Montiporic Acids A and B, Cytotoxic and Antimicrobial Polyacetylene Carboxylic Acids from Eggs of the Scleractinian Coral *Montipora digitata*[1]

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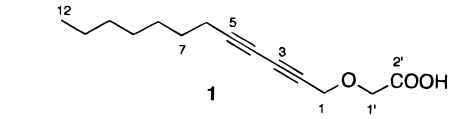
Two new polyacetylene carboxylic acids, montiporic acids A (**1**) and B (**2**), have been isolated from the eggs of the scleractinian coral *Montipora digitata* and their structures elucidated on the basis of spectroscopic data. They exhibited antimicrobial activity against *Escherichia coli* and cytotoxicity against P-388 murine leukemia cells.

Montipora digitata Dana, 1845 (Scleractinia: Coelenterata) is a hermaphroditic coral; its colonies release bundles of tangled eggs and sperm, which are disentangled prior to fertilization. Polyacetylene alcohols are released from the eggs to attract sperm.[2] Because large numbers of eggs are released during the full moon in early summer, it is likely that the eggs are capable of chemical defense against predators,[3−7] which prompted us to examine bioactive constituents of the released eggs of *M. digitata*. Indeed, the EtOH extract showed antibacterial and cytotoxic activities. Bioassay-guided isolation afforded two polyacetylene carboxylic acids. This paper describes the isolation and structure elucidation of these compounds.

The CH_2Cl_2-soluble portion of the aqueous EtOH extract of the frozen eggs (3.0 g) was partitioned between aqueous MeOH and *n*-hexane, followed by CH_2Cl_2. The aqueous MeOH layer, which showed antibacterial activity against *Escherichia coli*, was purified by repeated chromatography on Si gel and ODS to yield montiporic acids A (**1**, 0.5 mg) and B (**2**, 0.9 mg).



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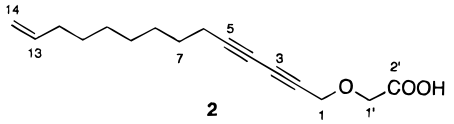

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Montiporic acid A (**1**) had a molecular formula of $C_{14}H_{20}O_3$ as established by HRFABMS. The IR spectrum exhibited bands of a disubstituted acetylene (2250 cm^{-1}) and a carboxyl (1730 cm^{-1}) group. The ^1H- and ^{13}C-NMR spectra (Table 1) revealed a terminal methyl (δ_H 0.86; δ_C 13.9), two oxygenated methylenes (δ_H 4.36, 4.22; δ_C 59.1, 65.8), two acetylenic units (δ_C 82.1, 72.9, 69.7, 65.8), and a carboxylic acid (δ_C 178.8). ^{13}C signals were reminiscent of a conjugated diyne system, which was supported by IR and UV data.[8,9] HMBC cross peaks H1/C2, H1/C3, H1/C4, H6/C3, H6/C4, and H6/C5 revealed that the conjugated diyne was connected to C1 and C6 methylene carbons. It must be noted that the fortuitous observation of four-bond couplings through the acetylenic bond allowed the unambiguous assignment of this system. HMBC cross peaks (H1/C1′, H1′/C1) and a NOESY correlation (H1/H1′) displayed further connection of C1 to C1′ through an ether oxygen. C1′ was a part of substituted acetic acid unit, because it was correlated with the C2′ carboxylate carbon in the HMBC spectrum. The remaining portion was an unbranched C_6-alkane linked to C6, which was supported by the 2D-NMR data. Thus, montiporic acid A is 2-*O*-(2,4-dodecadiynyl)ethanolic acid. The fragmentation pattern observed in the negative FABMS/MS spectrum was consistent with this structure (Figure 1).

Table 1. ^1H- and ^{13}C-NMR Data for Montiporic acids A (**1**) and B (**2**) in $CDCl_3$

	1		**2**	
position	^1H	^{13}C	^1H	^{13}C
1	4.36 (2H, s)	59.1 (t)	4.36 (2H, s)	59.1
2		69.7 (s)		69.7
3		72.9 (s)		73.0
4		64.2 (s)		64.2
5		82.1 (s)		82.3
6	2.26 (2H, t, J = 7.5 Hz)	19.0 (t)	2.22 (2H, t, J = 7.5 Hz)	19.2
7	1.52 (2H, quint., J = 7.5 Hz)	27.9 (t)	1.47 (2H, quint., J = 7.5 Hz)	28.0
8	1.34 (2H, qunit., J = 7.5 Hz)	28.4 (t)	1.35 (2H, quint., J = 7.5 Hz)	28.9
9	1.24 (m)	28.4 (t)	1.25 (m)	28.9
10	1.24 (m)	31.6 (t)	1.25 (m)	28.9
11	1.25 (m)	22.6 (t)	1.35 (m)	28.9
12	0.86 (3H, t, J = 7.0 Hz)	13.9 (q)	1.97 (2H, tdt, J = 8.0, 6.7, 1.5 Hz)	33.7
13			5.79 (1H, ddt, J = 17.0, 10.2, 6.7 Hz)	139.1
14a			4.98 (1H, ddt, J = 17.0, 2.1, 1.5 Hz)	114.2
14b			4.92 (1H, ddt, J = 10.2, 2.1, 1.2 Hz)	
1′	4.22 (2H, s)	65.8 (t)	4.22 (2H, s)	65.8
2′		178.8 (s)		172.9

Montiporic acid B (**2**) had a molecular formula of $C_{16}H_{22}O_3$, which was determined by HRFABMS. Its ^1H-NMR spectrum was superimposable on that of **1** (Table 1), except for the presence of a monosubstituted olefin (δ_H 5.79, 4.98, and 4.92) instead of a terminal methyl group. The presence of the conjugated diyne and

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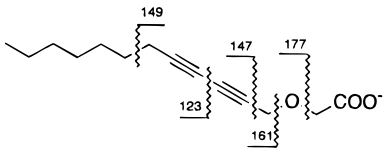

Figure 1. Prominent fragment ions in the FAB-MS/MS spectrum of **1**.

glycolate ether was straightforward from the HMBC spectrum. Therefore, montiporic acid B is 2-*O*-(13-tetradecane-2,4-diynyl)ethanolic acid.

Montiporic acids A and B were not only antibacterial against *Escherichia coli*, but also cytotoxic against P-388 murine leukemia cells with IC_{50} values of 5.0 and 12.0 μg/mL, respectively. These compounds are closely related to the methyl esters reported from an Okinawan *Montipora* sp.;[8] their spectral data are consistent with those of our compounds.

Experimental Section

General Experimental Procedures. NMR spectra were recorded either on a Bruker AM 600 or a Bruker AC 300 NMR spectrometer. Chemical shifts were referenced to solvent peaks: δ_H 7.24 and δ_C 77.0 for $CDCl_3$. MS were measured with a JEOL SX-102 mass spectrometer. Triethanolamine was used as a matrix in the FABMS, HRFABMS, and FABMS/MS.

Animal Material. Eggs of *M. digitata* were collected at Orpheus Island, Australia, as described previously.[10]

Extraction and Isolation. The frozen eggs (3.0 g) thus obtained were homogenized and extracted three times with EtOH/H_2O (7:3). The combined extracts were concentrated and partitioned between CH_2Cl_2 and H_2O. The organic layer was dissolved in MeOH/H_2O (9:1) and extracted with *n*-hexane, and the aqueous MeOH phase was further extracted with CH_2Cl_2. The aqueous MeOH layer was fractionated by ODS flash chromatography (H_2O/MeOH/$CHCl_3$, stepwise gradient). The fraction eluted with 100% MeOH was chromatographed on a Si gel column with a $CHCl_3$/MeOH system, followed by preparative TLC on Si gel with $CHCl_3$/MeOH/H_2O (70:30:5). The active band was finally purified by repeated HPLC on an ODS column (MeOH/H_2O, 9:1, MeCN/H_2O, 7:3) to afford montiporic acids A (**1**, 0.5 mg) and B (**2**, 0.9 mg).

Montiporic acid A (1): colorless oil; UV (MeOH) λ max 255 (ϵ 1059), 273 (799); IR (film) ν_{max} 2925, 2850, 2250, 1730 cm^{-1}; HRFABMS (negative) *m/z* found 235.1332 $(M - H)^-$, calcd for $C_{14}H_{19}O_3$ 235.1334; ^1H and ^{13}C NMR data, see Table 1.

Montiporic acid B (2): colorless oil; UV (MeOH) λ max 256 (ϵ 861), 273 (668); IR (film) ν max 3070, 2925, 2850, 2250, 1730, 1640 cm^{-1}; HRFABMS (negative) *m/z* found 261.1500 $(M - H)^-$, calcd for $C_{16}H_{21}O_3$ 261.1491; ^1H and ^{13}C NMR data, see Table 1.

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