Cytotoxic Acylated Spermidine from a Soft Coral, *Sinularia* sp.

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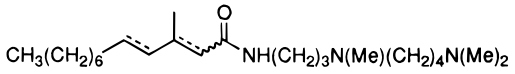
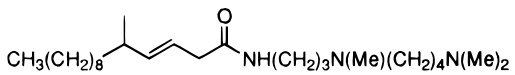
A cytotoxic acylated spermidine was isolated from a Pacific soft coral, *Sinularia* sp. and its structure determined by NMR and mass spectral analysis. The acyl portion corresponds to (3*E*)-5-methyltetradec-3-enoic acid.

Soft corals or alcyonaceans are prominent reef organisms in the western Pacific and are prolific sources of terpenoids, especially cembranoid diterpenes.[1] Members of the genus *Sinularia* have been the source of many terpenoids but have also yielded the spermidine derivatives **1**−**5**.[2−4] Additional spermidine and hydroxyspermidine derivatives in which the acyl moiety is much more complex have been isolated from sponges (e.g., the crambescidins,[5−7] ptilomycalin A,[8] the phloeodictines[9,10]) and starfishes (e.g., celeromycalin and fromiamycalin).[11] In an earlier condensed report[4] we presented the structure of the cytotoxic spermidine derivative **5** from a soft coral, *Sinularia* sp., without any experimental support; in this paper we describe details of this work.



1 saturated **2** Δ^2 (2E)
3 Δ^2 (2Z) **4** $\Delta^{2,4}$ (2Z,4E)



5

The *Sinularia* species, most likely *S. compacya* Tixier-Durivault 1970 (family Alcyoniidae, order Alcyonacea), see Experimental Section, was collected around the island of Nauru, frozen, and later extracted sequentially with MeOH and MeOH−$CHCl_3$ (1:1). The combined extracts were concentrated, and the residue (ED_{50} 0.4 μg/mL vs. P-388) was partitioned between organic solvents and aqueous MeOH to give a cytotoxic chloroform-soluble fraction. This fraction was subjected to high-speed centrifugal countercurrent chromatography using the upper phase of a $CHCl_3$−MeOH−*i*-PrOH−H_2O (9:12:1:8) mixture as the mobile phase. One of the fractions therefrom was further resolved by C18 reversed-phase HPLC using CH_3CN−MeOH−H_2O (2:5:4) as eluent to give a major component whose ^{1}H- and ^{13}C-NMR spectra were similar to those of acylated spermidines isolated earlier by our group from *S. brongersmai*.[2] Proton NMR signals corresponding to NMe groups appeared at δ 2.8−2.9, which indicated the amine groups were protonated and hence the fraction was partitioned between EtOAc and 10% aqueous KOH to give the free amine **5** in the organic layer.

The HREIMS of **5** supported the formula $C_{25}H_{51}ON_3$, and prominent ions were observed in the LREIMS at *m/z* 58 and 100 corresponding to $[CH_2N(CH_3)]^+$ and $[\text{cyclo}-(CH_2)_4-N(CH_3)_2-]^+$ ions, as might be expected from the methylated spermidine unit. The IR spectrum of **5** showed absorptions at 3294 (NH), 1645, and 1554 (C═O) cm^{-1} indicative of a secondary amide group. Only end absorption was noted in the UV spectrum. Signals in the ^{13}C NMR spectrum at δ 141.8 and 120.8 (both CH by DEPT analysis) confirmed the presence of one disubstituted double bond to account for all of the unsaturation in **5**. The amide carbonyl ^{13}C resonance was not observed in the spectrum of the free base, but was present in a spectrum of the salt form of **5**.

The ^{1}H NMR spectrum revealed the presence of three N−Me signals [2.18 (3H), 2.22 (6H)] which were shifted to ca. δ 2.8 (3H) and 2.9 (6H) in the native salt form of **5**. The ^{1}H−^{1}H COSY spectrum elucidated the spermidine moiety by revealing the following two spin-coupled systems: (a) NH signal at δ 6.72 (br t)/3.32 (CH_2 quart.)/1.74 (quint.)/2.43 (t) and (b) partially overlapping two-proton multiplets at δ 2.05 and 2.40, which were each coupled to a four-proton multiplet at δ 1.58−1.68.

The acyl portion of **5** was resolved from proton correlation spectra and single-frequency decouplings. The COSY spectrum revealed that a two-proton doublet at δ 2.92 was coupled to the olefinic two-proton multiplet at δ 5.48, but no correlation was observed from the olefinic proton signal to any other resonances, perhaps due to the very broad nature of the H-5 signal. In a relayed coherence transfer experiment, correlations were observed between the olefinic proton signal and a broad signal at ca. δ 2.1−2.2 as well as a methyl doublet signal at δ 0.97, consistent with the structure proposed for **5**. The δ 0.97 to 2.1−2.2 correlation was also obvious in the COSY spectrum. Addition of a small amount of C_6D_6 to the $CDCl_3$ solution induced a resolution of the olefinic signal into a more distinct set of lines such that when the doublet at δ 2.92 was irradiated, the residual olefinic signal was clearly recorded as a doublet (J = 15 Hz) at δ 5.48 and a double doublet (J = 15, 6.6 Hz) at δ 5.38. This confirms the *trans* configuration for the double bond. Irradiation of a broad, low-intensity signal overlapped by the NMe singlet at ca. δ 2.1 collapsed the methyl doublet resonance at δ 0.97 to a singlet and converted the olefinic signal to a broad doublet at δ 5.38 and complex multiplet at δ 5.48. The remaining proton signals in the NMR spectrum consisted of a methyl triplet at δ 0.88 and large, broad methylene singlet at δ 1.21−1.32 consistent with the *n*-C_9H_{19} unit in the final proposed structure **5**. In the protonated form of **5** not only are the ^{1}H NMR signals of the NMe protons shifted downfield, but all of the absorptions for methylene protons adjacent to amine nitrogens are overlapped at

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δ 3.20, while resonances for methylene protons in the spermidine unit that are not deshielded by a nitrogen appear as a broad peak at δ 2.0, and the amide NH occurs at δ 7.35. Minor shifts of proton resonances of methyl and methylene groups attached to amine nitrogens were noted in solution prepared at different times for NMR analysis. This is attributed to varying amounts of adsorbed water in the sample and DCl in the $CDCl_3$ used.

The carbon skeleton of the acid portion of **5** is elongated by one acetate unit compared to the acyl portion of **1**−**4**. The acid corresponding to the acyl group of **5** appears to be new, although the saturated analogue, 5-methyl tetradecanoic acid, has been reported.[12] Acylated spermidine **5** was cytotoxic to P-388 cells, ED_{50} 0.04 μg/mL, as were **1** and **2**.[2]

Experimental Section

General Experimental Procedures. NMR data were obtained at 300 MHz for ^{1}H and 75 MHz for ^{13}C in the solvents specified using a Varian XL-300 spectrometer. LRMS were obtained on a Hewlett-Packard 5985 mass spectrometer, and IR spectra were measured on a Bio Rad FTS-7 Fourier transform spectrometer. Countercurrent chromatography was carried out on a P.C. Inc., Ito Multi-Layer Coil instrument. Optical rotation was measured using a Rudolph Autopol III automatic polarimeter.

Animal Material. Specimens of sample 4-NA-85, probably *compacta*, Tixier-Durivault 1970, were collected off the island of Nauru in November 1985 at a depth of about 14 m and frozen shortly after collection. Voucher specimens are available at the Department of Chemistry and Biochemistry, University of Oklahoma (4-NA-85), and at the Museum and Art Gallery of the Northern Territory, Darwin, NT, Australia (voucher no. NMT 12423). The specimen was identified by Dr. P. Alderslade, Museum and Art Gallery of the Northern Territory, Darwin, NT, Australia. The colony morphology and the shape of the sclerites in both the surface and interior of the lobe indicate the sample could be from a colony of *S. compacta* Tixier-Durivault 1970. No material from the surface and interior of the base of the colony is present, so this cannot be confirmed. The holotype of *S. compacta* was collected in New Caledonia.

Extraction and Isolation. Freshly thawed specimens of the *Sinularia* sp. (1.3 kg, wet wt) were extracted sequentially with MeOH and MeOH−$CHCl_3$ (1:1). The combined extracts were concentrated *in vacuo* to give 68.7 g of residue that was cytotoxic to murine P-388 cells, ED_{50} 0.4 μg/mL. This extract was partitioned between *n*-hexane and 10% aqueous MeOH. The aqueous MeOH layer was diluted to 1:1 MeOH−H_2O and extracted with $CHCl_3$. A portion (1 g) of the cytotoxic $CHCl_3$ residue (7 g, ED_{50} 2.1 μg/mL vs. KB cells) was subjected to high-speed centrifugal countercurrent chromatography using the upper phase obtained from a $CHCl_3$−MeOH−*i*-PrOH−H_2O (9:12:1:8) mixture as the mobile phase. Fraction 11 (10-mL fractions) of 400 fractions was further resolved by C18 reversed-phase HPLC (9.4-mm × 250-mm column) using CH_3CN−MeOH−H_2O (2:5:4) as eluent, to give compound **5** as a salt. An EtOAc solution of this salt was washed with 10% aqueous KOH, the EtOAc layer dried over Na_2SO_4, and the solvent evaporated, to give **5** as a viscous oil (ca. 5 mg, ca. 0.002% of wet wt).

Compound 5: $[\alpha]_D$ + 7 (*c* 0.2, CH_2Cl_2); IR (film) ν 3294 (NH), 1645 (C=O), 1554 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J = 6.6 Hz), 0.97 (3H, d, J = 6.6 Hz), 1.21−1.32 (16H br s), 1.58−1.68 (4H, m), 1.68−1.80 (2H, quint., J = ~6 Hz), ~2.15 (br m, H-5), 2.18 (3H, s), 2.22 (6H, s), 2.25 (2H, m), 2.35 (2H, m), 2.43 (2H, t, J = 6.7 Hz), 2.92 (2H, br d, J = ~6 Hz, H-2), 3.32 (2H, quart., J = 6.6 Hz), 5.48 (2H, m, H-3,4), 6.72 (1H, br t); ^{13}C NMR ($CDCl_3$) δ (C=O not obsd) 141.77, 120.79, 58.61, 56.94, 55.31, 44.30, 41.42, 40.59, 38.07, 36.87, 36.65, 31.88, 29.76, 29.65, 29.32, 27.34, 25.94, 24.05, 23.92, 22.66, 20.37, 14.09; **5**-salt form δ 172.62 (s), 141.16 (d), 120.58 (d), 56.82 (t), 55.04 (t), 53.81 (t), 43.09 (2C, q), 40.13 (q), 40.09 (t), 36.82 (t), 36.49 (2C, d, t), 31.81 (t), 29.71 (t), 29.58 (2C, t), 29.25 (t), 27.23 (t), 24.17 (t), 22.58 (t), 21.83 (t), 21.33 (t), 20.23 (q), 14.03 (q); HREIMS *m/z* 409.40361 [M]$^+$ calcd for $C_{25}H_{51}ON_3$, 409.40321; LREIMS (70 ev) *m/z* (int) 409 (11), 394 (6), 365 (7), 351 (2), 337 (22), 323 (15), 310 (11), 280 (47), 157 (25), 143 (21), 129 (37), 100 (100), 85 (42), 84 (63), 83 (54), 71 (27), 58 (81).

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