Mycapolyols A–F, New Cytotoxic Metabolites of Mixed Biogenesis from the Marine Sponge *Mycale izuensis*[1]

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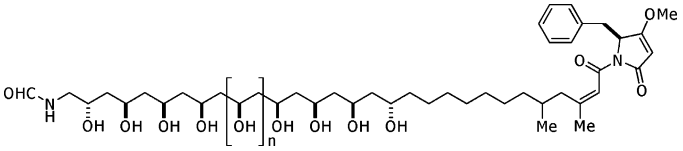
ABSTRACT



Mycapolyols A–F (1–6), six new unusual PKS/NRPS metabolites, were isolated from the marine sponge *Mycale izuensis.* The gross structures were elucidated by analysis of spectroscopic data, while the stereochemistry was established using chemical method and the universal NMR database.

A variety of bioactive metabolites have been isolated from marine sponges of the genus *Mycale*, including terpenoid peroxides,[2] steroid saponins,[3] nucleosides,[4] nitrogen-containing polyketides,[5] and macrolides.[6] Previously, we reported the isolation of mycalolides, tris-oxazole-containing macrolides, from *Mycale izuensis*[6d] collected in southern Japan. Further investigation of the same sponge resulted in the isolation of six unusual PKS/NRPS metabolites named mycapolyols A−F (**1**−**6**). This paper describes the isolation and structural determination of these new compounds.

The MeOH extract of the frozen sponge was partitioned according to the Kupchan's protocol to afford the cytotoxic $CHCl_3$ fraction, which was subsequently fractionated by ODS flash chromatography, Sephadex LH-20 gel filtration, and two rounds of reversed-phase HPLC to furnish mycalopyols A (**1**, 8.5×10^{-5} % yield based on wet wt), B (**2**, 1.7×10^{-4} %), C (**3**, 1.4×10^{-4} %), D (**4**, 1.1×10^{-4} %), E (**5**, 1.1×10^{-4} %), and F (**6**, 5.5×10^{-5} %).

Mycapolyol B (**2**)[7] had a molecular formula of $C_{53}H_{90}N_2O_{17}$ on the basis of HRFABMS and UV absorption at 250 nm attributable to α,β-unsaturated amides. Most ^1H and ^{13}C

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(7) White powder; $[\alpha]^{27}_D$ +104.7° (*c* 0.22, 80% aq MeOH); UV (80% aq MeOH) λ_{max} (log ϵ) 259 (4.60); ^1H and ^{13}C NMR data (DMSO-d_6), see Table 1; HRFABMS *m*/*z* 1027.6342 (M + H^+, calcd for $C_{53}H_{91}O_{17}N_2$ 1027.6318).

Table 1. ^{1}H and ^{13}C NMR Data for Mycapolyol B (**2**) in DMSO-d_6

position	δ_C		δ_H (mult, J in Hz)	COSY and TOCSY correlations	HMBC correlations
1	163.9				
2	119.5		6.82 (s)		C-1, 3, 4, CH_3-3
3	157.8				
CH_3-3	25.3		1.87 (s)		C-2, 3, 4
4	40.4	a	2.55 (dd, 8.5, 12.3)	H-5, CH_3-5	C-2, 3, 5, 6
		b	2.62 (dd, 6.5, 12.7)	H-5, CH_3-5	C-2, 3, 5, 6
5	31.3		1.75 (m)	H-4ab, 6a, 7ab, CH_3-5	
CH_3-5	19.4		0.82 (d, 6.9)	H-4ab, 5, 6a 7ab	C-4, 5, 6
6	36.5	a	1.15 (m)	H-7ab, 5, CH_3-5	C-4
		b	1.35[a]		
7	25.5	a	1.20[a]	H-5, 6a, CH_3-5	
		b	1.32[a]	H-5, 6a, CH_3-5	
8	29.2[b]		1.24[a]		
9	29.3[b]		1.24[a]		
10	29.4[b]		1.24[a]		
11	26.5	a	1.23[a]		
		b	1.35[a]		
12	37.9		1.27[a] (2H)	H-13, OH-13	C-10, 13
13	66.4		3.59 (m)	H-12, OH-13, H-14	
OH-13			4.19 (d, 5.4)	H-12, 13, 14	C-12, 13, 14
14	45.2		1.42[a] (2H)	H-13, OH-13, OH-15	
15	65.81		3.81 (m)	OH-15	
OH-15			4.43 (d, 4.6)	H-14, 15	C-14, 15
16−34 (see below)[c]					
35	65.35		3.80 (m)	OH-35	
OH-35			4.46 (d, 5.0)	H-35, 36	C-34, 35, 36
36	42.5		1.32[a] (2H)	OH-35, OH-37	
37	65.96		3.69 (m)	OH-37, H38ab	
OH-37			4.60 (d 5.4)	H-36, 37, 38ab	C36, 37, 38
38	44.1	a	2.96 (m)	H-36, 37, OH-37, NH	C-37
		b	3.11 (m)	H-36, 37, OH-37, NH	C-37
NH			7.94 (brt, 5.4)	H-38ab	C-38
CHO	161.1		7.99 (s)		C-38
1′	169.2				
2′	95.0		5.08 (s)		C-1′, 4′
3′	177.5				
OCH_3-3′	58.7		3.85 (s)		C-3′
4′	58.8		4.91 (dd, 2.7, 4.6)	H-5′ab	C-1′, 3′, 6′
5′	33.9	a	3.00 (dd, 2.3, 13.5)	H-4′	C-3′, 4′, 6′, 7′
		b	3.40 (dd, 5.0, 13.9)	H-4′	C-3′, 4′, 6′, 7′
6′	134.3				
7′, 11′	129.3		6.87 (d, 6.5)	H-8′, 9′, 10′	C-5′, 8′, 9′, 10′
8′, 10′	128.0		7.21 (m)	H-7′, 9′, 11′	C-7′, 11′
9′	126.8		7.18 (m)	H-7′, 8′, 10′, 11′	

[a] Envelop. [b] Interchangeable within column. [c] Following signals could not be individually assigned: Carbons 16, 18, 20, 22, 24, 26, 28, 30, 32, 34; δ_C 44.6 (8C), 45.25, 45.30; δ_H 1.35−1.50. Carbons 17, 19, 21, 23, 25, 27, 29, 31, 33; δ_C 67.48, 67.51 (6C), 67.63, 67.71; δ_H 3.78 (brs), 4.65 (brs, 9H, OH).

NMR signals were well dispersed, except for clusters of signals ascribable to methylenes and secondary alcohols. Interpretation of NMR data (Table 1) led to partial structures A−F (Figure 1).

Partial structure A contained a benzyl group attached to a nitrogenous methine (C-4′) that was connected to a carbon at δ 177.5 (C-3′) on the basis of an HMBC cross-peak, H-5′b/C-3′ (Figure 1). HMBC cross-peaks, *O*CH_3/C-3′ and H-2′/C-4′, together with HMBC correlations above and chemical shift values of C-1′ and C-4′, led to a 4-methoxypyrrolidone unit. In fact, the NMR data of this portion coincided well with those of the dolapyrrolidone unit in dolastatin 15.[8]

Partial structure B included the other α,β-unsaturated carbonyl moiety as inferred from HMBC cross-peaks H-2/C-1, C-3, C-4, and 3-Me. COSY correlations established the connectivities from C-4 to C-6, where C-5 was methylated. In the TOCSY spectrum, H-5 and H_2-6 were further correlated with methylene protons resonating between δ 1.24 and 1.32, thereby indicating linkage of C-6 to an aliphatic chain; however, the length of methylenes attached at C-6 could not be determined.

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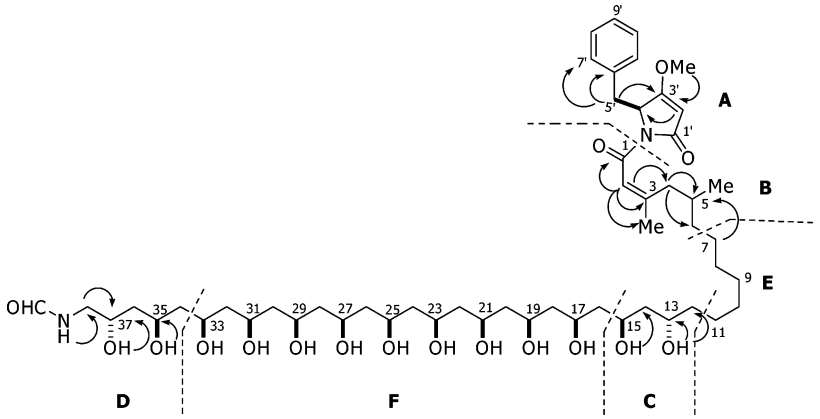


Figure 1. Partial structures and selected HMBC correlations of mycapolyol B (**2**).

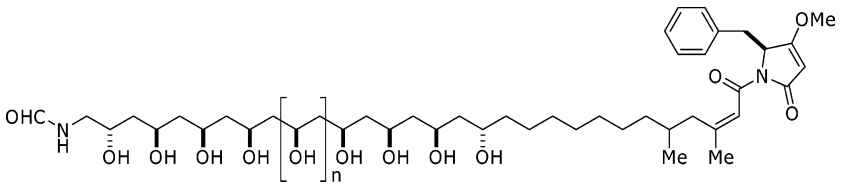
Partial structure C, the portion from C-12 to C-15, could be constructed mainly by HMBC cross-peaks, OH-13/C-12, C-14, and OH-15/C-14, although the heavily overlapped aliphatic proton signals prevented the precise analysis of COSY data. Because H-15 was barely separated from the cluster of oxymethine signals, we were not able to extend partial structure C further.

Partial structure D comprised a terminal portion of the molecule; COSY correlations from the NH proton of the terminal formamide were observed to a pair of methylene protons (H_2-38) that were in turn coupled to an oxymethine (H-37). Connectivities from C-37 to C-35 were evident from HMBC cross-peaks 37-OH/C-36, C-38; 35-OH/C-34, C-36. Because H-35 was scarcely separated from the cluster of oxymethine signals, further analysis of partial structure D was not possible.

Partial structure E, composed of five contiguous methylenes, was consistent with NMR data (δ_H/δ_C 1.20,1.32/25.5, 1.23,1.35/26.5, 1.24/29.2, 1.24/29.3, 1.24/29.4).

The remaining elements of $C_{18}H_{36}O_9$ contained nonequivalent methylenes (δ_H/δ_C 1.40, 1.50/45.2) and hydroxylated methine [δ_H/δ_C 3.78/67.5 (CH), 4.65 (OH)], which were coupled to each other. The highly homogeneous nature of the signals was reminiscent of an either isotactic or syndiotactic 1,3-polyol system (partial structure F). These partial structures were assembled by further interpretation of two-dimensional NMR data. The carbon chemical shift value of C-1 in partial structure B connected it to the nitrogen atom in partial structure A. TOCSY correlations from isolated signals assignable to H-5 and H-6a with signals at δ 1.24 suggested that the other end of partial structure B was connected to partial structure E, the other end of which was in turn connected to C-12, since H-13 was correlated with signals at δ 1.24 in the TOCSY spectrum. By default, partial structure F should be accommodated between partial structures C and D. The 2*Z* stereochemistry was deduced on the basis of the carbon chemical shift of 25.3 ppm for the 3-Me signal. The relative stereochemistry of the 1,3-polyol system was assigned on the basis of the Kishi's ^{13}C NMR database for the 1,3,5-polyol system.[9]

In mycapolyol B, the oxymethines located between C-17 and C-33 resonated at 67.5 ppm, whereas C-13, C-15, C-35, and C-37 resonated at around 66 ppm. The chemical shift rule of C-3 in the 1,3,5-triols system measured in DMSO-d_6 [δ 68 (syn/syn), 66 (anti/syn), 64 (anti/anti)] indicated that the hydroxy groups located between C-15 and C-35 were all syn, while C-13/C-15 and C-35/C-37 were anti. However, the latter two assignments were contradictory to those



mycapolyol A (**1**, n=6)
mycapolyol B (**2**, n=5)
mycapolyol C (**3**, n=4)
mycapolyol D (**4**, n=3)
mycapolyol E (**5**, n=2)
mycapolyol F (**6**, n=1)

Figure 2.

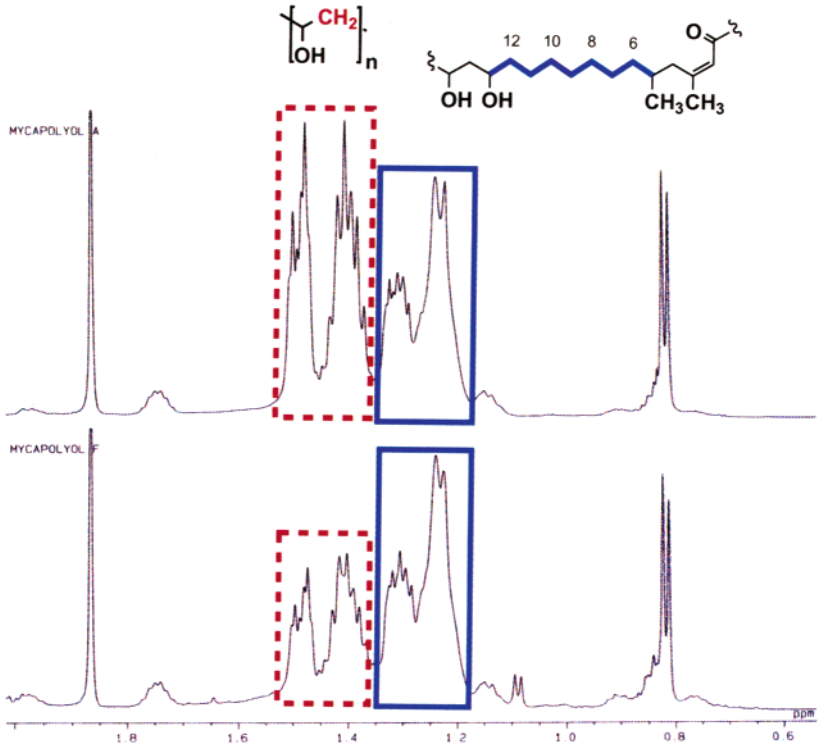


Figure 3. Aliphatic regions of ^{1}H NMR spectra of mycapolyols A (**1**, top) and F (**6**, bottom). Red dashed boxes indicate methylene signals in the polyol portion, while blue boxes indicate those of the alkyl chains.

obtained on the basis of proton signals flanked by methoxylated methines described by Mynderse and Moore[10] for polymethoxylated alkenes. All methylene protons in the skipped polyol portion of mycapolyol B were nonequivalent, differing by 0.1 ppm, which was consistent with the isotactic nature of all substituents between C-13 and C-37. At the moment, we cannot conclude which is correct, but the structure derived from the Kishi's rule is adopted here. The 4′*S* stereochemistry was inferred from the presence of L-Phe in the hydrolysate[11] of an ozonolysis product of mycapolyol B. Unfortunately, we were not able to determine the absolute stereochemistry of the polyol portion as well as that of C-5.

Mycapolyols exhibited ^{1}H and ^{13}C NMR spectra virtually superimposable on each other, except for the intensity of the clusters of secondary alcohol and nonequivalent methylenes in the polyol portion (Figure 3). In fact, the molecular formulas of mycapolyols A−F were different by units of C_2H_4O (Figure 2). Therefore, the structural determination of the remaining mycapolyols[12] was straightforward on the basis of two-dimensional NMR and HRFABMS data. It is likely that all mycapolyols share a common stereochemistry.

Mycapolyols are related to malyngamides[13] and dolastatin 15,[8] while the 1,3-polyol portion was reminiscent of isotactic polymethoxy-1-alkenes, metabolites of cyanobacteria,[10] and the marine sponge *Myriastra clavosa*.[14] Mycapolyols A−F exhibited cytotoxicity against HeLa cells with IC_{50} values of 0.06, 0.05, 0.16, 0.40, 0.38, and 0.90 μg/mL, respectively.

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Supporting Information Available: Experimental section and MS and NMR spectra of isolated compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Optical rotations of mycapolyols A (**1**) and C−F (**3**−**6**) were as follow. Mycapolyol A (**1**): white powder; $[\alpha]^{21}_D$ +101.1° (*c* 0.34, 80% aq MeOH); HRESIMS *m*/*z* 1071.6610 (M + H$^+$, calcd for $C_{55}H_{95}O_{18}N_2$, 1071.6580). Mycapolyol C (**3**): white powder; $[\alpha]^{27}_D$ +105.7° (*c* 0.32, 80% aq MeOH); HRESIMS *m*/*z* 983.6086 (M + H$^+$, calcd for $C_{51}H_{87}O_{16}N_2$, 983.6056). Mycapolyol D (**4**): white powder; $[\alpha]^{27}_D$ +100.6° (*c* 0.25, 80% aq MeOH); HRESIMS *m*/*z* 961.5638 (M + Na$^+$, calcd for $C_{49}H_{82}O_{15}N_2$-Na, 961.5613). Mycapolyol E (**5**): white powder; $[\alpha]^{21}_D$ +117.7° (*c* 0.27, 80% aq MeOH); HRFABMS *m*/*z* 895.5566 (M + H$^+$, calcd for $C_{47}H_{79}O_{14}N_2$, 895.5531). Mycapolyol F (**6**): white powder; $[\alpha]^{21}_D$ +120.1° (*c* 0.20, 80% aq MeOH); HRFABMS *m*/*z* 851.5259 (M + H$^+$, calcd for $C_{45}H_{75}O_{13}N_2$, 851.5269).

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