An Antimicrobial C_{14} Acetylenic Acid from a Marine Sponge *Oceanapia* Species[1]

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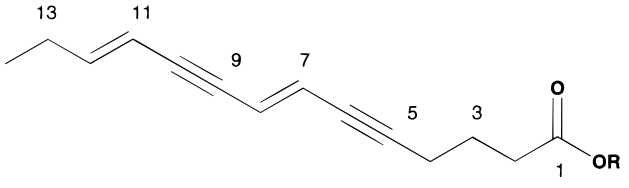
A C_{14} acetylenic acid has been isolated as an antimicrobial principle from a marine sponge *Oceanapia* sp. Its structure was determined on the basis of spectral data.

Brominated C_{16}, C_{18}, and C_{20} acetylenic acids have been reported from marine sponges of the genera *Xestospongia* and *Oceanapia*[2] and have been reported to possess antibacterial,[3a−c] antifungal,[4] cytotoxic,[3c] and HIV protease inhibitory[5] acivity. In our continuing search for bioactive metabolites from Japanese marine invertebrates, we found the lipophilic extract of a marine sponge, *Oceanapia* sp.,[2] inhibited several mutants of the yeast *Saccharomyces cerevisiae*. Bioassay-guided separation afforded an active substance, which was identified as an acetylenic acid. We report here the isolation, structure elucidation, and bioactivities of this compound.

The MeOH extract of the frozen sponge (120 g) was partitioned between H_2O and ether. The organic phase was further partitioned between 90% MeOH and *n*-hexane. The aqueous MeOH phase was fractionated by chromatography on ODS and silica gel to afford an active compound (**1**) (12 mg, 0.01% wet weight).



1 R=H
2 R=CH_3

Compound **1** had a molecular formula of $C_{14}H_{16}O_2$, as established by HRFABMS [*m/z* 215.1063 $(M - H)^-$, Δ −0.9 mmu]. NMR data revealed the presence of two vinyl, two acetylenic, four methylene, and one methyl functionality, in addition to a carboxylic acid moiety (Table 1), which accounted for all unsaturations in the molecule, thus indicating a linear structure of **1**. The presence of a carboxylic acid was also supported by an IR band at 1702 cm^{-1} and formation of the methyl ester **2**[7] upon treatment with CH_2N_2. Connectivities from C14 to C11 were straightforward by interpretation of $^1H-^1H$ COSY and HMBC[6] spectra (Table 1). A COSY cross-peak between H8 (δ 5.94) and H11 (δ 5.53) together with the upfield shift of C8 (δ 120.2) and C11 (δ 108.5) indicated that one of the acetylene groups could be placed between the two double bonds. This extended the structural unit from C14 to C7, and HMBC data (Table 1) substantiated this assignment. Similarly, the other acetylenic unit could be accommodated between C7 and C4 since H7 and H4 were weakly coupled, while C7 (δ 120.3) and C4 (δ 19.0) experienced upfield shifts. The presence of a CH═CH−C≡C−CH═CH−C≡C unit was also supported by intense UV absorption at 292 and 310 nm[8] and IR bands at 3023, 2212, and 2182 cm^{-1}. Connectivities from C2 to C4 were readily established by interpretation of 2D NMR data; the chemical shift value of H2 (δ 2.42) implied that C2 was adjacent to a carboxylic acid.

Table 1. NMR Data for **1** in $CDCl_3$

position	^1H	^{13}C	HMBC
1		178.1	
2	2.42 (2H, t, J = 7.3 Hz)	33.0	C1, C3, C4
3	1.83 (2H, tt, J = 7.2, 7.1 Hz)	23.6	C1, C2, C4, C5
4	2.39 (2H, t, J = 7.1 Hz)	19.0	C1, C2, C3, C5, C6, C8
5		94.3	
6		80.1	
7	5.84 (1H, d, J = 16.0 Hz)	120.3	C5, C8, C9
8	5.94 (1H, d, J = 16.0 Hz)	120.2	C6, C7, C10
9		86.6	
10		92.8	
11	5.53 (1H, d, J = 16.0 Hz)	108.5	C9, C13
12	6.15 (1H, dt, J = 16.0, 6.9 Hz)	147.0	C10, C13, C14
13	2.08 (2H, tt, J = 6.9, 7.4 Hz)	26.2	C10, C11, C12, C14
14	0.95 (3H, t, J = 7.4 Hz)	12.8	C12, C13

E-geometry of two double bonds was assigned on the basis of their coupling constants of 16.0 Hz. Therefore, compound **1** was determined to be 7*E*,11*E*-tetradecadiene-5,9-diynoic acid. The structure, especially positions of the two acetylenes, was confirmed by HMBC cross-peaks (Table 1).

Compound **1** exhibited some selectivity in antimicrobial activity (Table 2). It was moderately active against four mutants of *S. cerevisiae* and *Candida albicans*, but was inactive against *Penicillium chrysogenum* and *Mortierella ramanniana*. The compound was also inhibitory against both Gram-positive and Gram-negative bacteria. Amide and ester derivatives of acetylenic fatty acids have been previously isolated from Compositae,[9] and the ultraviolet-mediated antimicrobial activity of these compounds has also been reported.[10]

Previously reported C_{16}, C_{18}, and C_{20} acetylenic acids from marine sponges of the genera *Xestospongia* and *Oceanapia* possess one or two bromine atoms attached to sp^2 carbons.[2] Therefore, our compound is the first example of a midchain acetylenic acid without a bromine atom. Although C_{14} acetylene-containing glyceryl ethers occur in marine sponges,[2] a C_{14} acetylenic acid has not been reported. This is the first report of a marine acetylene containing a CH═CH−C≡C−CH═CH−C≡C unit.

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Table 2. Antimicrobial Activities of **1**

species/strain	50 μg[a]	100 μg[a]
yeast		
S. cerevisiae		
GT160-45C[c]		6.5[b]
cdc5[c]	7.0[b]	7.5
act1-1[c]	9.0	10.0
YAT2296[c]	6.5	7.0
fungi		
P. chrysogenum		
M. ramanniana		
C. albicans		8.5
bacteria		
E. coli	8.5	12.0
P. aeruginosa	8.5	13.0
B. subtilis		11.0
S. aureus	9.5	13.5

[a] Amounts loaded on a 6 mm ϕ disk. [b] Diameter of inhibitory zone in mm. [c] *S. cerevisiae* mutant.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a JEOL α-600 spectrometer. NMR chemical shifts were referenced to the $CDCl_3$ solvent (δ_H 7.24; δ_C 77.0 in $CDCl_3$). FAB mass spectra were obtained with a JEOL SX102 mass spectrometer using glycerol as a matrix. IR spectra were measured on a JASCO FT/IR-5300 spectrometer. UV spectra were obtained with a HITACHI 300 spectrometer.

Biological Material. The sponge was collected using scuba at a depth of 25 m in Kamagi Bay on the Sada Peninsula (33°24.96′ N, 132°08.14′ E). The sponge is a globular mass of 15 × 10 × 10 cm with a large number of thin limp fistules on the upper side. The fistules are irregular in outline and of unequal length and thickness, up to 5 cm long and 5 mm in diameter. Fistules and main mass have a detachable ectosome and have long stringy spicule tracts inside, between which the interior is pulpy. The color was pale yellowish brown. The ectosomal skeleton is a tangential reticulation of single spicules, forming an irregular somewhat halichondrioid crust. This surface skeleton is carried by a system of long spicule tracts, 30−50 μm in diameter, with 3−10 spicules in cross section. The fistules are not hollow but contain a core of parallel longitudinal spicule tracts. Between the tracts there are an irregular independent single spicule reticulation and abundant microscleres. Spicules are curved, abruptly pointed oxeas, 184−226 × 5−9 μm, and thin, shallow-curved, slightly angular sigmas, 14−22 μm. The species is assigned to *Oceanapia* sp. (order Haplosclerida, family Phloeodictyidae) because of possession of fistules in combination with a skeleton of long spicule tracts and independent single spicule reticulation. There is no matching description in the literature. A voucher was incorporated in the Zoological Museum of Amsterdam, reg. no. 14407

Antimicrobial Assay. Antifungal assay was carried out against four strains of *S. cereisiae* including three of its mutants, *P. chrysogenum*, *M. ramanniana*, and *C. albicans*. The following yeast strains were used: GT160-45C is the wild type; cdc5 and act1-1 have a mutation in the *cdc5* and *act1-1* gene, respectively; YAT2296 has mutations in the *mad1* and *erg6* genes. Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and Gram-positive bacteria *Bacillus subtillis* and *Staphylococcus aureus* were used in the antibacterial assay. A 50 or 100 μg portion of **1** was applied onto a paper disk of ϕ 6 mm, and the paper disk was air-dried. Then the disks were placed on agar plates that had been seeded with respective organisms. Diameters of inhibitory zones were measured after the plates were incubated at 26 °C for 24 h.

Extraction and Isolation. The frozen sponge (120 g) was extracted with MeOH (500 mL × 3); the combined extracts were concentrated and partitioned between ether and water. The organic phase which inhibited *S. cerevisiae* was further partitioned between *n*-hexane and MeOH/H_2O (9:1). The aqueous MeOH fraction was subjected to ODS column chromatography using stepwise elution from 50 to 100% MeOH; the fraction eluted with 90% MeOH was repeatedly separated by ODS HPLC with MeOH/100 mM $NaClO_4$ in H_2O (8:2). Final purification was achieved by silica gel column chromatography with $CHCl_3$, $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH/H_2O (6:4:1), and MeOH to yield compound **1** (12.0 mg, 0.01% wet wt).

Compound 1: pale-yellow solid; UV (1-PrOH) λ_{max} (log ϵ) 292 (4.56), 310 (4.53) nm; IR (neat) ν_{max} 3023, 2212, 2182, 1709, 1254, 1215, 1063, 953, 937, 941, 758 cm^{-1}; HRFABMS *m/z* 215.1063 (calcd for $C_{14}H_{15}O_2$, 215.1072); ^1H and ^{13}C NMR data, see Table 1.

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