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Amantelides A and B, Polyhydroxylated Macrolides with Differential Broad Spectrum Cytotoxicity from a Guamanian Marine Cyanobacterium

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Abstract

Cytotoxicity-guided fractionation of a Guamanian cyanobacterial collection yielded the new compounds amantelides A (**1**) and B (**2**). These polyketides are characterized by a 40-membered macrolactone ring consisting of a 1,3-diol and contiguous 1,5-diol units and a *tert*-butyl substituent. Amantelide A (**1**) displayed potent cytotoxicity with sub-micromolar IC₅₀ against HT29 colorectal adenocarcinoma and HeLa cervical carcinoma cell lines. Acetylation of the hydroxy group at C-33 in **2** caused a close to 10-fold decrease in potency. Exhaustive acetylation of the hydroxy groups abrogated the antiproliferative activity of amantelide A (**1**) by 20–67-fold. Further bioactivity assessment of **1** against *bacterial pathogens* and marine fungi indicated a broad spectrum of bioactivity.

Secondary metabolites from marine cyanobacteria have been dominated by peptide-based molecules, both from ribosomal and nonribosomal origins.^{1,2} Nonribosomal peptides often incorporate polyketide fragments that increase the chemical diversity of these small molecules, while purely polyketide synthase-derived compounds from cyanobacteria are less prevalent.² These polyketides include tolytoxin,³ scytophycins,⁴ oscillariolide,⁵ phormidolide⁶ and caylobolides A⁷ and B,⁸ with characteristic polyhydroxylation and/or polyunsaturation patterns. These compounds display antiproliferative effects towards eukaryotic cells, demonstrated using mammalian cells and/or model organisms.^{3–9} Tolyltoxin shows a wide range of biological activities including potent inhibition of human nasopharyngeal cancer cells, yeast and other fungal cells.⁹

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Supporting Information Available: NMR spectra for compounds **1–3** and Figure S1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The broad spectrum of activities of polyenes and polyols has been ascribed to their lipid permeabilizing effects, primarily due to their amphiphilic nature. The polyene and polyhydroxy moieties of the dinoflagellate compounds, amphidinols, have been shown to permeabilize phospholipid liposomes in the presence of cholesterol within the layer.¹⁰ In addition, the lipid permeabilizing effect of amphidinol was dependent on cholesterol content,¹⁰ suggesting the direct binding of these compounds to cholesterol leading to pore formation.¹⁰ Structure-activity relationship studies corroborated these results, with the polyene region being critical to binding to the lipid bilayer and the size of the pore being dictated by the polyhydroxy region.¹¹

Here we report the purification, structure elucidation and bioactivity studies of a new polyhydroxylated macrolactone, amantelide A (**1**), and its related compounds amantelide B (**2**) and peracetyl-amantelide A (**3**). Compound **1** showed cytotoxic and antifungal activities, with preliminary structure-activity relationship (SAR) indicating the importance of the polyhydroxy functionalities.

	R ¹ - R ⁷	R ⁸	R ⁹
Amantelide A (1)	H	H	H
Amantelide B (2)	H	Ac	H
Peracetyl-amantelide A (3)	Ac	Ac	Ac

A gray cyanobacterium collected near Puntan dos Amantes, Tumon Bay, Guam was extracted with CH₂Cl₂–MeOH (1:1). The resulting nonpolar extract exhibited

antiproliferative activity against HT29 cells at a concentration of 10 $\mu\text{g/mL}$. Solvent partitioning of the nonpolar extract gave the hexanes-, *n*-BuOH- and H_2O -soluble fractions. The antiproliferative *n*-BuOH fraction was further fractionated by silica column chromatography, with the bioactivity concentrated in the fraction eluting from 70% *i*-PrOH in CH_2Cl_2 . Reversed-phase HPLC purification, afforded two related polyketide-derived compounds, amantelides A (**1**) and B (**2**), as bioactive constituents.

The HRESIMS spectrum of amantelide A (**1**) suggested a molecular formula of $\text{C}_{44}\text{H}_{84}\text{O}_{11}$ based on the observed $[\text{M} + \text{Na}]^+$ ion at m/z 811.5927. The three degrees of unsaturation were partially accounted for by an α,β -unsaturated ester based on ^1H and ^{13}C NMR, HSQC and HMBC spectra, suggesting the presence of one ring system to fulfill the molecular formula requirements. HMBC correlations with the sp^2 C (δ_{C} 160.3) were observed for the CH_3 singlet (δ_{H} 1.85) and a vinyl group (δ_{H} 5.62), with the latter also having long-range correlations to a carbonyl group (δ_{C} 165.5), confirming the presence of an α,β -unsaturated ester (Table 1). The presence of an ester functionality was also corroborated by the presence of a deshielded methine ($\delta_{\text{C}}/\delta_{\text{H}}$ 76.6/4.93), which also showed HMBC correlations to C-1 (δ_{C} 165.5). In addition, a 1,3-methine carbinol and a *tert*-butyl moiety were also deduced from the NMR data. Using COSY and HMBC correlations, a partial structure (Figure 1) for amantelide A (**1**) was derived. This is reminiscent of the C-1 to C-9 and C-33 to C-40 moieties of caylobolide B.⁸ However, instead of an isohexyl pendant side chain, amantelide A (**1**) bears a *tert*-butyl moiety. ROESY analysis indicated that the C2-C3 double bond has a *Z*-configuration. Selective NOESY experiments showed correlation between H-2 (δ_{H} 5.62) and H-44 (δ_{H} 1.85). NOE analysis of caylobolide B indicated a *Z*-configuration as well (Supporting Information).

The overlapping ^1H and ^{13}C NMR signals only allowed for partial assignment of the structure of **1**. Comparison of the ^1H and ^{13}C NMR chemical shifts of caylobolide B and amantelide A (**1**) indicated that the latter lacks the distinctive 1,3,5-triol system present in caylobolides A⁷ and B⁸ (Table 1). Based on the ^1H and ^{13}C NMR chemical shifts as well as the remaining $\text{C}_{27}\text{H}_{52}\text{O}_6$ to be accounted for from the partial structure and molecular formula of **1**, a contiguous chain of 1,5-diols is proposed to form the macrocyclic structure of amantelide A (**1**). The observed degenerate ^{13}C NMR shifts in amantelide A (**1**) (Table 1) are in accordance with literature values for 1,5-diols in luteophanols¹² and caylobolides A⁹ and B.¹⁰ To verify the proposed structure, MS/MS fragmentation of amantelide A (**1**) was performed under negative ionization (Figure 2). Fragmentations were observed at α - and β -positions to the methine carbinols, analogous to the fragmentation pattern observed in caylobolide B and amphidinols. Combined MS/MS fragmentation and NMR analysis confirmed that amantelide A (**1**) has a closely related structure to the caylobolides.

HRESIMS data for amantelide B (**2**) showed $[\text{M} + \text{Na}]^+$ ion at m/z 853.6044, with a 42 amu mass difference with amantelide A (**1**), suggesting a molecular formula of $\text{C}_{46}\text{H}_{86}\text{O}_{12}$. ^1H and ^{13}C NMR, HSQC and HMBC spectra of amantelide B (**2**) suggested that this compound belong to the same structural class, with an additional acetyl group in amantelide B (**2**). This was corroborated by a singlet CH_3 ($\delta_{\text{C}}/\delta_{\text{H}}$ 20.7/1.97) that showed an HMBC correlation to a carbonyl group (δ_{C} 170.0) (Table 1). This acetyl group is proposed to modify a methine carbinol, which is evident from the appearance of a deshielded methine ($\delta_{\text{C}}/\delta_{\text{H}}$ 73.3/4.73)

that also showed an HMBC correlation to the carbonyl at δ_C 170.0 (Table 1). C-7 (δ_C/δ_H 68.6/3.58), C-9 (δ_C/δ_H 68.8/3.55) and C-37 (δ_C/δ_H 66.4/3.21) were eliminated as possible sites of acetylation since the characteristic 1H and ^{13}C NMR shifts of these moieties can still be clearly discerned (Table 1). A TOCSY correlation between δ_H 4.73 (H-33) and δ_H 3.21 (H-37) suggested that C-33 bears the additional acetyl group in **2**. Hence, amantelide B (**2**) is the C-33 monoacetylated analog of amantelide A (**1**). Verification by MS/MS fragmentation was, however, not successful due to the immediate loss of the acetyl group upon ionization, yielding a similar fragmentation pattern as amantelide A (**1**).

Amantelides A (**1**) and B (**2**) showed similarities to caylobolides A and B, with the presence of a polyhydroxylated macrolactone ring that contains a pendant aliphatic side chain. The C-1 to C-21 portions of the macrolactone ring are similar, with the characteristic 1,3-diol moiety (C-7 to C-9) flanked by a 1,5-diol moiety (C-10 to C-24) and an α,β -unsaturated ester (C-1 to C-3). Compounds **1** and **2** are distinguished by their 1,5-dihydroxylation pattern (C-25 to C-39), as well as a larger 40-membered macrolactone ring instead of a 36-membered macrocycle in caylobolides. The amantelides also possess a *tert*-butyl side chain instead of an isohexyl moiety present in the caylobolides. *Tert*-butyl bearing-natural products are rare and present only a small portion of secondary metabolites. Among the cyanobacterial metabolites, the cytotoxins apratoxins, laingolides, madangolide and bisbromoamide and the neurotoxins antillatoxins and palmyrolide A, bear a *tert*-butyl moiety.¹

The absolute configuration of the stereocenters in amantelides A (**1**) and B (**2**) was not determined. The relative configuration at C-7/C-9 of amantelides were assigned as *syn*, based on comparison with caylobolide B (Table 1) and also in agreement with the Kishi's Universal NMR Database for 1,3-diols (Figure 3).¹³ Assignment of the absolute configuration of 1,5-diols has been challenging and lacks the appropriate methods for analysis particularly due to the degenerate 1H and ^{13}C chemical shifts in this system. Because of this, potential methods for configurational analysis of 1,5-diols are likely to be NMR-independent methods. Circular dichroism and X-ray diffraction analysis of either the derivatized or natural macrolactone are possible chemical methods. In addition, since the configuration of the stereocenters in polyketides is genetically determined,^{14, 15} the absolute configuration of the stereocenters can also be inferred from genomic analysis of the polyketide biosynthetic gene clusters, particularly the ketoreductase domains.^{14, 15} It is beyond the scope of this current paper to develop methods for the assignment of the absolute configuration of 1,5-diols, and will be subject of future studies by our group.

The cytotoxic effects of amantelides A (**1**) and B (**2**) were evaluated against HT29 colorectal adenocarcinoma and HeLa cervical carcinoma cells. Amantelide A (**1**) exhibited potent antiproliferative activity in HT29 and HeLa cancer cell lines, with sub-micromolar IC_{50} values (Table 2). Compared to caylobolide B, amantelide A (**1**) showed superior cytotoxic activity. Using the same cell lines and assay conditions, **1** showed close to 5–14 fold improvement in potency compared to caylobolide B. Monoacetylation of amantelide A (**1**) at C-33, however, caused more than a 10-fold decrease in antiproliferative activity, as observed for amantelide B (**2**) (Table 2). This then suggested the importance of the C-33 hydroxy group for cytotoxicity and modulation of the biological activity by chemical modification, in

particular, acetylation. In order to gain insight into the role of acetylation in the antiproliferative activity of the amantelides, a semisynthetic derivative of **1** was prepared using acetic anhydride and pyridine to yield peracetyl-amantelide A (**3**). Antiproliferative activity testing of **3** indicated that peracetylation led to a dramatic decrease in potency, increasing the IC₅₀ values by 20- and 67-fold for HeLa and HT29 cells, respectively (Table 2). In addition to acetylation of the hydroxy groups, the difference in antiproliferative activities of caylobolides and amantelides may suggest that the size of the macrolide ring, hydroxylation pattern and aliphatic side chain may all contribute to the antiproliferative activity of these compounds.

Structural relatedness of amantelide A (**1**) with other polyhydroxylated compounds suggested possible broad spectrum activity against eukaryotic and prokaryotic cells. Further assays were performed to determine the anti-infective properties of amantelide A (**1**) against fungal and bacterial pathogens. The antifungal activity of **1** was tested against the marine fungi *Dendryphiella salina*, *Lindra thalassiae*, and *Fusarium* sp. Initial antifungal assays employed a concentration of 625 µg/mL, based on the estimated natural concentration of amantelide A (**1**) of 300–750 µg/mL wet cyanobacteria. Amantelide A (**1**) completely inhibited the growth of the three marine fungi (Table 3), suggesting a possible ecological role as chemical defense against these pathogens. To further characterize the potency and selectivity of amantelide A (**1**) against the three marine fungal pathogens, bioactivity was assessed using 10- and 100-fold lower concentrations and in comparison with the known antifungal agent amphotericin B. Using a 62.5 µg/mL concentration, **1** completely inhibited the growth of *L. thalassiae* and *Fusarium* sp., while *D. salina* was less potently inhibited (Table 3). Amphotericin B on the other hand, completely inhibited *D. salina* growth, while having minimal effect on the growth of *L. thalassiae* and *Fusarium* sp. (Table 3) using the same concentration of 62.5 µg/mL. A further 10-fold dilution rendered amphotericin B inactive against *L. thalassiae*, while still completely inhibiting *D. salina* growth (Table 3). In contrast, amantelide A (**1**) did not inhibit *D. salina* using a concentration of 6.25 µg/mL, while modest inhibition of *L. thalassiae* and *Fusarium* sp. was observed (Table 3). These indicated that **1** and amphotericin B may have differing selectivity towards selected marine fungal pathogens. Amphotericin B inhibited the proliferation of HT29 and HeLa cancer cells with an IC₅₀ value of 10 µM against both cell lines (Table 2). The antimicrobial activities of amantelide A (**1**) and amphotericin B were evaluated using the Gram (+) *S. aureus* and Gram (-) *P. aeruginosa* bacterial pathogens. Compound **1** showed weak antibacterial activity against both pathogen with minimum inhibitory concentration (MIC) of 32 µM. Consistent with published literature, amphotericin B did not inhibit either bacterial pathogen with MIC >100 µM.¹⁶

Bioactivity assessment of amantelide A (**1**) against mammalian cells, fungal and bacterial pathogens indicated that this compound exhibits a broad spectrum of activity, inhibiting the growth of both eukaryotic and prokaryotic cells with varying potencies. This may suggest the possible effects of amantelide A (**1**) on the cell membrane, similar to other polyhydroxylated compounds.¹⁰ Initial results suggest this possible mechanism of cell killing based on the rapid onset of the antiproliferative effects of amantelide A (**1**). The cytotoxic effects of **1** on HeLa and HT29 cells were observed within 1 h of incubation. This

led to a time independent cytotoxic effect of amantelide A, giving the same IC₅₀ value of 870 nM for all the six time points (1, 3, 6, 12, 24, 48 h) (Fig S1, Supplementary Information). A detailed investigation of the mechanism of action is ongoing in our laboratories.

Experimental Section

General Experimental Procedures

Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. UV absorbance was measured on SpectraMax M5 (Molecular Devices). ¹H and 2D NMR spectra were measured in DMSO-*d*₆ on a Bruker Avance II 600 MHz spectrometer equipped with a 5-mm TXI cryogenic probe using residual solvent signals (δ_{H} 2.50; δ_{C} 39.5) as internal standards. HSQC and HMBC experiments were optimized for $^1J_{\text{CH}} = 145$ and $^nJ_{\text{CH}} = 7$ Hz, respectively. HRESIMS data were obtained using an Agilent LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector. LRESIMS measurements and MS/MS fragmentation were done on an ABI 3200Q TRAP using direct syringe infusion.

Biological Material

The gray cyanobacterium belonging to the Family Oscillatoriaceae was collected on June 21, 2002 from Two Lover's Point (Puntan dos Amantes), Tumon Bay, Guam. The cyanobacterium forms a dense mat of fine gray hairs with a mucous base that can cover the substratum in coral reef habitats of Guam. The morphology of the filaments is fairly uniform with cells much wider than long. Cell width ($n = 4$) $26.4 \pm 1.1 \mu\text{m}$; cell length $3.1 \pm 0.6 \mu\text{m}$. Cells have a thin sheath, no cross-wall constriction, and rounded terminal cells with no calyptra. The morphological appearance is consistent with *Lyngbya majuscula*. However, we now know that many phylogenetically distinct cyanobacteria fall within this morphological classification and molecular methods are required for further taxonomic characterization. These specimens likely fall within the Clade II group defined by Engene et al.,¹⁷ now known as the genus *Okeania*,¹⁸ but molecular vouchers are lacking for this specimen, and further classification within the Oscillatoriaceae is not currently possible. Taxonomic voucher specimens, preserved in 5% formalin, for phenotypic analyses are maintained at Smithsonian Marine Station, Fort Pierce, FL. However, this is unsuitable for molecular analysis of 16S rRNA gene.

Extraction and Isolation

The cyanobacterial collection (22.0 g dry weight) was extracted with CH₂Cl₂-MeOH (1:1) to yield 3.4 g of the nonpolar extract. The lipophilic extract was further partitioned between hexanes:80% aqueous MeOH. The latter was concentrated and further partitioned between *n*-BuOH:H₂O. The *n*-BuOH fraction (0.488 g) was further purified on a silica column, eluting with increasing gradients of *i*-PrOH in CH₂Cl₂ until 100% *i*-PrOH, followed by 100% MeOH. The fraction from 70% *i*-PrOH elution was subjected to reversed-phase HPLC (semipreparative, Phenomenex Synergi-Hydro RP, 4 μm) using a linear gradient of MeOH-H₂O (40%–100% MeOH in 30 min and then 100% MeOH for 10 min) to yield amantelide A (**1**) (t_{R} 29.4 min, 13.3 mg) and amantelide B (**2**) (t_{R} 30.8 min, 5.7 mg).

Amantelide A (1): colorless, amorphous solid; $[\alpha]_{20}^D -5.0$ (*c* 0.06, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 220 (3.99); ^1H and ^{13}C NMR data, Table 1; HRESI/APCIMS m/z 811.5927 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{44}\text{H}_{84}\text{O}_{11}\text{Na}$, 811.5911).

Amantelide B (2): colorless, amorphous solid; $[\alpha]_{20}^D -68$ (*c* 0.02, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 218 (3.76); ^1H and ^{13}C NMR data, Table 1; HRESI/APCIMS m/z 853.6044 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{46}\text{H}_{86}\text{O}_{12}\text{Na}$, 853.6017).

Acetylation of Amantelide A (1)

Acetic anhydride (0.5 mL), pyridine (0.5 mL) and **1** (6.0 mg) was left to stir overnight. The reaction was terminated through solvent removal and further dried under N_2 to yield peracetyl-amantelide A (**3**).

Peracetyl-amantelide A (3): oily liquid; $[\alpha]_{20}^D -58$ (*c* 0.02, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 214 (4.19); ^1H NMR (CDCl_3 , 600 MHz) δ 5.64 (s, 1H), 4.92 (d, $J = 12$ Hz, 1H), 4.88 (m, 2H), 4.81 (m, 6H), 4.75 (m, 1H), 2.70 (m, 1H), 2.45 (m, 1H), 2.03 (m, 27H), 1.88 (s, 3H), 1.85 (m, 1H), 1.74 (m, 1H), 1.60 (m, 8H), 1.51 (m, 26 H), 1.32 (m, 8H), 1.25 (m, 6H), 0.88 (2, 9H); ^{13}C NMR (CDCl_3 , 600 MHz) δ 170.0, 165.5, 160.3, 116.5, 74.6, 73.6, 71.1, 70.3, 38.5, 34.1, 33.7, 33.5, 32.9, 25.8, 25.0, 23.6, 21.1, 20.9; HRESI/APCIMS m/z 1169.6891 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{60}\text{H}_{102}\text{O}_{20}\text{Na}$, 1169.6857).

ESIMS/MS Fragmentation of Amantelide A (1)

A methanolic solution of **1** was directly infused into the mass spectrometer using a syringe driver. MS fragmentation was obtained by positive and negative ionization using the enhanced product ion (EPI) and MS2 scan. The $[\text{M} + \text{H}]^+$ and $[\text{M} - \text{H}]^-$ ions were fragmented by ramping the collision energy through the possible allowed range. Compound-dependent and source gas parameters used were as follows: DP ± 65.0 , EP ± 10.0 , CUR 10.0, CAD High, IS ± 4500 , TEM 0, GS1 10, GS2 0.

Antifungal Assay

The activity of amantelide A (**1**) was tested against three marine fungal strains, *L. thalassiae*, *D. salina*, and *Fusarium* sp. using the antifungal assay described by Engel et al.¹⁹

Amantelide A (**1**) was dissolved in MeOH, serially diluted and $100 \mu\text{L mL}^{-1}$ was added to molten YPM P/S media (2 g yeast, 2 g peptone, 4 g D-mannitol, 8 g agar, 250 mg penicillin G, 250 mg streptomycin sulfate in 1 L seawater) to final concentrations of 625, 62.5 and $6.25 \mu\text{g/mL}$. A known antifungal compound, amphotericin B was prepared in the same manner at final concentrations of 62.5 and $6.25 \mu\text{g/mL}$. Solvent controls were prepared with MeOH only. Six hundred microliters of each treatment was added to each of three wells in a sterile 24-well culture plate. An additional three wells contained only media. A 1 mm^2 section of fungal culture growing on YPM P/S media was transferred to each well. Plates were incubated at 26°C . Fungal growth was evaluated when the media controls were completely covered by fungal hyphae. Window screening was held behind the plate and the number of squares containing fungal hyphae was counted. Percent inhibition of amantelide A (**1**) and amphotericin B was calculated relative to the solvent control $((\text{Control} - \text{Test Sample})/\text{Control}) \times 100\%$.

Cell Viability Assay

HT29 colorectal adenocarcinoma and HeLa cervical carcinoma cells were cultured in Dulbecco's modified Eagle medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Hyclone) under a humidified environment with 5% CO₂ at 37 °C. HeLa (3,000) and HT29 (12,500) cells were seeded in 96-well plates and treated with varying concentrations of **1–3**, amphotericin and solvent control (DMSO) 24 h after seeding. The cells were incubated for an additional 48 h before the addition of the MTT reagent. In addition, time course analysis of the cell viability was carried using 1, 3, 6, 12 h incubation of amantelide A (**1**) with HeLa and HT29 cells. Cell viability was measured according to the manufacturer's instructions (Promega). IC₅₀ calculations were done by GraphPad Prism 5.03 based on duplicate experiments.

Antimicrobial Assay

The antimicrobial activity was assessed using the broth microdilution method as adapted from Wiegand et al.,²⁰ with modifications. Glycerol stocks of *S. aureus* ATCC 6538, and *P. aeruginosa* ATCC 27853 were thawed before streaking in a Mueller Hinton Agar (MHA) plate. The plate was sealed and incubated at 37 °C and shaken at 150 rpm for 24 h. A single colony of each pathogen from the MHA plate was obtained and transferred into 50 mL Mueller Hinton broth (MHB). The broth was incubated for 6–8 h using the same condition indicated above. The turbidity of the broth was then adjusted to match the 0.5 MacFarland Standard, which approximates a cell density of 1×10^8 cells/mL. The adjusted broth culture was diluted 100-fold and used as the final inoculum for the assay. A 100 µL-aliquot of the test organism was dispensed in each well of the 96-well microtiter plate. The microbial pathogens were treated with the test sample and incubated at 37 °C and 150 rpm for 24 h. At the end of incubation, 20 µL of 0.02% v/v resazurin was added into each well and the fluorescence signal was measured using 530 nm excitation and 590 nm emission filters using the Biotek Synergy HT microplate reader. Oxacillin was used as positive control for *S. aureus* while nalidixic acid was used as positive control for *P. aeruginosa*. Sterility control contained MHB media only. The percent inhibition of amantelide A (**1**), amphotericin B, oxacillin and nalidixic acid was calculated relative to the solvent control. MIC endpoint is defined as the lowest concentration of antibiotic or sample at which there is no detectable microbial growth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Partial structure of amantelide A (**1**).

Figure 2.
Fragmentation pattern of amantelide A (**1**) induced by ESI-MS (negative mode).

Figure 3.

Predicted ^{13}C NMR shifts of 1,3-diols based on Kishi's Universal NMR database and corresponding chemical shifts of the 1,3-diol moiety in amantelide A (**1**) and caylobolide B. ^{13}C NMR shifts for C-7 and C-9 in **1** are in accordance with *syn* configuration and comparable to those of caylobolide B.

Table 1NMR Data of Amantelide A (**1**) and Amantelide B (**2**) in DMSO-*d*₆

Position	Amantelide A (1)		Amantelide B (2)	
	δ_C^a	δ_H (J in Hz) ^b	δ_C^a	δ_H (J in Hz) ^b
1	165.5, C		165.8, C	
2	116.2, CH	5.62, s	116.1, CH	5.67, s
3	160.3, C		160.0, C	
4	32.9, CH ₂	2.54, m	32.4, CH ₂	2.56, m
5a	23.6, CH ₂	1.54, m	23.4, CH ₂	1.52, m
5b		1.40, m		1.40, m
6	37.0, CH ₂	1.27, m	37.0, CH ₂	1.23, m
7	68.7, CH	3.58, m	68.6, CH	3.58, m
7-OH		4.54, br d (3.20)		4.50, br d (3.2)
8	44.1, CH ₂	1.37, m	44.2, CH ₂	1.37, m
9	68.5, CH	3.55, m	68.8, CH	3.55, m
9-OH		4.52, br d (3.0)		4.55, br d (3.0)
10	37.0, CH ₂	1.22, m	37.0, CH ₂	1.23, m
11	21.3, CH ₂	1.21, m	21.2, CH ₂	1.22, m
12	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
13	69.4, CH	3.34, m	69.4, CH	3.34, m
13-OH		4.23, m		4.23, m
14	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
15	20.8, CH ₂	1.31, m	20.8, CH ₂	1.32, m
16	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
17	69.4, CH	3.34, m	69.4, CH	3.34, m
17-OH		4.23, m		4.23, m
18	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
19	20.8, CH ₂	1.31, m	20.8, CH ₂	1.32, m
20	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
21	69.4, CH	3.34, m	69.4, CH	3.34, m
21-OH		4.23, m		4.23, m
22	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
23	20.8, CH ₂	1.31, m	20.8, CH ₂	1.32, m
24	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
25	69.4, CH	3.34, m	69.4, CH	3.34, m
25-OH		4.23, m		4.23, m
26	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
27	20.8, CH ₂	1.31, m	20.8, CH ₂	1.32, m
28	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
29	69.4, CH	3.34, m	69.4, CH	3.34, m

Position	Amantelide A (1)		Amantelide B (2)	
	δ_C^a	δ_H (J in Hz) ^b	δ_C^a	δ_H (J in Hz) ^b
29-OH		4.23, m		4.23, m
30	37.0, CH ₂	1.31, m	36.9, CH ₂	1.30, m
31	20.8, CH ₂	1.31, m	<i>c</i>	<i>c</i>
32	37.0, CH ₂	1.31, m	<i>c</i>	<i>c</i>
33	69.4, CH	3.34, m	73.3, CH	4.73, m
33-OH		4.23, m		
34	37.0, CH ₂	1.31, m	<i>c</i>	<i>c</i>
35	20.8, CH ₂	1.31, m	<i>c</i>	<i>c</i>
36	37.0, CH ₂	1.31, m	<i>c</i>	<i>c</i>
37	66.4, CH	3.21, m	66.4, CH	3.21, m
37-OH		4.26, br d (5.8)		4.27, br d (5.2)
38	37.1, CH ₂	1.43, m	37.1, CH ₂	1.41, m
39	76.6, CH	4.93, br d (10.0)	76.2, CH	4.93, br d (10.4)
40	34.1, C		34.2, C	
41–43	26.3, CH ₃	0.82, s	25.7, CH ₃	0.83, s
44	24.6, CH ₃	1.85, s	24.3, CH ₃	1.86, s
45			170.0, C	
46			20.7, CH ₃	1.97, s

^a 125 MHz.

^b 600 MHz.

^c Not assigned due to significant overlap of signals.

Table 2

Cytotoxic (IC₅₀, μ M) and Antimicrobial Activities (MIC, μ M) of the Isolated Cyanobacterial Polyketides (**1–3**)

Compound	HT29 ^a	HeLa ^a	<i>S. aureus</i> ^b	<i>P. aeruginosa</i> ^b
Amantelide A (1)	0.87 $\pm$ 0.02	0.87 $\pm$ 0.07	32 $\pm$ 0	32 $\pm$ 0
Amantelide B (2)	12 $\pm$ 1.6	9.9 $\pm$ 0.05	NT ^c	NT ^c
Peracetyl-amantelide A (3)	58 $\pm$ 6.7	18 $\pm$ 1.6	NT ^c	NT ^c
Amphotericin B	10 $\pm$ 2.7	10 $\pm$ 4.4	>100	>100

^aData are presented as mean $\pm$ SD (n = 2).

^bData are presented as mean $\pm$ SD (n = 3).

^cNot tested.

Table 3

Mean Percent Growth Inhibition ($\pm$ SE, $n = 3$) of Marine Fungal Strains *D. salina*, *L. thalassiae*, and *Fusarium* sp. by Amantelide A (**1**) and the Known Antifungal Compound, Amphotericin B

Fungi strains	% Inhibition					
	Amantelide A (1)			Amphotericin B		
	62.5 $\mu\text{g/mL}$	62.5 $\mu\text{g/mL}$	6.25 $\mu\text{g/mL}$	62.5 $\mu\text{g/mL}$	6.25 $\mu\text{g/mL}$	6.25 $\mu\text{g/mL}$
<i>D. salina</i>	100 $\pm$ 0	60 $\pm$ 5	0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0
<i>L. thalassiae</i>	100 $\pm$ 0	100 $\pm$ 0	40 $\pm$ 3	21 $\pm$ 5	0	0
<i>Fusarium</i> sp.	100 $\pm$ 0	100 $\pm$ 0	35 $\pm$ 8	6 $\pm$ 6	12 $\pm$ 4	12 $\pm$ 4