHz, CD_3OD), see Table 1; HRMS (ESI): *m*/*z* 1369.7250 $[M+H]^+$ (calcd for $C_{65}H_{101}N_{12}O_{20}$, 1369.7250), HRMS (ESI): *m*/*z* 1391.7067 $[M+Na]^+$ (calcd for $C_{65}H_{100}N_{12}O_{20}Na$, 1391.7069).

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Supplementary data

^{1}H NMR, HSQC, HMBC and HSQC-TOCSY spectra for papuamides E and F. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.08.100.

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