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Malyngamide 3 and Cocosamides A and B from the Marine Cyanobacterium *Lyngbya majuscula* from Cocos Lagoon, Guam

Sarath P. Gunasekera † , Chivas S. Owle † , Rana Montaser ‡ , Hendrik Luesch ‡ , and Valerie J. Paul *,†

Smithsonian Marine Station at Ft. Pierce, 701 Seaway Drive, Ft. Pierce, FL 34949, and Department of Medicinal Chemistry, University of Florida, 1600 SW Archer Road, Gainesville, Florida 32610

Abstract

Malyngamide 3 (1) and cocosamides A (2) and B (3) were isolated from the lipophilic extract of a collection of *Lyngbya majuscula* from Cocos Lagoon, Guam. The planar structures of compounds 1–3 were determined by spectroscopic methods. The absolute configuration of 1 was determined by modified Mosher's method, NOESY data and comparison with lyngbic acid (4). The absolute configurations of 2 and 3 were assigned by enantioselective HPLC analysis and comparison with the closely related compound pitipeptolide A (5). Compounds 1–3 showed weak cytotoxicity against MCF7 breast cancer and HT-29 colon cancer cells.

Marine cyanobacteria of the genus *Lyngbya* are a prolific source of chemically diverse bioactive secondary metabolites. Malyngamides are small amides first discovered in the late 1970s and early 1980s from *L. majuscula* by Richard E. Moore's research group. There are now over 30 known examples of malyngamides and the majority are reported from cyanobacteria. Recently, William Gerwick's group reported the newest addition malyngamide 2 isolated from *L. sordida* collected from Papua New Guinea. Malyngamides are characterized by a fatty acid side chain, which is most commonly 7*S*-methoxytetradec-4(*E*)-enoic acid (lyngbic acid). The other part of the malyngamides usually encloses a cyclic unit. In one notable example (malyngamide J) the cyclic ketone has a pendant 2,4-dimethoxyxylose. Malyngamides O and P are the only examples of an acyclic molecules in this series. Malyngamide 3 (1) described here is the next example of an acyclic malyngamide.

Cyclic depsipeptides containing a unique 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya), 2,2-dimethyl-3-hydroxy-7-octenoic acid (Dhoea) or 2,2-dimethyl-3-hydroxyoctanoic acid (Dhoaa) were first reported by Scheuer's group from a marine mollusk. 11,12 Subsequently, Richard Moore's 13 and William Gerwick's 14 groups have isolated several of these unique cyclic depsipeptides from *L. majuscula*. Here, we report the isolation, structure determination and biological activity determination of two new cyclic depsipeptides that possess these distinctive moieties (Dhoea/Dhoya), namely cocosamides A (2) and B (3), from *L. majuscula* collected from Cocos Lagoon, Guam. Interestingly, this is the first report

To whom correspondence should be addressed. Tel: (772) 462-0982. Fax: (772) 461-8154. paul@si.edu.

[†]Smithsonian Marine Station

Department of Medicinal Chemistry, University of Florida

of cyclic depsipeptides in this series with one ester linkage, while other related compounds reported thus far with these unique acids have two or more ester linkages. 11-14

The sample of the marine cyanobacterium *L. majuscula* was collected from a patch reef near Cocos Island, Guam in February 2001. The freeze-dried material was extracted with a mixture of EtOAc–MeOH (1:1) to afford a lipophilic extract, which was subsequently partitioned between EtOAc and H₂O. The EtOAc-soluble portion was repeatedly fractionated by SiO₂ chromatography followed by reversed-phase C18 HPLC to give three new compounds malyngamide 3 (1), and cocosamides A (2) and B (3) in addition to the known compounds malyngamide A,⁴ malyngamide B,³ and an unresolved mixture of majusculamides A and B.¹⁵

Malyngamide 3 (1) was obtained as a colorless, amorphous powder. The molecular formula $C_{28}H_{47}ClN_2O_7$ was determined from HRESIMS data. Its infrared spectrum contained absorption due to amide proton at 3320 cm⁻¹, ester carbonyl at 1725 cm⁻¹, and an amide carbonyl at 1636 cm⁻¹. The ¹H and ¹³C NMR spectra (Table 1) showed signature signals for the presence of the characteristic 7-methoxy-tetradec-4(*E*)-enoic acid moiety suggesting compound 1 to be an analogue of the malyngamides.

Following the interpretation of DQF COSY and edited HSQC experiments, the ^{1}H and ^{13}C NMR signals were assignable to three partial structures C-1 to C-4, C-8 to C-10, C-2′ to C-14′ and an isolated C-6 methylene group. In addition, the ^{1}H , ^{13}C and edited HSQC spectra indicated the presence of signals for two O-Me groups (C-12, δ_{H} 3.68, δ_{C} 51.9 and C-15′, δ_{H} 3.30, δ_{C} 56.5), one N-Me (C-13, δ_{H} 2.93, δ_{C} 35.5) and four carbonyl groups (C-5, δ_{C} 202.2; C-7, δ_{C} 166.6; C-11, δ_{C} 172.4 and C-1′, δ_{C} 174.0). The chemical shift values for C-2 (δ_{C} 131.4, C) and C-3 (δ_{H} 6.08, s; δ_{C} 120.7, CH) indicated the presence of a chloromethylene moiety in the C-1 to C-4 partial structure as in other malyngamides^{4,5,16} and accounted for the chlorine atom in the molecular formula. HMBC correlations (Table 1) from H-3 (δ_{H} 6.08) to C-1 (δ_{C} 45.9), C-2 (δ_{C} 131.4) and C-4 (δ_{C} 46.8) confirmed the position of the chloromethylene moiety. Similarly, HMBC correlations from H-4a and H-4b (δ_{H} 3.20, 3.15) to C-5 (δ_{C} 202.2) and H₂-6 (δ_{H} 3.39) to the C-5 and C-7 (δ_{C} 166.6) carbonyl

groups extended the carbon chain to the amide carbonyl group. The HMBC correlations N-H ($\delta_{\rm H}$ 7.26) to C-7 and C-8 ($\delta_{\rm C}$ 45.2), H-9 ($\delta_{\rm H}$ 4.17) to C-8, C-10 ($\delta_{\rm C}$ 38.9) and C-11 ($\delta_{\rm C}$ 172.4), and H_3 -12 (δ_H 3.68) to the C-11 carbonyl group established the planar structure for the right hand end of the molecule. The HMBC correlations from H_3 -13 (δ_H 2.93) to the C-1' carbonyl ($\delta_{\rm C}$ 174.0) and to C-1 connected the fatty acid chain to the right hand end of the molecule via an amide linkage, resembling other malyngamides. An E configuration was assigned for the C-4'/C-5' olefin on the basis of the coupling constant (15.7 Hz). ¹⁷ The NOESY spectrum of 1 did not show any cross-peaks between H-3 and H₂-1, nor between H-3 and H₃-13. However, the presence of a strong cross-peak between H-3 (δ_{H} 6.08) and H-4b ($\delta_{\rm H}$ 3.15) established a Z configuration for the chloromethylene moiety in 1 as in isomalyngamides A and B.¹⁷ In order to determine the configuration at C-7', compound 1 was hydrolyzed under basic conditions to give lyngbic acid (4). The observed specific rotation of 4 ($[\alpha]^{25}$ _D -12) was comparable to the reported value for 7(S)methoxytetradec-4(E)-enoic acid ($[\alpha]_D$ –12.6), ¹⁶ and thus, established a 7(S) configuration at the C-7' position in 1. The absolute configuration at C-9 of 1 was determined by the modified Mosher's method. ¹⁸ Compound **1** was converted to (S)- and (R)-MTPA esters. The $\delta (= \delta_S - \delta_R)$ values of (S)- and (R)-MTPA esters (-0.078 for Ha-8; -0.031 for Hb-8; +0.028 for H₂-10; +0.072 for CH₃-12) revealed the R configuration at C-9. These data confirmed the structure 1 for malyngamide 3.

Cocosamides A (2) and B (3) were obtained as white solids. The molecular weights of 2 and 3 differ by two mass units on the basis of HRESI/TOFMS analysis. The ¹H and ¹³C NMR spectra were indicative of depsipeptides (Table 2).

Following the interpretation of DQF COSY, edited HSQC and HMBC experiments, the ¹H and ¹³C NMR signals of 2 and 3 were assignable to six partial structures, which accounted for all atoms in both molecules. These partial structures consisted of the amino acids valine, proline, glycine, two N-Me-phenylalanines, besides 2,2-dimethyl-3-hydroxy-7-octenoic acid (Dhoea) in 2 and 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya) in 3. The ¹H NMR spectra showed the presence of three olefinic protons ($\delta_{\rm H}$ 5.76, H-7; $\delta_{\rm H}$ 4.96, 5.04, H-8a, b) in the spectrum of 2, while no olefinic protons were seen in the spectrum of 3, which instead revealed a characteristic acetylenic proton ($\delta_{\rm H}$ 1.96, t, J = 2.7 Hz, H-8). The ¹³C spectrum of 2 indicated olefinic signals (δ_C 138.0, CH, C-7; δ_C 115.2, CH₂, C-8), while the ¹³C spectrum of 3 indicated acetylenic signals (δ_C 83.6, C, C-7; δ_C 69.2, CH, C-8). These data together with other data presented in Table 2 confirmed the presence of a 2,2-dimethyl-3-hydroxy-7octenoic acid in 2 and 2,2-dimethyl-3-hydroxy-7-octynoic acid in 3, respectively. The residue sequences for 2 and 3 were determined from HMBC data which showed linkages: Val-NH to C-1, Me-25 to C-11, CH₂-30 to C-16, Me-40 to C-26, Gly-NH to C-31 and H-3 to C-41, and these connections were confirmed by NOESY correlations. These data established the residue sequences as 1,6-anhydro[Dhoea-Val-N-Me-Phe(1)-Pro-N-Me-Phe(2)-Gly] for 2 and 1,6-anhydro[Dhoya-Val-N-Me-Phe(1)-Pro-N-Me-Phe(2)-Gly] for 3. The absolute configurations of the amino acids were determined by enantioselective HPLC analysis of the acid hydrolysates of 2 and 3. The analysis revealed L-configurations for valine, proline and both N-Me-phenylalanines in compounds 2 and 3. Because we have isolated only small quantities of 2 and 3, the configuration at C-3 of the hydroxy acids (Dhoea and Dhoya) was investigated by comparison of NOE data with pitipeptolide A (5). 13 Pitipeptolide A (5) is a cyclic depsipeptide that has a (S)-Dhoya moiety, which is connected to L-valine and glycine forming the amide and ester linkages similar to compounds 2 and 3. The NOE data for 5 were not previously reported, ¹³ therefore, we used 5 that we isolated from another Lyngbya sample for NOE comparison studies. The ¹H NMR spectrum, HRMS and specific rotation data for this sample matched that reported in the literature. ^{13,19} The NOESY spectrum of **5** showed a strong correlation between H-3 ($\delta_{\rm H}$ 4.94) and H₃-10 ($\delta_{\rm H}$ 1.15) and another correlation between H-4a ($\delta_{\rm H}$ 1.80) and H₃-10. Similarly, strong

correlations were observed between H-4b ($\delta_{\rm H}$ 1.58) and H₃-9, $\delta_{\rm H}$ 1.29), and methyl (H₃-9 and L-Val-NH ($\delta_{\rm H}$ 6.08). There was no correlation seen between H-3 and H₃-9. These data clearly indicated that in **5** the methine (H-3) is closer to methyl H₃-10 and away from methyl H₃-9. Cocosamides A (**2**) and B (**3**) showed the same patterns of NOE correlations for H-3, H₃-9 and H₃-10. These data suggested a 3*S* configuration at C-3 in compounds **2** and **3**.

Compounds 1–3 were tested for antiproliferative activity against MCF7 breast cancer and HT-29 colon cancer cells and found to be weakly active. Malyngamide 3 (1) showed cytotoxic activity against MCF7 and HT-29 cells with IC $_{50}$ values of 29 and 48 μ M, respectively. This is an about 10-fold weaker activity than reported for the closely related analogue malyngamide O. 10 Cocosamides A (2) and B (3) showed cytotoxic activity against HT-29 cells with IC $_{50}$ values of 24 μ M and 11 μ M, respectively. MCF7 cells were slightly less susceptible to both compounds with IC $_{50}$ values of 30 μ M for 2 and 39 μ M for 3. The closely related pitipeptolides A and B exert similar activity against cancer cells. 13

Experimental Section

General Experimental Procedures

The optical rotations were recorded on a Perkin Elmer model 343 polarimeter. UV spectrophotometric data were acquired on a Hitachi U-3010 spectrophotometer. IR spectroscopic data were obtained on a Bruker Vector 22 FT-IR spectrometer. NMR data were collected on a JEOL ECA-600 spectrometer operating at 600.17 MHz for 1 H and 150.9 MHz for 13 C. The edited-HSQC experiment was optimized for $J_{\rm CH}$ = 140 Hz and the HMBC spectrum was optimized for $^{2/3}J_{\rm CH}$ = 8 Hz. 1 H NMR chemical shifts (referenced to residual CHCl₃ observed at δ 7.25) were assigned using a combination of data from 2D DQF COSY and multiplicity-edited HSQC experiments. Similarly, 13 C NMR chemical shifts (referenced to CDCl₃ observed at δ 77.0) were assigned on the basis of multiplicity-edited HSQC experiments. The HRMS data were obtained using an Agilent 6210 LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector at the Mass Spectrometer Facility at the University of California, Riverside, California. Silica gel 60 (EMD Chemicals, Inc. 230–400 mesh) was used for column chromatography. All solvents used were of HPLC grade (Fisher Scientific).

Collection, Extraction and Isolation

The sample of cyanobacterial assemblage of Lyngbya majuscula for this study was collected in February 2001 from a patch reef near Cocos Island, Guam. This was a collection of ECO 27, first collected in March 1999. This chemotype of L. majuscula grew during winter months on Guam (January – March) and consistently produced malyngamides A and B, and majusculamides A and B. The samples were identified by one of us (VJP) based on morphological characteristics of the genus, and a voucher specimen (VP-ECO 27) is maintained at the Smithsonian Marine Station, Fort Pierce, FL. The freeze dried material (328 g) was extracted with EtOAc-MeOH (1:1). This lipophilic extract was partitioned between EtOAc and H₂O and the aqueous portion subsequently partitioned between n-BuOH and H₂O. Concentration of these extracts furnished 9.56 g (2.9%) of EtOAc-soluble fraction and 2.24 g (0.6%) of BuOH-soluble material. The EtOAc-soluble fraction (9.56 g) was chromatographed on a column of SiO₂ (100 g) using a hexanes-EtOAc step gradient system followed by an EtOAc-MeOH step gradient to give thirteen sub-fractions. The combined sub-fractions 7 to 9 (2.0 g), eluting with hexanes-75% EtOAc was further chromatographed on a Si-column (100 g) using a hexanes-EtOAc step gradient system to give eight sub-fractions. Sub-fraction 6 (36 mg), eluting with hexanes-25% EtOAc was further purified by reversed-phase HPLC (semi-prep 250 × 10 mm, 5 μm, RP-18, flow 3.0 mL/min) using 15% H₂O-MeOH to give 3 mg of impure cocosamide B, 20 mg of

majusculamides A and B, 4.0 mg of malyngamide 3 (1, yield, 0.001% dry wt), and 1.4 mg of cocosamide A (2, yield, 0.0004% dry wt). The impure cocosamide B fraction was further separated by reversed-phase HPLC using 30% H_2O –MeOH to give 2.0 mg of cocosamide B (3, yield 0.0006% dry wt).

Malyngamide 3 (1)

colorless, amorphous powder; $[\alpha]^{25}_D$ = 10.1 (c 0.36, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.19), 274 (3.40) nm; IR (film) ν_{max} 3320, 2932, 1725, 1636 cm⁻¹; 1 H and 13 C NMR data, see Table 1, assignments were made by interpretation of 2D DQF COSY, edited-HSQC, HMBC and NOESY data; HRESI/TOFMS m/z 559.3146 [M + H]⁺ (calcd for $C_{28}H_{48}^{35}$ ClN₂O₇, 559.3145).

Cocosamide A (2)

white solid; $[\alpha]^{25}_D$ –77.7 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.36), 260 (3.26) nm; IR (film) ν_{max} 3330, 2920, 1665, 1634, 1527, 1197 cm⁻¹; ¹H and ¹³C NMR data, see Table 2, assignments were made by interpretation of 2D DQF COSY, edited-HSQC, HMBC and NOESY data; HRESI/TOFMS m/z 744.4330 [M + H]⁺ (calcd for C₄₂H₅₈N₅O₇, 744.4331).

Cocosamide B (3)

white solid; $[\alpha]^{25}_D$ –103 (c 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.54), 260 (3.35) nm; IR (film) ν_{max} 3416, 2950, 1665, 1634, 1541, 1032 cm⁻¹; ¹H and ¹³C NMR data, see Table 2, assignments were made by interpretation of 2D DQF COSY, edited-HSQC, HMBC and NOESY data; HRESI/TOFMS m/z 742.4186 [M + H]⁺ (calcd for C₄₂H₅₆N₅O₇, 772.4174).

Preparation of (R)-MTPA and (S)-MTPA Esters of 1

Compound 1 (1.0 mg) was dissolved in CHCl₃ (50 μ L) and added pyridine (50 μ L) and a catalytic amount of 4-DMAP. The solution was treated with S(+)-MTPA chloride (1.0 μ L) and stirred at room temperature for 12 h. The reaction was terminated with the addition of MeOH (200 μ L) and the solvent was evaporated to give the (R)-MTPA ester of 1. Similarly, the (S)-MTPA ester of 1 was prepared with R(-)-MTPA chloride using the same procedure. Both esters were subjected to HPLC (semi-prep 250 × 10 mm, 5 μ m, SiO₂, flow 3.0 mL/min) using EtOAc– 3% MeOH to yield the pure (R)-MTPA ester of 1 (0.4 mg) and (S)-MTPA esters of 1 (0.3 mg).

R-MTPA ester of 1

¹H NMR δ (only key resonances are listed) 5.921 (1H, t, J = 5.4 Hz, NH), 5.292 (1H, m, H-9), 3.584 (3H, s, OMe-12), 3.536 (2H, s, H₂-6), 2.624 (2H, d, J = 6.9 Hz, H₂-10); ESIMS m/z 777.4 [M + H]⁺; HRESI/TOFMS m/z 777.3404 [M + H]⁺ (calcd for C₃₈H₅₇³⁵ClF₃N₂O₉, 777.3422).

S-MTPA ester of 1

¹H NMR δ (only key resonances are listed) 5.732 (1H, t, J = 5.4 Hz, NH), 5.292 (1H, m, H-9), 3.656 (3H, s, OMe-12), 3.535 (2H, s, H₂-6), 2.652 (2H, d, J = 6.9 Hz, H₂-10); ESIMS m/z 777.4 [M + H]⁺; HRESI/TOFMS m/z 777.3443 [M + H]⁺ (calcd for C₃₈H₅₇³⁵ClF₃N₂O₉, 777.3422).

Base Hydrolysis of Malyngamide 3

Compound **1** (1.9 mg) was dissolved in a 0.5 mL solution of 10% KOH in 80% aqueous EtOH and refluxed for 12 h. The hydrolysate was concentrated in vacuo and partitioned between H₂O and CH₂Cl₂. The H₂O layer was separated, acidified, and extracted with CH₂Cl₂ to yield lyngbic acid (**5**, 0.4 mg): colorless oil; $[\alpha]^{25}_D$ –12 (c 0.04, CHCl₃) [lit. –12.6 (c 0.8, MeOH)¹⁶]; ¹H NMR (600 MHz, CDCl₃) δ 5.48 (2H, m), 3.31 (3H, s, *O*Me), 3.15 (1H, quin, J = 5.5 Hz), 2.42 (2H, t, J = 7.5 Hz), 2.34 (2H, m), 2.18 (2H, m), 1.43 (2H, m), 1.27 (10H, m), 0.87 (3H, t, J = 7.0 Hz); HRESI/TOFMS m/z 257.2113 [M + H]⁺ (calcd for C₁₅H₂₉O₃, 257.2114).

Acid Hydrolysis and Enantioselective HPLC analysis

Compounds **2** and **3** (0.1 mg each) were suspended in 6 N HCl (0.3 mL) and heated at 115 °C for 18 h in two sealed tubes. The hydrolysates were concentrated to dryness. The residues were reconstituted in 0.3 mL of H₂O and analyzed by enantioselective HPLC, comparing the retention times with those of authentic standards [Phenomenex Chirex ($_{\rm D}$) Penicillamine, 4.6 × 250 mm, 5 μ m]; solvent mixtures of 2.0 mM CuSO₄– CH₃CN (85:15 or 90:10); detection at 254 nm. Using 2.0 mM CuSO₄ – CH₃CN (90:10) with a flow rate of 0.8 mL/min, the retention times ($t_{\rm R}$ min) for authentic standards were L-Pro (10.0) and D-Pro (19.6) and with a flow rate of 1.0 mL/min the retention times ($t_{\rm R}$ min) for authentic standards were L-Val (17.0) and D-Val (22.9). Using 2.0 mM CuSO₄–CH₃CN (85:15) with a flow rate of 1.0 mL/min, the retention times ($t_{\rm R}$ min) for authentic standards were N-Me-L-Phe (34.2) and N-Me-D-Phe (36.6). The retention times in min (and respective HPLC conditions) of the amino acids in the hydrolysates of **2** and **3** were 10.0 (90:10, 0.8 mL/min), 17.0 (90:10, 1.0 mL/min), and 34.2 (85:15, 1.0 ml/min), indicating the presence of L-Pro, L-Val and N-Me-L-Phe.

Cell Viability Assays

Cells were propagated and maintained in DMEM (Invitrogen) supplemented with 10% FBS (Hyclone) at 37 $^{\circ}$ C humidified air and 5% CO₂. Cells were seeded in 96-well plates (MCF7 10,500 cells/well; HT-29 13,000 cells/well). After 24 h, cells were treated with various concentrations of the test compound, or solvent control (1% EtOH). After 48 h of incubation, cell viability was measured using MTT according to the manufacturer's instructions (Promega). Paclitaxel was used as a positive control; IC₅₀ values were 7 nM and 6 nM in HT-29 and MCF7 cell lines, respectively. Experiments were done in duplicate. IC₅₀ values were determined using non-linear regression in GraphPad Prism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

.50 MHz)

position	$\delta_{ m C}$ mult.	$\delta_{ m H} (J { m in} { m Hz})$	$\cos \lambda_d$	HMBC	$NOESY^b$
1a	45.9, CH ₂	4.24, d (-15.1)		2, 3, 4, 1'	13
1b		4.17, d (-15.1)	3	2, 3, 4, 1'	13
	131.4, C				
	120.7, CH	6.08, s	1,4	1, 2, 4	4b
4a	46.8, CH ₂	3.20, d (17.1)	3	1, 2, 3, 5	3,6
		3.15, d (17.1)	3	1, 2, 3, 5	1b, 3, 6
	202.2, C				
	49.7, CH ₂	3.39, s		5,7	4, NH
	166.6, C				
8a	$45.2, CH_2$	3.52, ddd (14.2, 7.0, 5.4)	NH, 9	7, 9, 10	9, NH
98		3.21, ddd (14.2, 9.0, 5.4)	NH, 9	7, 9, 10	9, NH
	67.2, CH	4.17, m	8, 10	8, 10, 11	8a, 8b, 10
10	38.9, CH ₂	2.50, d (6.9)	6	8, 9, 11	8a, 8b, 9
11	172.4, C				
12	$51.9, CH_3$	3.68, s		11	
13	35.5, CH ₃	2.93, s		1, 1'	1a, 1b, 2'
H-N		7.26, t (5.4)	∞	7,8	6, 8a, 8b
	174.0, C				
	$33.1, \mathrm{CH}_2$	2.35, m	3,	1', 3', 4'	13, 4'
	28.1 , CH_2	2.28, m	2', 4'	1', 2', 4', 5	5,
	130.8, CH	5.43, dt (15.7, 3.5)		3', 5'	2', 6'
	127.6, CH	5.49, dt (15.7, 3.5)		3', 4', 6'	3', 7', 15'
	36.4, CH ₂	2.17, m	5,7	4', 5', 7', 8'	4', 7'
	80.8, CH	3.14, m	8,9	5′, 9′, 15′	5', 6', 15'
	33.4, CH ₂	1.41, m	7, 9	6', 7', 9', 10'	
	25.4, CH ₂	1.32, m 1.26, m	8, 10	8', 10'	
10,	20 A CU.	1.27			

position	$\delta_{ m C}$ mult.	position $\delta_{ m C}$ mult. $\delta_{ m H}$ (J in Hz)	COSYa HMBC	HMBC	$NOESY^b$
11'	29.9, CH ₂ 1.27, m	1.27, m			
12′	31.9, CH ₂ 1.27, m	1.27, m			
13′	22.7, CH ₂ 1.27, m	1.27, m			
14′	14.2, CH ₃	0.86, t (6.9)	13	12', 13'	
15′	56.5, CH ₃ 3.30, s	3.30, s		7,	5', 7'

 $^{a_1}\mathrm{H-}^{1}\mathrm{H\,cosy}$

 $[\]ensuremath{^{h}\text{NOESY}}$ correlations are from proton(s) stated to the indicated proton(s).

NMR Spectroscopic Data for Cocosamides A (2) and B (3) in CDCl $_3$ (1 H 600 MHz, 13 C 150 MHz) Table 2

unit	position	$\delta_{ m C}$ mult.	$\delta_{ m H} (J { m in} { m Hz})$	HMBC^d	$NOESYb^b$	$\delta_{ m C}$ mult.	$\delta_{ m H} (J m in Hz)$
Dhoea ^c /Dhoya ^d	1	176.5, C				176.5, C	
	2	48.9, C				46.3, C	
	3	77.8, CH	5.19, dd (11.0, 2.1)	1, 9, 41	4a, 4b, 10	77.3, CH	5.20, br. d (11.0)
	4a	27.9 , CH_2	1.54, m	5	3, 10	$27.6, \mathrm{CH}_2$	1.75, m
	4b		1.48, m	S	3,9		1.58, m
	5	$25.0, \mathrm{CH}_2$	1.34, m	7	3,6	24.6, CH ₂	1.51, m
	9	33.3, $CH2$	2.06, m		5,7	$18.1,\mathrm{CH}_2$	2.21, m
	7	138.0, CH	5.76, ddt (17.0, 11.6, 6.9)	9	6, 8a	83.6, C	
	8a	$115.2, CH_2$	4.96, dd (11.6, 3.4)	9	7	69.2, CH	1.96, t (2.7)
	8b		5.04, dd (17.0, 3.4)	9			
	6	17.5, CH ₃	1.25, s	1, 3, 10	4a, 4b, NH (Val)	17.6, CH ₃	1.28, s
	10	$23.5, CH_3$	1.17, s	1, 3, 9	3, 4a, 9	$23.5, CH_3$	1.20, s
Val	11	172.2, C				172.3, C	
	12	55.5, CH	4.33, dd (7.7, 7.5)	1, 11, 14, 15	13, NH (Val), 25	55.6, CH	4.32, dd (7.7, 7.5)
	13	30.5, CH	1.91, m	12	12, 14, 15	30.6, CH	1.93, m
	14	$19.1, CH_3$	0.97, d (6.9)	12, 13, 15	12, 13, 15	19.2, CH ₃	0.97, d (6.9)
	15	$18.7, CH_3$	0.88, d (6.9)	12, 13, 14	12, 13, 14	18.8, CH ₃	0.90, d (6.9)
	NH		5.82 d (7.5)	1, 12	9, 12, 13, 14		5.83 d (7.6)
N-Me-Phe-1	16	168.9, C				169.0, C	
	17	54.2, CH	5.08, dd (12.4, 3.9)		18a, 18b, 25, 27	54.3, CH	5.03, br.d (12.4)
	18a	37.7, CH ₂	3.18, dd (12.4, 12.3)	16, 19, 20/24	17, 20/24, 25	37.8, CH ₂	3.18, dd (12.4, 12.3)
	18b		3.02, dd (12.4, 3.9)	16, 19, 20/24	17, 20/24, 25		3.02, dd (12.4, 3.9)
	19	137.6, C				137.6, C	
	20/24	129.8, CH	7.41, d (7.6, 2.7)	18	17, 18, 21/23	129.9, CH	7.40, d (7.6, 2.7)
	21/23	128.5, CH	7.24, m	19	20/24, 22	128.6, CH	7.24, m
	22	127.0, CH	7.18, m	20/24	21/23	127.0, CH	7.18, m

			Cocosamide A (2)	ide A (2)		Coc	Cocosamide B (3)
unit	position	position $\delta_{ m C}$ mult.	$\delta_{ m H} (J m in Hz)$	HMBC^{a}	$NOESYb^b$	$\delta_{ m C}$ mult.	$\delta_{ m H} (J m in Hz)$
	25	32.3, CH ₃	3.58, s	11, 17	12, 17, 18a	32.3, CH ₃	3.58, s
Pro	26	171.5, C				171.6, C	
	27	56.0, CH	3.08, dd (8.2, 1.8)	28, 29, 30	17, 28a, 32	56.1, CH	3.06, dd (8.2, 1.8)
	28a	29.8 , CH_2	0.46, m	26	27, 28b, 29b	$29.9, CH_2$	0.47, m
	28b		-0.18, m		28a, 32		-0.19, m
	29a	21.9 , CH_2	1.31, m		28b, 29b, 30a	$22.0, \mathrm{CH}_2$	1.31, m
	29b		1.21, m		28a, 29a, 30b		1.21, m
	30a	46.2 , CH_2	3.38, m	16	29a, 30b	$46.0, \mathrm{CH}_2$	3.37, m
	30b		3.22, m	16	29b, 30a		3.21, m
N-Me-Phe-2	31	169.4, C				169.5, C	
	32	63.5, CH	3.93, dd (9.7, 3.5)	31, 33, 40	27, 33, NH (Gly)	63.6, CH	3.94, dd (9.7, 3.5)
	33a	34.9, $CH2$	3.68, dd (12.0, 3.5)	32, 34, 35/39	32, 33b, 35/39	$35.0, \mathrm{CH}_2$	3.68, dd (12.0, 3.5)
	33b		2.75, dd (12.0, 9.7)	32, 34, 35/39	32, 33a, 35/39		2.75, dd (12.0, 9.7)
	34	138.1, C				138.2, C	
	35/39	129.4, CH	7.05, d (7.6)	33, 37	33, 36/38	129.5, CH	7.05, d (7.6)
	36/38	128.8, CH	7.23, m	34	35/39, 37	128.9, CH	7.23, m
	37	127.0, CH	7.22, m	35, 39	36/38	127.1, CH	7.22, m
	40	$31.0, \mathrm{CH}_3$	2.82, s			$31.0, CH_3$	2.82, s
Gly	41	168.3, C				168.4, C	
	42a	$41.9, CH_2$	4.80, dd (16.8, 8.9)	41	42b, NH (Gly)	41.9, CH ₂	4.78, dd (16.8, 8.9)
	42b		3.66, dd (16.8, 1.0)	41	42a, NH (Gly)		3.68, dd (16.8, 1.0)
	HN		8.91, dd (8.9, 1.0)	31	32, 40, 42b		8.91, dd (8.3, 1.0)

^aHMBC correlations, optimized for $^{23}J_{CH} = 8$ Hz, are from proton(s) stated to the indicated carbon.

 $^{^{}b}$ NOESY correlations are from proton(s) stated to the indicated proton(s).

 $^{^{}c}$ Dhoea moiety in cocosamide A.

 $[^]d$ Dhoya moiety in cocosamide B.