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Malyngolide Dimer, a Bioactive Symmetric Cyclodepside from the Panamanian Marine Cyanobacterium *Lyngbya majuscula*

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Abstract

Fractionation of the crude extract of the marine cyanobacterium *Lyngbya majuscule* collected from Panama led to the isolation of malyngolide dimer (**1**). The planar structure of **1** was determined using 1D and 2D NMR spectroscopy, and HRESI-TOF MS. The absolute configuration was established by chemical degradation followed by chiral GC-MS analyses and comparisons with an authentic sample of malyngolide seco acid (**4**). Compound **1** showed moderate *in vitro* antimalarial activity against chloroquine resistant *Plasmodium falciparum* (W2) (IC_{50} = 19 μM) but roughly equivalent toxicity against H-460 human lung cell lines. Furthermore, because the closely related cyanobacterial natural product 'tanikolide dimer' was a potent SIRT2 inhibitor, compound **1** was evaluated in this assay, but found to be essentially inactive.

Malyngolide (**2**) and tanikolide (**3**) are natural δ-lactones obtained from different strains of the marine cyanobacterium *Lyngbya* and characterized by a hydroxymethyl group and a long aliphatic chain attached to the δ-position of a six-membered lactone ring. The main differences between compounds **2** and **3** are the presence of a secondary methyl group at C-2 of malyngolide, the number of carbons in the aliphatic chain, and perhaps most interestingly, the absolute configuration of the δ-carbon (**2** = 5*S*; **3** = 5*R*). Compounds **2** and **3** were isolated from *L. majuscula* collected in Hawaii and Madagascar, respectively.[1,2] Malyngolide (**2**) displayed antibacterial activity against *Mycobacterium smegmatis* and *Streptococcus pyogenes*, while tanikolide (**3**) showed cytotoxicity against brine shrimp as well as antifungal and molluscicidal activity. Since malyngolide (**2**) was synthesized for the first time in 1980,[3] more than 66 synthetic studies have been published for this compound, and 23 for the more recently isolated tanikolide (**3**), providing evidence of the remarkable interest by the synthetic community in these compounds.[4]

Here we report the isolation, structural determination and bioactivity of malyngolide dimer (**1**) from *L. majuscula* (Oscillatoriaceae, Gomont ex Gomont 1892), collected at Coiba National Park off the Pacific coast of Panama as a part of our International Cooperative

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Supporting Information Available. ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra in $CDCl_3$ for malyngolide dimer (**1**) and malyngolide seco acid (**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

Biodiversity Group (ICBG) drug discovery program.[5] Interestingly, we recently discovered and reported the structure of the naturally occurring dimer of tanikolide, tanikolide dimer (**5**), from a Malagasy collection of this same cyanobacterium,[6] and thus the work herein reported indicates an emergent trend in the lipophilic natural products chemistry of these organisms. Furthermore, as tanikolide dimer showed remarkable activity as a SIRT2 inhibitor,[7] an HDAC-associated protein with potential as a target for anticancer therapy, isolation of malyngolide dimer (**1**) provided an opportunity to expand on structure-activity information in this compound class.

HRESI–TOF MS spectra of compound **1** showed a pseudomolecular ion $[M + H]^+$ at *m/z* 541.4453 and its sodium adduct $[M+Na]^+$ at *m/z* 563.4286 which corresponded with the molecular formulas $C_{32}H_{61}O_6$ and $C_{32}H_{60}O_6Na$, respectively. However, ^{13}C-NMR resonances were observed for only 16 carbon atoms, indicating that **1** must be a symmetric dimer. The three degrees of unsaturation implied by the molecular formula were accounted for by two carbonyl groups, and one ring. DEPT experiments in combination with HSQC data revealed that each monomer of compound **1** possessed two methyl groups at δ_C 14.03 (C-14) and 16.9 (C-16), 11 methylenes at δ_C 67.5 (C-15), 36.6, 31.76, 29.9, 29.42, 29.36, 29.2, 23.5, 22.6 (C-6 to C-13), 25.1, 26.1 (C-3 and C-4), one methine at δ_C 35.4 (C-2) and two quaternary carbons at δ_C 175.4 (C-1) and 86.9 (C-5).

A key structural feature revealed by the ^{1}H NMR analysis was a pair of doublets at δ_H 3.64 and 3.44 that were assigned as the methylene protons H-15a and H-15b, respectively. A multiplet located at δ_H 2.41 (m, H-2) a methine proton, was coupled to the secondary methyl group at C-16 and a methylene at C-3. A group of six diastereotopic methylene protons observed between 1.5 and 2.00 ppm and were assigned as δ_H 1.91 (m, H-3a), δ_H 1.59 (m, H-3b), 1.99 (ddd, *J* = 4.0, 14.0, 17.5 Hz, H-4a), 1.74 (dt, *J* = 4.0, 14.0 Hz, H-4b), 1.68 (m, ^{1}H, H-6a), 1.53 (m, H-6b), by ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC (Figure 1). A group of overlapped prominent signals between 1.23 and 1.30 ppm were assigned to the aliphatic methylene chain (H-7 to H-13) and the secondary methyl group H-16 using gHSQC and COSY experiments. Finally a triplet located at δ_H 0.85 (t, *J* = 6.5 Hz, H-14) corresponded with the terminal methyl group of the aliphatic chain. The position of the methyl group H_3-16 as well as the connectivity of the oxygen-bonded methylene at C-15 and the spin systems formed by protons H-2 to H-4 and H-6 to H-14, to carbon C-5 was determined based on ^2J and ^3J HMBC correlations (Figure 1). Thus, the overall analysis of the 1D-NMR (^{1}H, ^{13}C, DEPT) and 2D-NMR (gCOSY, gHSQC, gHMBC) data indicated that **1** was structurally related to malyngolide (**2**).

The absolute configurations at carbons C-2 and C-5 were determined by chiral GC-MS analysis. Compound **1** was hydrolyzed under mild basic conditions using barium hydroxide in aqueous methanol to obtain malyngolide seco-acid (**4**).[1] Compound **4** was analyzed directly by chiral GC-MS and gave a single peak at 28.64 min indicating that both monomers of dimer **1** had the same absolute configuration at C-2 and C-5. Compound **4** was then analyzed by co-injection using chiral GC-MS and an authentic sample of natural malyngolide seco-acid obtained from a Papua New Guinea collection of *Lyngbya* sp.; the chiral standard was shown to have the configuration of 2*R* and 5*S* by NMR and optical rotation ($[\alpha]_D$ -10.7, *c* 0.44, CH_2Cl_2; lit. $[\alpha]_D$ - 14.6, *c* 1, CH_2Cl_2).[1] GC-MS analysis gave only a single peak at 28.67 min indicating that both the authentic and malyngolide dimer-derived synthetic malyngolide seco-acid (**4**) possessed the same 2*R*,5*S* absolute configuration. Therefore, we deduce that **1** possesses a 2*R*,2'R,5*S*,5'*S* stereoconfiguration.

Malyngolide dimer (**1**) was evaluated for *in vitro* activity against the chloroquine resistant *Plasmodium falciparum* strain W2 and showed an IC_{50} of 19 μM.[8] However, compound **1** showed toxicity when evaluated against the H-460 human lung cell line at approximately the

same level (9 μM, 110% survival; 55 μM, 10% survival). Because tanikolide dimer (**5**) had shown considerable potency as a SIRT2 inhibitor (176 nM to 2.4 μM in two different assay formats),[6] malyngolide dimer (**1**) was similarly evaluated, but gave only 30% inhibition at 50 μM. Thus, it appears that one or a combination of the three structural differences between these two dimeric molecules [a secondary methyl group at C-2 and C-2' in **1**, a 5*S*,5'*S* configuration in **1** versus a 5*R*,5'*R* configuration in **5**, and an alkyl chain length of 14 carbons in **1** versus 16 carbons in **5**] prevents the effective binding of **1** to an inhibitory site on the SIRT2 protein. Biosynthetically, isolation of malyngolide dimer is intriguing, especially in light of our recent isolation and characterization of the related compound, tanikolide dimer (**5**),[6] suggesting that cyanobacteria possess a generalized capacity to dimerize these polyketide-derived natural products. Indeed, precedence exists for such polyketide dimerization in cyanobacterial natural products as shown by the isolation of swinholide and ankaraholide A from two field collections, both of which are macrocyclic dimeric products of polyketide synthases (PKS).[9] Whether the two halves of such dimers are produced by a PKS, released and then dimerized in a separate enzymatic reaction, or dimerization occurs coincidentally with release from the PKS, is an intriguing and interesting area for future investigation. Finally, it is also interesting to note the conservation of configurational fidelity in the tanikolide monomer and dimer series (5*R* in both cases) versus the malyngolide monomer and dimer series (5*S* in both cases).

Experimental Section

General Experimental Procedures

Optical rotations were measured with a Jasco P-2000 polarimeter. UV spectra were measured on a Beckman Coulter DU-800 spectrophotometer and IR spectra were recorded on a Nicolet IR 100 FT-IR spectrophotometer. NMR spectra were acquired on Varian Inova 500 MHz and 300 MHz spectrometers and referenced to residual solvent ^{1}H and ^{13}C signals (δ_H 7.26, δ_C 77.0 for $CDCl_3$). Low resolution ESIMS spectra were acquired on a Finnigan LCQ Advantage Max mass spectrometer while high accuracy mass measurements were obtained on an Agilent ESI-TOF mass spectrometer. Purification of the compound was carried out on a Phenomenex reversed-phase C-18 solid phase extraction cartridge.

Biological Material Collection and Identification

Reddish filaments of the marine cyanobacterium *Lyngbya majuscula* were collected from a sandy bottom in 2 m of water by snorkeling near Coiba Island at Coiba National Park, Panama (N 07° 23' 38.5", W 81° 40' 16.5"). The samples were stored in 1:1 EtOH/H_2O and frozen at −20 °C. Voucher specimens are available from WHG as collection number PAC-03/12/06D37. The cyanobacterium was microscopically identified as *L. majuscula* Harvey *ex* Gomont based on the current taxonomic systems of Hoffmann 1994[10] and Komárek *et al*. 2005.[11] The filaments of PAC-03-12-06-D37 were cylindrical, approximately 25 μm in width with cells organized in trichomes enclosed by distinct sheaths. The cells were disk-shaped, approximately 20 μm in width and 3 μm in length, and displayed no constrictions at the cell cross-walls. The terminal cells were rounded without the presence of calyptras.

Extraction and Isolation Procedures

A 4 L collection of *L. majuscula* was extracted with 2:1 CH_2Cl_2/MeOH and concentrated to dryness in vacuo to give 5.5 g of crude extract. VLC fractionation of the extract using a gradient with 0–100% EtOAc in hexanes followed by 0–100% of MeOH in EtOAc, yielded nine fractions (A– I). Fraction E, eluted with 60% MeOH in EtOAc (200 mg) was further purified using a reversed-phase C-18 solid phase extraction cartridge eluted with 50–100%

MeOH in H_2O to yield 7 fractions. Fraction E3 eluted with 70% MeOH and gave 66.0 mg of malyngolide dimer (**1**) as a glassy yellow solid.

Malyngolide dimer (1)

Yellow glassy solid; $[\alpha]^{25}_D$ – 9.2 (*c* = 3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (3.73); IR (film) ν_{max} 3415 (br), 2930, 2855, 1714, 1461, 1377, 1332, 1251, 1210, 1068 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.64 (2H, d, *J* = 12.0 Hz, H-15a and H-15a'), 3.44 (2H, d, *J* = 12.0 Hz, H-15b and H-15b'), 2.41 (2H, m, H-2 and H-2'), 1.99 (2H, ddd, *J* = 4.0, 14.0, 17.5, H-4a and H-4a'), 1.91 (2H, m, H-3a and H-3a'), 1.74 (2H, dt, *J* = 4.0, 14.0, H-4b and H-4b'), 1.68 (2H, m, H-6a and H-6a'), 1.59 (2H, m, H-3b and H-3b'), 1.53 (2H, m, H-6b and H-6b'), 1.24 (34H, m, H_2-7 to H_2-13, H_2-7' to H_2-13', H_3-16 and H_3-16'), 0.85 (6H, t, *J* = 6.5 Hz, H_3-14 and H_3-14'); ^{13}C NMR ($CDCl_3$, 125 MHz, * indicates these signals may be interchanged) δ 175.4 (C, C-1 and C-1'), 86.9 (C, C-5 and C-5'), 67.5 (CH_2, C-15 and C-15'), 36.6 (CH_2, C-6 and C-6'), 35.4 (CH, C-2 and C-2'), 31.7 (CH_2, C-12 and C-12'), 29.9* (CH_2, C-8 and C-8'), 29.42* (CH_2, C-9 and C-9'), 29.36* (CH_2, C-10 and C-10'), 29.2* (CH_2, C-11 and C-11'), 26.14 (CH_2, C-4 and C-4'), 25.13 (CH_2, C-3 and C-3'), 23.5 (CH_2, C-7 and C-7'), 22.6 (CH_2, C-13 and C-13'), 16.99 (CH_3, C-16 and C-16'), 14.0 (CH_3, C-14 and C-14'); ESIMS *m/z* (%) 239 (77), 211 (56), 155 (41), 143 (75), 115 (31); HRESI-TOFMS *m/z* $[M + H]^+$ 541.4453 (calcd for $C_{32}H_{60}O_6$, 541.4468), $[M + Na]^+$ 563.4286 (calcd for $C_{32}H_{60}O_6Na$, 563.4288).

Basic hydrolysis of compound 1

A solution of 5.5 mg of **1** and 30 mg of $Ba(OH)_2$ in 1 mL of 20% aq MeOH was reacted for 72 h at 4 °C. The MeOH was evaporated and the aqueous suspension acidified to pH 4 with dilute HCl. Extraction with $CHCl_3$ gave 5.5 mg of malyngolide seco acid (**4**), isolated as a white glassy solid; $[\alpha]^{25}_D$ – 7.9 (*c* = 0.3, CH_2Cl_2); ^1H NMR ($CDCl_3$, 500 MHz) δ 3.48 (2H, m, H_2-15), 2.48 (1H, m, H-2), 1.48 (6H, m, H_2-3, H_2-4, H_2-6), 1.27 (14H, m, H_2-7 to H_2-13), 1.21 (3H, d, *J* = 7.0 Hz, H_3-16), 0.88 (3H, t, *J* = 6.9 Hz, H_3-14); HRESI-TOFMS *m/z* $[M + Na]^+$ 311.2192 (calcd for $C_{16}H_{32}O_4Na$, 311.2193).

Biological Activity

Antiplasmodial activity was determined in a chloroquine-resistant *P. falciparum* strain (W2) utilizing a microfluorimetric assay to measure the inhibition of the parasite growth based on the detection of the parasitic DNA by intercalation with PicoGreen.[7] *P. falciparum* was cultured according to the methods described by Trager and Jensen.[12] The parasites were maintained at 2% haematocrit in flat-bottom flasks (75 mL) with RPMI 1640 medium (GibcoBRL) supplemented with 10% human serum.

Cytotoxicity was measured in NCI H-460 human lung tumor cells with cell viability being determined by MTT reduction.[13] Cells were seeded in 96-well plates at 6000 cells/well in 180 μL of medium. After 24 h, the test chemicals were dissolved in DMSO and diluted into medium without fetal bovine serum and then added at 20 μg/well. DMSO was less than 0.5% of the final concentration. After 48 h, the medium was removed and cell viability determined.

The measurement of SIRT2 inhibitory activity followed the procedures outlined in Heltweg et al.[14] except that the SIRT2 enzyme was overexpressed and its activity assayed as described in Uciechowska et al.[15]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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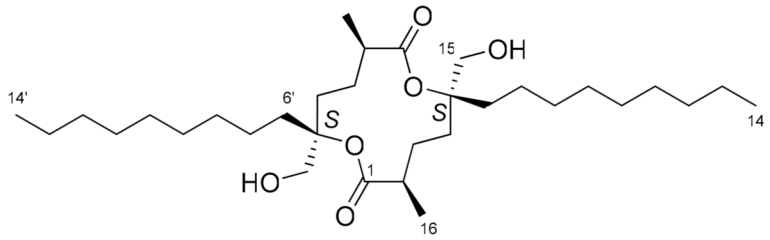

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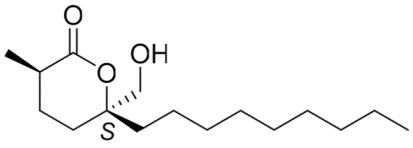

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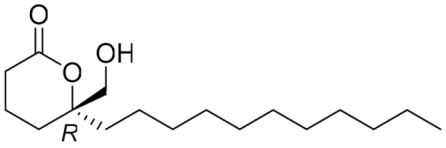

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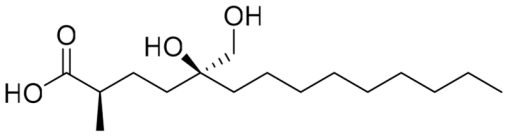

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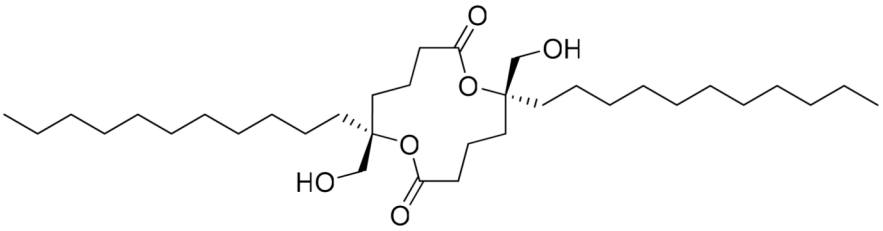

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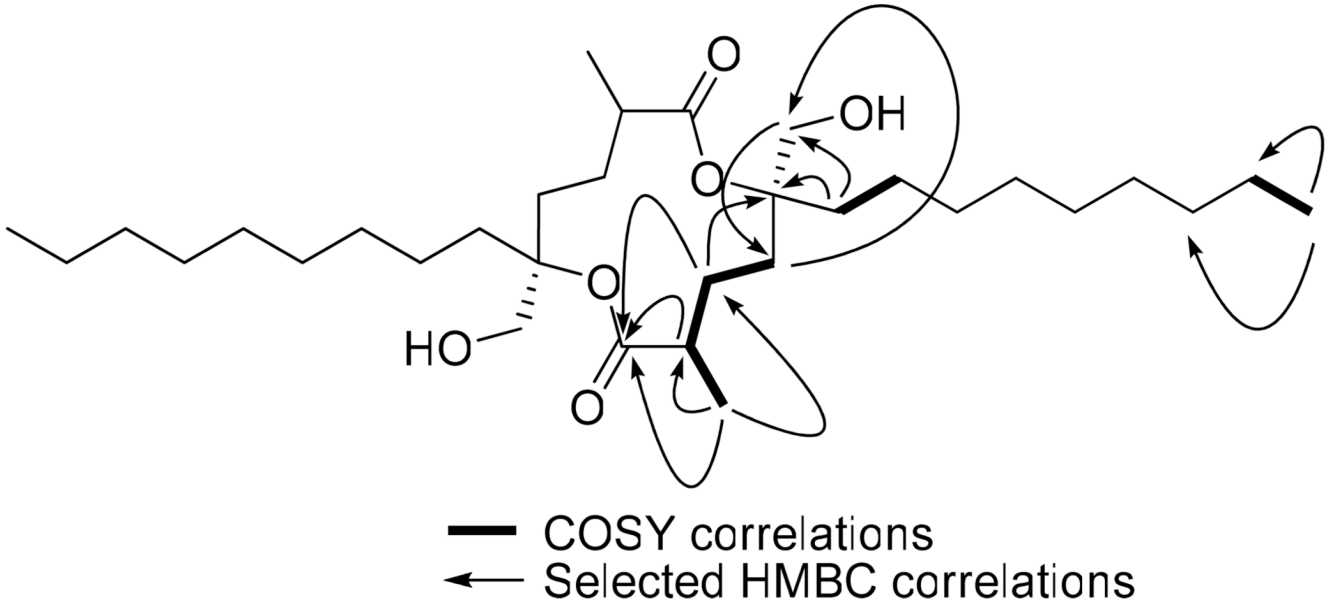

Figure 1.
Selected 2D NMR correlations for malyngolide dimer (**1**).