Granulatamides A and B, Cytotoxic Tryptamine Derivatives from the Soft Coral *Eunicella granulata*

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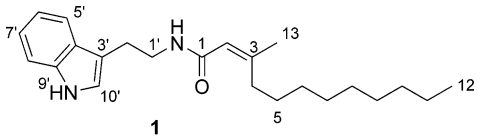
Two new tryptamine derivatives, granulatamides A (**1**) and B (**2**), were isolated from the 2-propanol extract of the soft coral *Eunicella granulata*. Their structures were determined on the basis of detailed spectroscopic analysis and comparison with previously published data for similar compounds. Both compounds showed moderate in vitro cytotoxicity against a panel of 16 human tumor cell lines.

Gorgonians have proved to be a rich source of structurally and pharmacologically diverse natural products.[1] Previous studies carried out on species of the genus *Eunicella* have revealed that the chemistry of this genus is dominated by the presence of eunicellin-type diterpenes, containing a cladiellane skeleton and a C-2, C-9 ether bridge. The presence of nitrogenated compounds in this genus is represented only by the isolation of two nucleosides with antiviral properties from *Eunicella cavolini*[2] and two eunicellin derivatives, labiatamides A and B, from *Eunicella labiata*.[3]

On the other hand, spermidine and tyramine amides of both the *E*- and *Z*-isomers of 3-methyldodec-2-enoic acid have been isolated from various samples of soft corals of the genus *Sinularia*.[4−7] Among the biological properties exhibited by these compounds, a marked cardiotonic activity for the tyramine derivatives[5] and antibacterial activity against *Pseudomonas aeruginosa* for the spermidine amides[6] have been described.

In the course of our screening program for the isolation of novel compounds with antitumor properties from marine sources, we have isolated the tryptamine derivative of the *Z*-isomer of the above-mentioned acid (**1**) together with a second structurally related compound (**2**) from a sample of *Eunicella granulata* Grasshoff (1992) (family Gorgoniidae, order Alcyonacea, suborder Holaxonia), collected by hand using scuba in Senegal.



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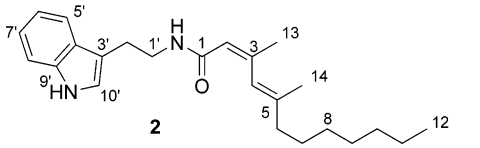

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Both compounds were obtained by bioassay-guided fractionation of the 2-propanol extract of specimens of this organism, using RP-18 column chromatography and semipreparative HPLC. Their structures were established by spectroscopic methods (mainly 1D and 2D NMR) and comparison with data reported for similar compounds. This contribution constitutes the first report of a (2*Z*,4*E*)-3,5-dimethyldodeca-2,4-dienoic acid derivative isolated from marine natural sources.

(+)-HRFABMS ($[M + H]^+$ *m*/*z* 355.2742) and NMR spectra accounted for a molecular formula of $C_{23}H_{34}N_2O$ for compound **1**. The presence of a tryptamine moiety in the molecule was immediately inferred by analysis of the signals and multiplicity patterns of the low-field region in the ^{1}H NMR spectrum [δ 7.61 (dd), 7.38 (dd) 7.19 (ddd), 7.12 (ddd), 7.04 (d)], two additional CH_2 signals at δ 3.63 (td) and 2.99 (t), signals for two NH protons at δ 8.10 (br s) and 5.41 (m) ppm, and correlations observed in the COSY and HMBC spectra. Closely related chemical shifts have been reported for other tryptamine derivatives isolated from natural sources.[8] The rest of the signals in the ^{1}H NMR were attributable to an olefinic proton at δ 5.43 (H-2), a vinyl methyl and an allylic methylene group [δ 1.78 (d) and 2.61 (t), respectively], and protons of a long aliphatic chain. An α,β-unsaturated amide carbonyl signal (δ_C 166.7), which correlated with H-2 in the HMBC spectrum, confirmed the "fatty acid" nature of this portion. The observed signals in both the ^{1}H and ^{13}C NMR spectra for this moiety were indicative of a 3-methyldodec-2-enoic acid derivative.[5] Indeed, HMBC correlations between Me-13 and C-2, C-3, and C-4 fixed the position of the methyl group at C-3. The geometry of the double bond was determined as *Z* by a NOESY correlation between H-2 and Me-13. In addition, the chemical shifts of the vinyl methyl (Me-13) and the allylic methylene group (H_2-4) in the ^{1}H NMR spectrum of granulatamide A were also in agreement with the stereochemistry proposed.[5] Finally, correlations from H_2-1′ and the NH proton at δ 5.41 ppm to the amide carbonyl signal at δ_C 166.7 in the HMBC spectrum established the placement of the tryptamine moiety as drawn in **1**.

(+)-HRFABMS of granulatamide B (**2**) gave an $[M + H]^+$ peak at *m*/*z* 367.2744, consistent with a molecular formula of $C_{24}H_{34}N_2O$ with nine degrees of unsaturation. Low-field ^{1}H NMR signals together with signals at δ_H 3.63 and 2.96 ppm confirmed the presence of a tryptamine moiety in the molecule as in **1**, accounting for six of the degrees of unsaturation. The other three unsaturations were attributed to a carbonyl group (δ_C 166.8) and two trisubstituted double bonds [δ_C 122.5 CH, 145.6 C, 122.9 CH, and 142.3 C; δ_H 5.67 (s) and 5.82 (br s)]. The rest of the signals in the ^{1}H NMR were assigned to two vinyl methyl groups (δ_H 1.85 and 1.55 ppm) and an aliphatic chain. The structural elucidation of this part of the molecule was accomplished by analysis of the HMBC and NOESY spectra (Figure 1). HMBC correlations from Me-13 to C-2, C-3, and C-4 and from Me-14 to C-4, C-5, and C-6 placed both methyl groups at C-3 and C-5, respectively. On the other hand, the stereochemistry of both double bonds was determined on the basis of correlations observed between H-2 and Me-13 and between H-4 and CH_2-6 in the NOESY spectrum. Although no HMBC correlation was observed to locate the carbonyl group, its chemical shift (δ_C 166.8), indicative of an α,β-unsaturation, fixed its position at

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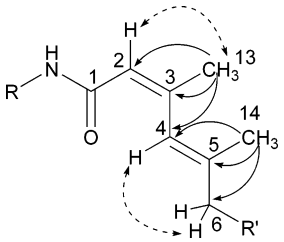

Figure 1. Key HMBC (H to C, solid line) and NOESY (dashed line) correlations for compound **2**.

Table 1. NMR Data for Compounds **1** and **2** ($CDCl_3$)[a]

	1		**2**	
no.	δ_C mult	δ_H mult (*J* in Hz)	δ_C mult	δ_H mult (*J* in Hz)
NH		8.10 br s		8.04 br s
1	166.7 C		166.8 C	
2	118.6 CH	5.43 d (1.5)	122.5 CH	5.67 br s
3	154.8 C		145.6 C	
4	33.0 CH_2	2.61 t (7.5)	122.9 CH	5.82 br s
5	28.3 CH_2	1.44 m	142.3 C	
6	29.8 CH_2	1.25 m	39.5 CH_2	1.82 t (7.3)
7	29.6 CH_2	1.25 m	27.8 CH_2	1.26 m
8	29.6 CH_2	1.25 m	29.3 CH_2	1.26 m
9	29.3 CH_2	1.25 m	29.2 CH_2	1.26 m
10	31.9 CH_2	1.25 m	31.8 CH_2	1.26 m
11	22.7 CH_2	1.25 m	22.7 CH_2	1.26 m
12	14.1 CH_3	0.88 t (7.0)	14.1 CH_3	0.88 t (7.0)
13	24.7 CH_3	1.78 d (1.5)	25.4 CH_3	1.85 s
14			17.7 CH_3	1.55 s
NH		5.41 m		6.16 m
1′	39.3 CH_2	3.63 td (6.7, 6.3)	39.5 CH_2	3.63 td (6.8, 5.9)
2′	25.4 CH_2	2.99 t (6.7)	25.4 CH_2	2.96 t (6.8)
3′	113.2 C		113.3 C	
4′	127.4 C		127.4 C	
5′	118.8 CH	7.61 dd (8.0, 1.0)	118.8 CH	7.58 br d (7.1)
6′	119.5 CH	7.12 ddd (8.0, 7.0, 1.0)	119.5 CH	7.11 ddd (8.1, 7.1, 1.0)
7′	122.2 CH	7.19 ddd (8.0, 7.0, 1.0)	122.2 CH	7.20 ddd (8.1, 7.3, 1.0)
8′	111.4 CH	7.38 dd (8.0, 1.0)	111.1 CH	7.37 dd (7.3, 1.0)
9′	136.4 C		136.4 C	
10′	122.0 CH	7.04 d (2.0)	121.8 CH	7.02 d (2.1)

[a] ^1H at 500 MHz, ^{13}C at 125 MHz.

C-1. Finally, the connection between the two moieties of the molecule was supported by an HMBC correlation between H_2-1′ and C-1.

Cytotoxicity assays were performed for granulatamides A and B against a panel of 16 human tumor cell lines.[9] Both compounds showed cytotoxicity in the μM range against all the lines tested (Table 2). Similar values have been reported for the structurally related compound hermitamide B,[8] confirming the cytotoxic properties of this structural class.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 881 infrared spectrophotometer. NMR spectra were recorded on a Varian "Unity 500" spectrometer at 500/125 MHz (^1H/^{13}C). Chemical shifts were reported in ppm using residual $CDCl_3$ (δ 7.26 for ^1H and 77.0 for ^{13}C) as internal reference. HMBC experiments were optimized for a $^3J_{CH}$ of 8 Hz. NOESY spectra were measured with a mixing time of 600 ms. (+)-HRFABMS was performed on a VGAutoSpec spectrometer employing a *m*-NBA matrix. ESIMS were recorded using an Agilent 1100 Series LC/MSD spectrometer.

Animal Material. *Eunicella granulata* was collected in April 2002 by scuba at a depth between 18 and 22 m at Madeleine Islands (Senegal) (14°39′04″ N, 17°28′28″ W). The material was identified by Dr. Pablo López from the University of Seville (Spain). A voucher specimen is deposited at PharmaMar (ORMA 005712).

Table 2. GI_{50} (μM) of Compounds **1** and **2** against Tumor Cell Lines

	GI_{50} (μM)	
cell line	**1**	**2**
DU-145	1.7	7.7
LN-caP	4.7	3.5
SK-OV-3	11.6	n.d.[a]
IGROV	6.7	8.2
IGROV-ET	12.7	6.7
SK-BR3	2.7	6.0
SK-MEL-28	3.9	10.6
HMEC1	6.2	n.d.
A549	6.7	8.9
K-562	6.8	4.3
PANC1	10.4	6.5
HT29	2.2	13.8
LOVO	10.5	8.6
LOVO-DOX	12.0	10.0
HeLa	n.d.	9.7
HeLa-APL	n.d.	9.0

[a] n.d. = not determined.

Extraction and Isolation. The frozen organism (500 g) was triturated and exhaustively extracted with 2-propanol (3 × 1.5 L). The extract was concentrated to yield an orange crude of 9.06 g. This material was partitioned in *n*-hexane against H_2O to yield a hexane active fraction of 4.05 g. This fraction was subjected to VLC on Lichroprep RP-18 with a stepped gradient from H_2O to MeOH.

A 240 mg portion of a fraction eluted with MeOH (557 mg) was subjected to semipreparative HPLC (SymmetryPrep C-18, 7.8 × 150 mm, H_2O/MeCN (3:7) for 5 min, then gradient to 100% CH_3CN in 15 min, UV detection, 2.3 mL/min). Compounds **1** (37.5 mg) and **2** (5.6 mg) were isolated from selected cytotoxic fractions of this chromatography by semipreparative HPLC (X-Terra RP-18 10 × 150 mm, isocratic H_2O/MeCN (4:6), UV detection, 3.7 mL/min).

Granulatamide A (1): pale yellow oil; IR (KBr) ν_{max} 3395, 3232, 2927, 2857, 1664, 1638, 1519, 1451, 1378, 1352, 1251, 1220 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz), see Table 1; ESIMS *m*/*z* 377 $[M + Na]^+$, 355 $[M + H]^+$; (+)-HRFABMS *m*/*z* 355.2742 $[M + H]^+$ (calcd for $C_{23}H_{35}N_2O$ 355.2749).

Granulatamide B (2): pale yellow oil; IR (KBr) ν_{max} 3301, 2925, 2855, 1641, 1599, 1526, 1456, 1375, 1299, 1257, 1213 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz), see Table 1; ESIMS *m*/*z* 389 $[M + Na]^+$, 367 $[M + H]^+$; (+)-HRFABMS *m*/*z* 367.2744 $[M + H]^+$ (calcd for $C_{24}H_{35}N_2O$ 367.2749).

Biological Activity. A colorimetric assay using sulforhodamine B reaction was adapted for a quantitative measurement of cell growth and viability following a literature description.[9] The in vitro activity of the compounds was evaluated against a panel of 16 tumor cell lines, including prostate (DU-145 and LN-caP), ovary (SK-OV-3, IGROV, and IGROV-ET), breast (SK-BR3), melanoma (SK-MEL-28), endothelium (HMEC1), NSCL (A549), leukemia (K-562), pancreas (PANC1), colon (HT29, LOVO, and LOVO-DOX), and cervix (HeLa and HeLa-APL).

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