Isolation of a New Mycalolide from the Marine Sponge *Mycale izuensis*[1]

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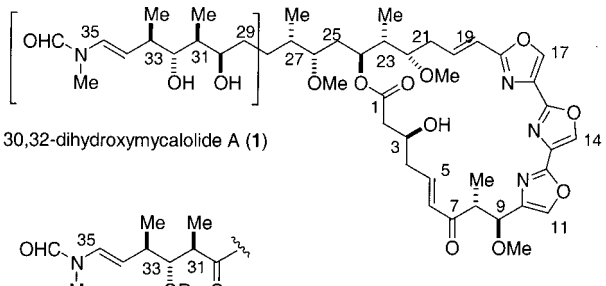
Bioassay-directed fractionation of the lipophilic extract of the marine sponge *Mycale izuensis* led to the isolation of cytotoxic mycalolides including a new compound, 30,32-dihydroxymycalolide A (**1**). Its structure including absolute stereochemistry was deduced by spectroscopic and chemical methods. Compound **1** was cytotoxic against HeLa cells with an IC_{50} value of 2.6 ng/mL.

The mycalolides are macrocyclic lactones containing a tris-oxazole moiety and a side chain terminating with a *N*-methylformamide group. Closely related sponge metabolites such as kabiramides, ulapualides, halichondramides, and jaspisamides[2] as well as distantly related macrolides such as swinholides,[3] bistheonellides (misakinolides),[4] and sphinxolides[5] from marines sponges, scytophycins[6] from blue-green algae, and aplyronines[7] from a sea hare exhibit potent cytotoxic activity by disrupting cellular microfilaments.[8] The absolute stereochemistry of mycalolides determined on the basis of chemical degradation was confirmed by a total synthesis of mycalolide A (**5**).[9]

In our continuing program of potential leads from Japanese marine invertebrates, we found that the marine sponge *Mycale izuensis* showed potent cytotoxicity in the lipophilic extract. Bioassay-directed fractionation of the extract afforded a new mycalolide along with five known mycalolides. We describe isolation and structure elucidation of the new compound.

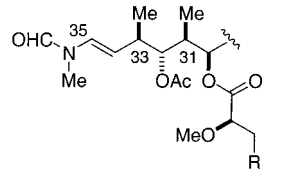
The alcoholic extract of the sponge *Mycale izuensis* collected in the Amakusa Islands 1700 km southwest of Tokyo was subjected to a solvent partitioning to afford the active $CHCl_3$ fraction. This was fractionated by ODS flash column chromatography followed by ODS HPLC to afford 30,32-dihydroxymycalolide A (**1**) together with five known mycalolides (**2**−**6**).

The ^{1}H NMR spectrum exhibited signals characteristic of the mycalolide class of compounds, e.g., three low-field heteroaromatic protons (δ 8.75, 8.49, and 8.04), a pair of doublet formamides (δ 8.30 and 8.05), three *E*-olefins (δ 6.68, 6.46, and 6.17), three *O*-methyls (δ 3.35, 3.34, and 3.14), a doublet *N*-methyl (δ 3.08 and 2.99), and five doublet methyls (δ 1.11, 0.93, 0.87, 0.87, and 0.83). Comparison of the ^{1}H NMR spectrum with that of mycalolide A readily indicated the absence of an acetoxyl group and the presence of an additional secondary alcohol, while comparison of the ^{13}C NMR spectrum with that of mycalolide A indicated the absence of the C-30 ketone and the acetyl groups. The gross structure of **1** was eventually assigned on the basis of 2D NMR data. The COSY spectrum exhibited spin systems very similar to those in 30-hydroxymycalolide A (**2**), except for the chemical shift values for H-32 (3.47 vs 4.93 ppm in **2**), thereby suggesting that the acetoxyl group in 30-hydroxymycalolide A is replaced by a hydroxyl group. All the ^{1}H and ^{13}C signals were assigned by interpretation of HMQC and HMBC data (Table 1).

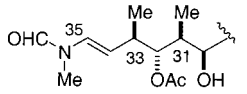


30,32-dihydroxymycalolide A (**1**)

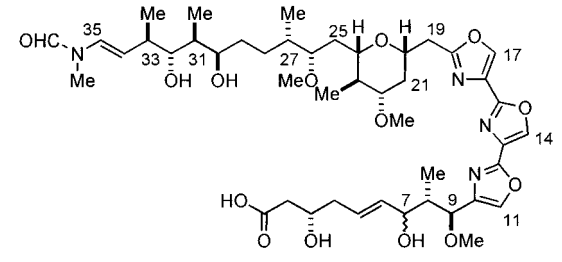
32-hydroxymycalolide A (**2**) R = H
mycalolide A (**5**) R = Ac



38-hydroxymycalolide B (**3**) R = OH
mycalolide B (**6**) R = OMe



30-hydroxymycalolide A (**4**)



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The absolute stereochemistry of **1** was determined by chemical conversion. Compound **1** was reduced with $NaBH_4$ followed by basic hydrolysis to afford compound **7**. This material was identical with compound **7** prepared from mycalolide B (**6**) in the same manner. Thus, the relative stereochemistry of all stereogenic centers was identical with that of other mycalolides. The modified Mosher analysis[10] of **1** resulted in the 3*S*-configuration as in the case of other mycalolides.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a JEOL A600 NMR spectrometer. ^{1}H and ^{13}C NMR chemical shifts were referenced to CD_3OD residue: δ_H 3.30 and δ_C 49.0. FABMS was carried out with a JEOL JMX-SX102/

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Table 1. ^{1}H and ^{13}C NMR Data of 30,32-Dihydroxymycalolide A (**1**) in CD_3OD

position	^{1}H	^{13}C	position	^{1}H	^{13}C
1		173.4	22	3.47 m	81.2
2	2.69 dd (3.6, 1.5)	43.3	22-OMe	3.35 s	58.0
3	4.41 m	68.6	23	1.93 m	41.4
4	2.47 m, 2.64 m	41.6	23-Me	0.93 d (7.2)	9.1
5	7.39 dt (16.8, 6.6)	148.3	24	5.28 m	74.6
6	6.17 d (16.2)	134.5	25	1.57 m	33.5
7		205.6	26	3.14 m[a]	83.0
8	4.23 m	44.3	26-OMe	3.34 s	58.2
8-Me	0.87 d (6.6)	14.4	27	1.78 m	36.4
9	4.35 d (9.0)	78.4	27-Me	0.87 d (6.6)	16.1
9-OMe	3.14 s	56.7	28	0.96 m, 1.69 m	29.3
10		139.8	29	1.48 m	33.5
11	8.04 s	139.9	30	3.90 m	73.1
12		157.3	31	1.57 m	41.6
13		132.0	31-Me	0.83 d (7.2)	9.1
14	8.57 s	139.9	32	3.47 m	78.3
15		158.1	33	2.42 m	38.9
16		131.1	33-Me	1.11 d (7.2)	19.8
17	8.49 s	139.9	34	5.20 dd (13.8, 9.0)	113.7
18		164.2	35	6.68 d (13.8)	130.2 [125.4][b]
19	6.46 d (16.2)	117.6	35-NMe	2.99 s [3.08 s]	27.7[33.5]
20	7.13 ddd (15.6, 7.5, 6.0)	141.3	35-NCHO	8.30s [8.05s]	164.6 [163.2]
21	2.76 m, 2.49 m	34.7			

[a] Overlapped with the 9-OMe signal. [b] Chemical shifts for the minor conformer are bracketed.

SX102 with 3-NBA as a matrix. UV spectra were measured on a Hitachi 330 spectrometer.

Animal Material. The sponge was collected by hand using scuba diving at depths of 5−25 m off Nagashima Island in the Amakusa Islands (32°12′ N, 130°08′ E) and identified as *Mycale izuensis*. Clusters of digitations rise from a common base. Digitations bear small apical oscules and may be up to 6 cm high and 1.5 cm in diameter. A skeleton of plumose spicule tracts diverges and thins out toward the periphery. No special surface skeleton is found in the surface membrane; spicule styles with the usual subterminal constriction (mycalostyles), 252−282 × 2−4 μm, anisochelae in three size categories (36−40, 23−26, and 13−15 μm), sigmas in two size categories (25−28 and 12−16 μm). These characteristics conform closely to the description of a type of this species. A voucher specimen (POR16160) was deposited at the Zoological Museum of the University of Amsterdam.

Extraction and Isolation. The frozen sponge (2 kg) was extracted with EtOH (5 × 3L) and MeOH (1 × 3L). The combined extracts were evaporated and partitioned between H_2O and ether. The ether fraction was evaporated and partitioned between MeOH/H_2O (9:1) and *n*-hexane. The former layer was diluted with water to MeOH/H_2O (6:4) and extracted with $CHCl_3$. The active $CHCl_3$ layer was fractionated by ODS flash column chromatography with MeOH/H_2O and MeCN/H_2O. The active MeCN/H_2O (7:3) fraction was gel filtered on Sephadex LH 20 (MeOH) followed by successive ODS HPLC with MeOH/H_2O (77:23) and MeCN/H_2O (53:47) to afford 30,32-dihydroxymycalolide A (**1**, 6 mg), 32-hydroxymycalolide A (**2**, 23 mg), 38-hydroxymycalolide B (**3**, 64 mg), 30-hydroxymycalolide A (**4**, 39 mg), mycalolide A (**5**, 5 mg), and mycalolide B (**6**, 38 mg).

30,32-Dihydroxymycalolide A (1): $[\alpha]^{22}_D$ −68.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 231 (ϵ 33055); ^{1}H and ^{13}C NMR data in CD_3OD, see Table 1; HRFABMS *m*/*z* 891.4371 (calcd for $C_{45}H_{64}N_4O_{13}Na$, 891.4368).

Preparation of 7. To a solution of 30,32-dihydroxymycalolide A (**1**, 1.4 mg) in MeOH (0.5 mL) was added $NaBH_4$ (2.5 mg), and the mixture was stirred at 0 °C until the reaction was complete. The reaction mixture was diluted with 5% AcOH (2 mL), concentrated, and separated by ODS HPLC with MeCN/H_2O (1:1), and the major peak was collected. The fraction obtained was dissolved in a mixture of 1 M LiOH in MeOH (2:1, 1 mL), and the mixture was stirred overnight at room temperature. To the reaction mixture was added 100 μL of AcOH, and the resulting mixture was concentrated and separated by ODS HPLC with MeCN/H_2O (2:3) to yield **7** (0.7 mg). Mycalolide B (**6**) was treated in the same manner to afford **7**, whose ^{1}H NMR and FABMS were identical to those of **7** prepared from **1** and those reported.[11]

Modified Mosher Analysis of 1. To a solution of **1** (0.2 mg) in two drops of pyridine was added (−)-MTPA chloride (7 μL), and the mixture was left at room temperature for 10 min. After addition of 2 mL of 1 M $NaHCO_3$, the reaction mixture was extracted with EtOAc, washed with brine, dried on anhydrous $MgSO_4$, and evaporated. The residue was purified on a short silica gel column to afford tris-(*S*)-(−)-MTPA derivative. The tris-(*R*)-(+)-MTPA derivative was prepared in the same way. $\Delta\delta H_{SR}$ values: H-2 = +0.049; H-4a = −0.414; H-5 = −0.012; H-6 = −0.223; H-8 = −0.153; H-27 = +0.009; H-29a = +0.049; 31-Me = −0.042; 33-Me = +0.056; H-34 = +0.042.

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