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Novel Polyketide Metabolites from a Species of Marine Fungi

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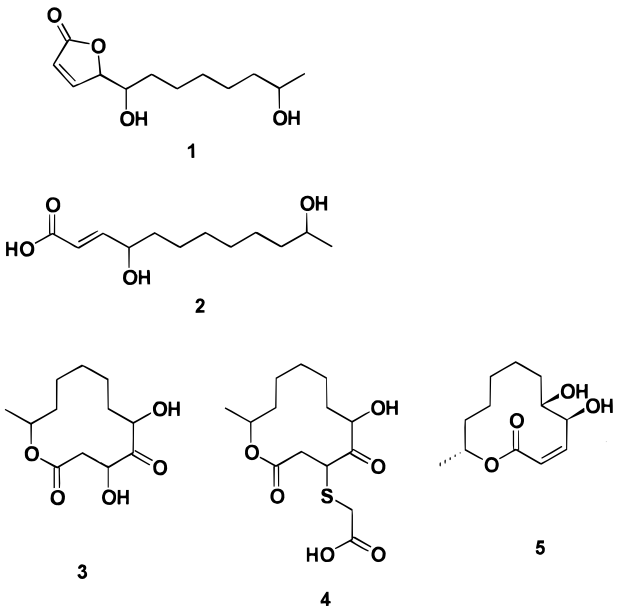
Fermentation of a marine fungal species obtained from a tissue sample of a marine sponge collected in Indonesia in October 1996, yielded the novel hexaketide compounds *iso*-cladospolide B (**1**); *seco*-patulolide C (**2**); the 12-membered macrolides, pandangolide 1 (**3**) and pandangolide 2 (**4**); and the known terrestrial fungal metabolite, cladospolide B (**5**).

Historically, terrestrial fungi have been a rich source of pharmaceutically important compounds. More recently, these studies have expanded to include marine species.[1] Fermentation of a marine fungal species obtained from a tissue sample of a marine sponge collected in Indonesia in October 1996, yielded the novel hexaketide compounds *iso*-cladospolide B (**1**); *seco*-patulolide C (**2**); the 12-membered macrolides, pandangolide 1 (**3**) and pandangolide 2 (**4**); and the known terrestrial fungal metabolite, cladospolide B (**5**).[2] Other known 12-membered macrolides produced from terrestrial fungi include cladospolides A[2,3] and C;[2a] patulolides A,[4] B,[4a] and C,[4a] and recifeiolide.[5]

Fungal isolate I96S215 was fermented in a 1-L flask. The fermentation broth was exhaustively extracted with EtOAc to give a crude extract. This extract was partitioned between hexane, $CHCl_3$, and aqueous MeOH. The $CHCl_3$-soluble material was further fractionated by gradient C_{18}-silica flash chromatography (50% aqueous CH_3CN−100% CH_3CN), resulting in the separation of a number of colorless bands. These fractions were inspected by ^1H NMR and TLC and purified further by gradient C_{18}-silica HPLC (90% aqueous CH_3CN−100% CH_3CN) to give *iso*-cladospolide B (**1**), *seco*-patulolide C (**2**), pandangolide 1 (**3**), pandangolide 2 (**4**), and cladospolide B (**5**).[2]

iso-Cladospolide B (**1**) was obtained as an amorphous solid that showed a protonated molecular ion in the positive ion FABMS spectrum at *m*/*z* 229 $(MH)^+$. This ion was measured to be 229.14269 Da by HRFABMS, which corresponded to the molecular formula $C_{12}H_{21}O_4$. The mass spectrum appeared to be identical to that of cladospolide B (**5**), indicating that the structures were isomeric. The ^1H NMR spectrum (Table 1), however, was significantly different from that of cladospolide B (**5**). The most significant differences were that the two olefinic resonances were shifted farther downfield (δ 7.63, 6.16) relative to those in cladospolide B (**5**) (δ 6.36, 5.68), and the coupling constant between these signals was 5.8 Hz for *iso*-cladospolide B (**1**) versus 12.2 Hz for cladospolide B (**5**). The ^{13}C NMR spectrum (Table 2) for *iso*-cladospolide B (**1**) contained 12 resolved signals in accordance with the molecular formula. A resonance at δ 175.75 combined with the observance of an absorption at 1747 cm^{-1} in the IR spectrum implied the presence of an ester functionality in the molecule. The



frequency of the carbonyl absorption combined with the vicinal coupling constant observed between the two olefinic protons implied that the ester functionality was part of a five-membered α,β-unsaturated lactone moiety. The ^{13}C NMR spectrum also contained three signals arising from sp^3 carbons bound directly to oxygen at δ 88.22, 71.65, and 68.54, accounting for the four oxygens present in the molecular formula. An HMQC experiment established the one-bond proton to carbon connectivities. A gradient multiple quantum COSY experiment established that the proton at δ 5.07 (carbon δ 88.22) was in an allylic position relative to the double bond. A gradient HMBC experiment confirmed this relationship and that the ester functionality was at the other end of the double bond. Because the chemical shift of the allylic proton and its carbon were the most downfield of the oxygenated carbons, it was assigned as the carbon attached directly to the ester oxygen confirming the five-membered α,β-unsaturated lactone moiety. The remaining two oxygenated carbons were assigned as hydroxyl-bearing carbons by virtue of their chemical shifts. A broad absorption centered at 3400 cm^{-1} in the IR spectrum also suggested the presence of hydroxyl groups in the molecule. The COSY and HMBC data allowed the assignment of the signal at δ 68.54 to a secondary hydroxyl-

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Table 1. ^{1}H NMR Data (CD_3OD) (δ, Mult, J) for Compounds **1**−**4**

atom	1	2	3	4
2a	6.16 (dd, 5.8, 1.9)	5.95 (dd, 15.6, 1.5)	3.30 (dd, 19.2, 3.5)	3.43 (dd, 19.4, 12.6)
2b			3.15 (dd, 19.2, 5.1)	3.00 (dd, 19.4, 3.7)
3	7.63 (dd, 5.8, 1.4)	6.87 (dd, 15.6, 5.0)	4.40 (dd, 5.1, 3.5)	3.72 (dd, 12.6, 3.7)
4	5.07 m	4.20 m		
5	3.77 m	1.54 m	4.09 (dd, 7.9, 2.9)	4.01 (dd, 8.1, 2.9)
6a	1.56 m	1.42 m	1.89 m	1.83 m
6b			1.78 m	
7a	1.36[a]m	1.33[a]m	1.48 m	1.57 m
7b			1.27 m	1.18 m
8	1.35[a]m	1.33[a]m	1.23 m	1.22 m
9a	1.56[a]m	1.34 m	1.43 m	1.41 m
9b			1.14 m	1.21 m
10a	1.42 m	1.40 m	1.65 m	1.60 m
10b			1.37 m	1.43 m
11	3.70 m	3.69 m	4.83 m	4.80 m
12	1.13 (d, 6.2)	1.13 (d, 6.3)	1.19 (d, 6.2)	1.18 (d, 6.1)
13				3.55 (d, 15.8)
14				3.35 (d, 15.8)

[a] Assignments within the same column may be interchanged.

Table 2. ^{13}C NMR Data (CD_3OD) for Compounds **1**−**4**

atom	1	2	3	4
1	175.75 (2, 3)[b]	170.46 (2, 3)	175.27 (2a, 2b, 3)	171.93 (3)
2	122.71 (3, 4)	121.49 (3, 4)	43.87 (3)	41.66 (3)
3	157.13 (2, 4, 5)	152.20 (4)	66.85 (2a, 2b)	43.31 (2a, 13a, 13b)
4	88.22 (2, 3, 6)	71.65 (3, 5)	213.09 (2a, 2b, 3, 5, 6a, 6b)	214.86 (2a, 3, 5, 6)
5	71.65 (4, 6)	37.64 (3, 4)	77.40 (6b, 7b)	77.81 (6)
6	34.22 (5)	26.39 (4, 5)	31.58 (5, 7b, 8)	32.41 (5)
7	30.61[a]	30.72[a]	21.59 (5, 6a, 6b, 8, 9a, 9b)	21.71 (6)
8	263.52[a]	30.61[a]	28.22 (6b, 7a, 7b, 9a, 9b, 10a, 10b)	28.97 (6)
9	26.78[a]	26.80 (11)	23.52 (7b, 8, 10a, 10b)	23.67
10	40.12 (11, 12)	40.18 (12)	34.63 (8, 9a, 12)	34.10 (12)
11	68.54 (10, 12)	68.58 (12)	74.60 (8, 9a, 10b, 12)	74.43 (12)
12	23.50	23.48	20.58 (10b)	20.00
13				34.65 (3)
14				173.51 (13a, 13b)

[a] Assignments may be interchanged. [b] Numbers in parentheses are protons to which the carbon correlated in HMBC NMR experiments.

bearing sp^3 carbon adjacent to a terminal methyl group. The rest of the structure was assigned using data from the 2D NMR experiments described above to give the structure shown for *iso*-cladospolide B (**1**).

seco-Patulolide C (**2**) was obtained as an oil that showed a protonated molecular ion in the positive ion FABMS at m/z 231 $(MH)^+$. This ion was measured to be 231.15919 by HRFABMS, which corresponded to the molecular formula $C_{12}H_{23}O_4$. The ^{13}C NMR spectrum (Table 2) again contained 12 signals. The ^{1}H NMR spectrum contained a pair of coupled olefinic resonances at δ 6.87 and 5.95 (J = 15.6 Hz), indicating that the double bond had trans geometry. There were only two resonances indicative of methine-bearing carbons bound directly to oxygen, at δ 4.20 and 3.69 in the ^{1}H NMR and at δ 71.65 and 68.58 in the ^{13}C NMR spectra. The ^{13}C NMR spectrum also showed a resonance at δ 170.46, consistent with a carboxylic acid. The IR spectrum contained an absorption at 1698 cm^{-1}, which provided further evidence for a carboxylic acid that was α,β-unsaturated. Analysis of the data from a gradient HMBC experiment showed that the carboxylic acid functionality was indeed directly attached to the olefinic carbon at δ 121.49. A doublet at δ 1.13 in the ^{1}H NMR spectrum could be attributed to a methyl group adjacent to a hydroxyl-bearing methine carbon. Inspection of gradient multiple quantum COSY and HMBC data facilitated the assignment of this carbinol group and also an allylic carbinol group to δ 3.69 and 4.20 in the ^{1}H NMR and δ 68.58 and 71.65 in the ^{13}C NMR spectra, respectively. The rest of the signals could be attributed to methylene groups in a saturated chain. The structure was, therefore, assigned as **2**, the open chain form of patulolide C.[4a]

Pandangolide 1 (**3**) was obtained as an oil that showed a protonated molecular ion in the positive ion FABMS spectrum at m/z 245 $(MH)^+$. This ion was measured to be 245.14063 by HRFABMS, which corresponded to the molecular formula $C_{12}H_{21}O_5$. Analogous to the previous two molecules, the ^{13}C NMR spectrum (Table 2) contained 12 signals. Again, a signal at δ 175.27 indicated the presence of an ester functionality plus a new signal at δ 213.09 arising from a keto-carbonyl functionality. The IR spectrum contained absorbances at 1732 and 1716 cm^{-1} that were consistent with the presence of ester and keto-carbonyl groups, respectively. A broad absorbance centered at 3444 cm^{-1} indicated the presence of hydroxyl group(s). The ^{1}H NMR spectrum (Table 1) contained three methine signals at δ 4.83, 4.40, and 4.09, indicating three oxygenated carbons. Also present were two signals at δ 3.30 and 3.15 that coupled to one another with a magnitude of 19.2 Hz and, on analysis of an HMQC experiment, were shown to be attached to the same carbon at δ 43.87. Inspection of a gradient multiple quantum COSY experiment showed the presence of two spin systems. One contained the two geminally coupled signals and the signal at δ 4.40; the second encompassed all the remaining protons. The data also allowed the assignment of a terminal methyl functionality (δ 1.19) adjacent to an oxygen-bearing methine (δ 4.83). This methine could also be assigned as part of a lactone functionality by virtue of its proton and carbon chemical shifts relative to those of cladospolide B (**5**) and

the two open-chain compounds, **1** and **2**. Analysis of a gradient HMBC experiment verified the assignment of the spin systems. The observance of a number of key HMBC correlations from H2, H3, H5, and H6 with the keto group (C4) allowed the assignment of its position between the two spin systems. Two 12-membered macrolides with keto groups at C4, patulolide A and patulolide B, have previously been isolated from terrestrial fungi.[4] The rest of the carbons were again assigned to a long methylene chain to complete the macrolide. The HMQC experiment showed that nearly all of the methylene protons had different chemical shifts from their geminal partner, providing further evidence for a macrocycle. This is in contrast to the straight-chain compounds **1** and **2**, which did not show diastereotopic methylene protons. Further analysis of the COSY and HMBC allowed assignment of structure **3**.

Pandangolide 2 (**4**) was obtained as an oil that showed a protonated molecular ion in the positive ion FABMS spectrum at *m/z* 319 $(MH)^+$. This ion was measured to be 319.12221 by HRFABMS, which corresponded to the molecular formula $C_{14}H_{23}O_6S$. The NMR spectral data for pandangolide 2 (**4**) was very similar to that of pandangolide 1 (**3**) (Table 1), except that it contained an extra two carbons and an extra spin system. The third spin system contained diastereotopic methylene protons (δ 3.55 and 3.35 ppm, 15.8 Hz) adjacent to a carboxylic acid group based on analysis of gradient multiple quantum COSY, HMQC, and gradient HMBC experiments. The resolution of the IR spectrum recorded precluded the observance of three absorptions consistent with the presence of ester, keto, and carboxylic acid carbonyls in the molecule, but the observance of peaks at 1711 and 1720 cm^{-1} is consistent with the presence of these three functionalities. The NMR data of **3** and **4** showed remarkable similarity except for the region of C3 where, in pandangolide 1 (**3**), the proton and carbon chemical shifts were δ 4.40 and 66.85, respectively, and in pandangolide 2 (**4**) were δ 3.72 and 43.31. The carbon chemical shift differential of C3 was consistent with substitution of sulfur for oxygen at this position. A gradient HMBC experiment showed correlations into the keto-carbonyl group from H2, H3, H5, and H6, again fixing its position at C4. A key correlation was observed from H3 to the methylene carbon at δ 34.65. Correlations were also observed from the two methylene protons at δ 3.55 and 3.35 to the carbon at C3. This connected the macrolide portion of the structure to the third spin system via a thioether linkage between C3 and the methylene carbon (C13) of the acetate spin system. The structure of pandangolide 2 (**4**) was therefore assigned as shown.

All of the compounds were tested for activity against a panel of Gram-positive and Gram-negative bacteria and yeast at a concentration of (250 μg/well). No activity was observed in these assays.

Experimental Section

General Experimental Procedures. Specific optical rotations were recorded with a JASCO DIP-370 digital polarimeter. IR spectra were recorded with a Perkin−Elmer 1600 Series FTIR spectrophotometer. ^1H and ^{13}C NMR experiments were performed with a Varian Unity 500 MHz spectrometer with a deuterium lock in the solvent indicated at 26 °C. Spectra were referenced to residual undeuterated solvent peaks or solvent ^{13}C signals. HRMS and LRMS were performed with a Finnigan MAT 95 high-resolution gas chromatography/mass spectrometer. C_{18}-Si gel used for flash chromatography was Merck LiChroprep RP_{18}, particle size 0.040−0.063 mm. HPLC was performed using a Beckman System Gold HPLC system with a Rainin Dynamax C_{18} column, 10 × 250 mm, particle size 8 μm, pore size 60 Å.

Collection and Fermentation. The fungal strain I96S215 was isolated from a bright orange, encrusting sponge collected in the waters surrounding Cordenamine Island, Indonesia. The strain grew well at both 22 and 28 °C, but no growth was observed at 37 °C. At 28 °C, all colonies consisted of smooth-walled, acutely branched septate hyphae with smooth colony margins. No pigment production was observed under any of the growth conditions. I96S215 was grown on several media, and its growth characters described below. The five media are listed in the *ATCC Catalog of Fungi/Yeasts*, (17th ed., Rockville, MD, 1991): malt extract agar (ATCC no. 323), Czapek's dox agar, (ATCC no. 312), yeast extract peptone dextrose (YePD) agar (ATCC no. 1245), cornmeal agar (ATCC no. 307), and potato dextrose (PD) agar (ATCC no. 336). Colony colors were matched with the Color Standards and Nomenclature; (Robert Ridgway, Washington, DC, 1912). Colonies grown on YePD agar for 7 days had an average size of 9.8 mm diameter; convex and densely felted with furrows and convolutions to colony margin; colony color, light gray to olive, reverse color, dark green olive; hyphae septate was 0.5−3.0 μm. Colonies grown on malt agar for 7 days had an average size of 14.2 mm diameter; velvety with furrows to colony margin; colony color, light gray to olive with tufts of aerial hyphae lacking pigmentation, reverse color, dark green olive; hyphae septate was 0.5−3.0 μm. Colonies grown on Czapek's agar for 7 days had an average size of 20.0 mm diameter; velvety with furrows to colony margin; colony color, yellow-gray center with light gray olive fringe, reverse color, dark green olive; hyphae septate was 0.5−3.0 μm. Colonies grown on cornmeal agar had an average size of 7.8 mm diameter; velvety to powdery to colony margin; colony color, olive gray, reverse color, dark green olive; hyphae septate was 0.5−3.0 μm. Colonies grown on PD agar for 7 days had an average size of 12.5 mm diameter; velvety with furrows to colony margin; colony color, light gray olive, reverse color, dark gray olive; hyphae septate was 0.5−3.0 μm.

The culture grown on Bennett's agar (10 g/L dextrose, 1 g/L beef extract [Difco], 1 g/L yeast extract [Difco], 2 g/L N−Z-amine A [Quest International], 15 g/L agar) was inoculated into 10 mL of potato−dextrose broth (PDB, Difco) in a 25 × 150 mm test tube and incubated at 22 °C, 160 rpm, for 7 days. A second stage seed was prepared from this culture by transferring the 10-mL fermentation to a 250-mL Erlenmeyer flask containing 50 mL of PDB; the seed flask was incubated at 22 °C, 200 rpm, for 4 days.

Production fermentations were performed in a 2.8-L Fernbach flask containing 1 L PDB. Each flask was inoculated with 50 mL of second-stage seed and incubated at 22 °C, 200 rpm, for 7 days.

Isolation and Purification. The fermentation broth was combined with an equal volume of ethyl acetate and shaken at 200 rpm for 1 h. The aqueous and ethyl acetate phases were separated by centrifugation, and the ethyl acetate extract was removed and concentrated in a rotary evaporator (Buchi Rotavapor model R110). The dried extract (64.3 mg) was redissolved in 10% aqueous MeOH (50 mL) and extracted with hexane (2 × 100 mL). The concentration of the aqueous MeOH was adjusted to 40% by the addition of water (25 mL), and the resulting solution was extracted with $CHCl_3$ (2 × 100 mL). All three phases were concentrated *in vacuo* and inspected by TLC and ^1H NMR spectroscopy. The $CHCl_3$ extract (38.5 mg) was further purified by flash silica chromatography (C_{18}, 20 × 260 mm, 50% aqueous CH_3CN−100% CH_3CN gradient) to give a number of fractions that were analyzed by TLC and ^1H NMR. The interesting fractions were further purified by HPLC (C_{18}, 10 × 250 mm, 90% aqueous CH_3CN−100% CH_3CN gradient) to afford cladospolide B (6.0 mg), *iso*-cladospolide B (7.6), *seco*-patulolide C (1.6 mg), pandangolide 1 (2.5 mg), and pandangolide 2 (0.6 mg).

***iso*-Cladospolide B (1):** $[\alpha]_D = -90°$ (*c* 0.23, MeOH); IR (film) ν_{max} 3400, 1747 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; FABMS *m/z* 229 $[MH]^+$ (100), 211 (48); HR-FABMS *m/z* 229.14269 (calcd for $C_{12}H_{21}O_4$, 229.14398).

***seco*-Patulolide C (2):** $[\alpha]_D = -10°$ (*c* 0.088, MeOH); IR (film) ν_{max} 3355, 1698 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; FABMS *m*/*z* 231 $[MH]^+$ (100), 213 (15), 195 (25); HRFABMS *m*/*z* 231.15919 (calcd for $C_{12}H_{23}O_4$, 231.15963).

Pandangolide 1 (3): $[\alpha]_D = -31°$ (*c* 0.19, MeOH); IR (film) ν_{max} 3444, 1732, 1716 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; FABMS *m*/*z* 245 $[MH]^+$ (100), 227 (24), 209 (14); HRFABMS *m*/*z* 245.14063 (calcd for $C_{12}H_{21}O_5$, 245.13890).

Pandangolide 2 (4): $[\alpha]_D = -30°$ (*c* 0.067, MeOH); IR (film) ν_{max} 3452, 1720, 1711 cm^{-1}; ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; FABMS *m*/*z* 319 $[MH]^+$ (100), 301 (18); HR-FABMS *m*/*z* 319.12221 (calcd for $C_{14}H_{23}O_6S$, 319.12153).

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