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# MARINE NATURAL PRODUCTS: CYTOTOXIC SPERMIDINE DERIVATIVES FROM THE SOFT CORAL *SINULARIA BRONGERSMAI*[1]

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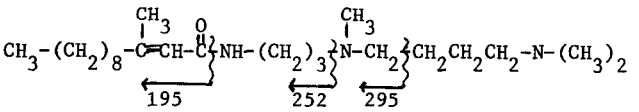
ABSTRACT: Two new cytotoxic spermidine derivatives isolated from the Pacific soft coral Sinularia brongersmai have been identified as $CH_3(CH_2)_8$-C($CH_3$)=CH-CO-NH$(CH_2)_3$-N$(CH_3)$-$(CH_2)_4$-N$(CH_3)_2$ and its dihydro analog by spectral methods combined with chemical conversions. Acid hydrolysis of the saturated amide followed by esterification yielded methyl 3-methyldodecanoate.

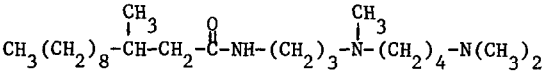
Soft corals (alcyonaceans) common in the Pacific Ocean have become noted for yielding sesqui- and diterpenoid metabolites, and a variety of new terpenoid skeletal classes have emerged from studies of these organisms.[2] Extracts of a number of these abundant coral reef coelenterates have also been found to exhibit interesting levels of cytotoxicity in a survey aimed at identifying marine sources of potential tumor-inhibitory agents.[3] This cytotoxic activity has been traced in several cases to cembranolides,[4] the most common diterpenoids found in soft corals and in a related order of octocorals, the gorgonians.[2] In contrast, we have found that the cytotoxicity of extracts of the alcyonacean *Sinularia brongersmai* from Korolevu, Fiji, is not due to cembranolides or other terpenoids, but rather to two new spermidine derivatives, 1 and 2. This letter describes their isolation and structure determination.

Partitioning of the aqueous alcohol extract of *S. brongersmai* resulted in concentration of the cytotoxic activity, successively, in the aqueous phase of a dichloromethane-water partition, the 1-butanol layer of a 1-butanol-water partition, and the chloroform layer of a chloroform-50% aqueous methanol partition. The chloroform-soluble material was triturated with acetone and the acetone-insoluble fraction was partitioned between 1-butanol-10% aqueous NaOH. The 1-butanol-soluble material was chromatographed over silica gel using methanol-conc. aqueous ammonia (9:1) to give one major cytotoxic fraction. This was distilled (130°C/10 microns) to give a clear, viscous oil that was shown by gc/ms analysis to be a ~9:1 mixture of two components (m/e 381, 383, respectively). After hydrogenation a single compound was obtained which corresponded by gc/ms analysis to the minor component (m/e 383) in the original mixture.

High resolution mass spectral analysis of the mixture established the formulas $C_{23}H_{47}N_3O$ and $C_{23}H_{49}N_3O$[5] for the two compounds, 1 and 2, respectively, and infrared data (3420, 3300, 1660, 1630 $cm^{-1}$) confirmed the presence of amide functionality. The $^1H$ NMR spectrum of the




1 (E isomer)

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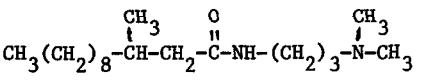


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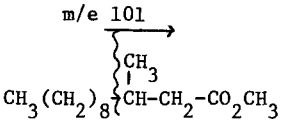


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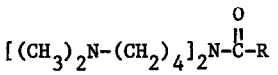
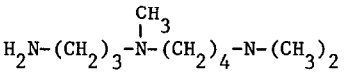



6 R = $-(CH_2)_{14}-CH_3$

7 R = $-CH=CH-(CH_2)_{12}-CH_3$

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mixture showed signals for an aliphatic chain, $CH_3-(CH_2)_{\sim 8}$ (0.90, t, 3H; 1.28, s, ∿13H), an isolated olefinic proton (δ5.55, s), a vinyl methyl group (2.16 and three N-methyl groups [δ2.2 in $CDCl_3$; shifted to δ2.88 upon addition of trifluoroacetic acid-d (TFA)]. The presence of three methylene groups attached to basic nitrogens was indicated by the shift of several multiplets (∿6H) from δ2.0-2.6 to 3.0-3.3 upon addition to TFA to the sample. The low field position of the vinyl methyl and olefinic proton signals suggested the partial structure $-C(CH_3)=CH-\overset{\beta}{\underset{}{C}}-$ and this could be expanded to $-C(CH_3)=CH-\overset{\beta}{\underset{}{C}}-NH-CH_2-CH_2-$ on the basis of confirmed coupling between the amide proton, triplet at δ6.92, and two methylene protons, quartet at δ3.38. Upon addition of $D_2O$ the amide proton signal disappeared and the δ3.38 signal was reduced to a triplet. A high resolution mass spectral fragment at m/e 195.17521 corresponding to $C_{13}H_{23}O$ (calcd 195.17489) indicated that the complete acyl group could be formulated as $CH_3-(CH_2)_8-C(CH_3)=CH-\overset{O}{\underset{}{C}}-$. Prominent fragment ions at m/e 252 and 295 containing 1 and 2 nitrogens, respectively, suggested the total structure 1 for the unsaturated amide (see proposed fragmentation indicated on the formula). The E configuration for the double bond is indicated by the position of the vinyl methyl signal, δ2.16, which is in good agreement with data observed[6] for a number of model α,β- unsaturated acids and esters.

Hot aqueous methanolic acid hydrolysis of the pure saturated amide[7] obtained by catalytic hydrogenation ($PtO_2$, EtOAc, 1 atm) of the mixture afforded an acid which was esterified ($CH_2N_2$) and identified as methyl 3-methyldodecanoate (3) by its $^1H$ NMR spectrum and comparison of its mass spectrum with published data, in particular the prominent peaks at m/e 73, 74 and 101 (weak m/e 87).[8] The base obtained from the hydrolysis exhibited a molecular ion at m/e 187 as expected for the N,N,N-trimethylspermidine 4[9] and was not further characterized.

The results of a Hofmann degradation of 2 confirmed the conclusion based on mass spectral evidence that the primary amide nitrogen is bonded to the trimethylene chain of the unsymmetrical spermidine molecule. Thus, the major Hofmann degradation product showed $M^+$ 298 corresponding to 5 as expected from loss of a N,N-dimethyltetramethylene segment from 2.

Comparable cytotoxicity was observed for the ∿9:1 mixture of 1 and 2 and for pure 2. The $ED_{50}$'s determined in three of the National Cancer Institutes' standard cell culture bioassays[10] are as follows: for the mixture, KB 1.0; PS 0.40; LE 0.30; for pure 2, KB 1.0; PS 0.37; LE 0.31.

This appears to be the first report of spermidine derivatives from marine organisms. A number of spermidine alkaloids, most incorporating spermidine into a macrocyclic lactam ring, have been isolated from plants in recent years.[11] Interestingly, the acyclic *sym-homo*-spermidine derivatives solapalmitenine (6) and solapalmitine (7),[12] like 1 and 2, are cytotoxic, whereas no cytotoxicity is reported for any of the macrocyclic lactam spermidine alkaloids.

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