11-Deoxyfistularin-3, a New Cytotoxic Metabolite from the Caribbean Sponge *Aplysina fistularis insularis*

Reinaldo S. Compagnone,*,† Ramona Avila,†,1 Alírica I. Suárez,‡ Odette V. Abrams,† Héctor R. Rangel,§ Francisco Arvelo,§ Ivette C. Piña,† and Elizabeth Merentes§

Centro de Química Orgánica, Escuela de Química Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47102, Caracas, Venezuela, Facultad de Farmacia, Universidad Central de Venezuela, Apartado 40109, Caracas, Venezuela, and Laboratorio de Cultivo de Tejido Animal, Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47114, Caracas, Venezuela

Received April 26, 1999

11-Deoxyfistularin-3 (**1**), a new bromotyrosine derivative, was isolated among other known compounds such as fistularin-3 (**2**), aerothionin (**3**), and 11-oxoaerothionin (**4**) from the Caribbean sponge *Aplysina fistularis* (Aplysinellidae). The structure of **1** was determined by spectroscopic analysis and showed in vitro activity against the human breast carcinoma cell line MCF-7.

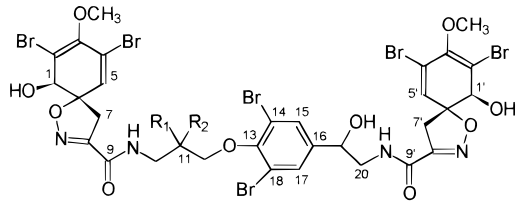
Sponge of the *Verongida* genera including *Verongula*, *Pseudoceratina*, *Ianthella*, and *Psammaplysilla* have been intensively studied due the presence of alkaloids with one, or more bromotyrosine residues.[2] Many of these metabolites show interesting antibiotic[3] and cytotoxic properties in cell lines.[4] Recently, particular interest has been given to the chemical analysis of these species because the presence of the bromotyrosine derivatives seems to be peculiar to the order and can be used as a chemical marker to support taxonomic work.[5] Previous reports on *Aplysina fistularis insularis* (Duchassaing & Michelotti) (family Aplisinidae) have documented the presence of a wide number of interesting metabolites, mainly of the bromotyrosine type. Examples of these brominated metabolites are the fistularins,[6] aerothionins,[7] ceratinamines,[8] aplysamines,[9] anamonian,[10] and psammaplysin[3].

Continuing a program of searching for new metabolites with potential biomedical interest from marine species collected in the Caribbean, we report in this paper the isolation of a new metabolite: 11-deoxyfistularin-3 (**1**) from *A. fistularis*. This sponge was collected by hand using SCUBA in Chichiriviche de la Costa, located on the central coast of Venezuela, at 20 m depth.

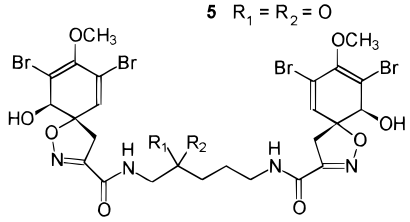
The MeOH/CH_2Cl_2 extract of *A. fistularis* was chromatographed on silica gel using a mixture of hexane/EtOAc. After preparative TLC purification of selected, combined fractions, 11-deoxyfistularin-3 (**1**) was isolated as a solid and identified by analysis of spectral data. Other known metabolites such as fistularin-3 (**2**),[6] aerothionin (**3**),[7] and 11-oxoaerothionin (**4**)[11] were also isolated and identified.

HRFABMS suggested the molecular formula $C_{31}H_{30}N_4O_{10}Br_6$ for **1**. The UV spectrum (λ_{max} 209, 235, 283 nm; ϵ 42 400, 21 600, 11 200) was similar to fistularin 3 (**2**). The IR spectrum of **1** was closely related to fistularin-3 and showed bands at 3417 cm^{-1} characteristic of NH and OH groups and at 1656 cm^{-1} typical of an amide group.

The ^1H NMR (Table 1) spectrum of **1** was very similar to that of **2**,[6] except for the absence of the signal corresponding to 11-CHOH group. Instead of the 11-CHOH group the presence of a multiplet at 2.11 ppm with J = 6.5 Hz due to methylene signals on 11-CH_2 was observed.



1 $R_1 = R_2 = H$
2 $R_1 = OH, R_2 = H$
5 $R_1 = R_2 = O$



3 $R_1 = R_2 = H$
4 $R_1 = R_2 = O$

The H−H COSY spectrum showed a correlation of 10-CH_2 and 12-CH_2 with 11-CH_2 indicating the presence of a 3 carbon chain between a tyrosine residue and the dibromophenoxy group. A similar H−H COSY correlation was found between 19-CHOH and 20-CH_2. The ^{13}C NMR spectrum of **1** was also quite similar to that of **2**[6] and differs only in the presence of a signal at 30.37 ppm as a triplet in the DEPT spectrum, corresponding to the 11-CH_2 instead of the 11-CHOH signal at 69.47 ppm. Additionally, chemical shifts were shifted from 76.13 and 43.95 ppm in **2**, to 71.51 and 37.13 ppm in **1**, corresponding to 12-CH_2 and 10-CH_2, respectively. Further proof to assign the position of the OH group was carried out by reduction of an authentic sample of the known compound 11-ketofistularin-3 (**5**)[6] isolated by us from *A. lacunosa*. The reduction of **5** was carried out using tosylhydrazine and sodium borohydride to give compound **1**. The spectral data of the reduction product of **5** matched our spectral data found for **1**. Combination of all the data allowed us to establish the structure of **1** as 11-deoxyfistularin-3 (Table 1).

1 was tested against the following cell lines: X-17, Hela, Hep-2, RD, Lovo, and MCF-7. Among all these cell lines, compound **1** was most cytotoxic against MCF-7 (human

* To whom correspondence should be addressed. Tel.: +58 2 6052236. Fax: 58 2 7534193. E-mail: rcompag@strix.ciens.ucv.ve.
† Centro de Química Orgánica.
‡ Facultad de Farmacia.
§ Instituto de Biología Experimental.

10.1021/np9901938 CCC: $18.00
Published on Web 09/03/1999

Table 1. ^{1}H (270 MHz, Pyridine-d_5) and ^{13}C NMR (67.5 MHz, Ppyridine-d_5) NMR Data[a] for 11-Deoxyfistularin (**1**) and Comparison with ^{13}C NMR Shift of Fistularin-3 (**2**) in Pyridine-d_5

	1[c]		**2**[b]
position	δ ^{1}H	δ ^{13}C	δ ^{13}C
1,1′	4.69 (1H, d, J = 7.8)	74.60 (d)	74.73 (d)
	4.66 (1H, d, J = 7.8)	74.67 (d)	
2,2′		121.66 (s)	121.80 (s)
3,3′		147.92 (s)	148.06 (s)
4,4′		115.16 (s)	115.20 (s)
5,5′	6.60 (1H, s)	132.15 (d)	132.36 (d)
	6.70 (1H, s)	132.31 (d)	
6,6′		91.72 (s)	91.87 (s)
		91.78 (s)	91.93 (s)
7,7′	4.50, 3.47 (2H, q, J = 18.3)	40.27 (t)	40.30 (t)
	4.44, 3.40 (2H, q, J = 18.3)		
8,8′		155.10 (s)	155.17 (s)
		155.23 (s)	155.25 (s)
9,9′		160.05 (s)	160.52 (s)
		160.44 (s)	
10	3.85 (2H, m)	37.13 (t)	43.95 (t)
11	2.27 (2H)	30.37 (t)	69.47 (d)
12	4.08 (2H, t)	71.51 (t)	76.13 (t)
13		152.27 (s)	152.29 (s)
14,18		118.35 (s)	118.42 (s)
15,17	7.92 (1H, s)	130.90 (d)	131.09 (d)
	7.91 (1H, s)		
16		143.35 (s)	143.52 (s)
19	5.32 (1H, dd, J = 7.4, 4.2)	70.70 (d)	69.47 (d)
20	3.99 (1H, m), 3.85 (1H,m)	47.99 (t)	48.15 (t)
OCH_3	3.64 (6H, s)	59.75 (q)	59.86 (q)
NHa	9.35 (1H, t, J = 5.7)		
NHa′	9.82 (1H, t, J = 5.9)		

[a] Table entries are chemical shifts, ppm from solvent (multiplicity, J in Hz). [b] See ref 6. [c] Assigments based on DEPT and ^{1}H−^{1}H COSY.

breast carcinoma) with a LD_{50} of 17 μg/mL. For the rest of the cell lines the LD_{50} of **1** exceeded 50 μg/mL.

Experimental Section

General Experimental Procedures. IR spectra (film) were recorded on a Nicolet Magna 560 spectrometer, UV spectra in MeOH were recorded on a Milton Roy Spectronic 3.000 array, and NMR spectra of CD_3OD and CD_5N solutions were recorded with a JEOL Eclipse 270 spectrometer operating at 270 MHz. Low-resolution mass spectra were measured by Dr. Robert B. Cody using a JEOL GCmat spectrometer at JEOL USA (11 Dearborn, Peabody, MA 01960); high-resolution mass spectrometry measurements were performed at UCR Mass Spectrometry Facility, Department of Chemistry, University of California—Riverside. Optical rotation was measured in a Carl Zeiss instrument, Na lamp, 590 Å.

Animal Material. The marine sponge *A. fistularis insularis* was collected by hand using SCUBA in a locality named Chichiriviche de la Costa on the Central Coast of Venezuela at a depth of 20 m and immediately frozen. The sponge was identified as *A. fistularis insularis* by Prof. Sheila Marques-Pauls of Instituto de Zoologia Tropical (IZT), Facultad de Ciencias, Universidad Central de Venezuela. A voucher of this species is kept in IZT Museum (no. 96004).

Extraction and Identification. Small pieces (187 g) of the marine sponge *A. fistularis insularis* previously lyophilized were extracted three times with (1 L) $MeOH/CH_2Cl_2$. The extracts were combined and concentrated under vacuum to give a green, oily residue (29.4 g). A portion of this residue (6.05 g) was chromatographed on a silica gel column (200 g) using a hexane/AcOEt step gradient ranging from 90:10 to 0:100 followed by a AcOEt/MeOH step gradient ranging from 100:0 to 30:70 to give many (15 mL) fractions. The first collected fractions were mostly complex mixtures of fatty acids and lipids. The fraction (800 mg) eluted with 30:70 hexane/AcOEt was subjected to a preparative TLC ($CHCl_3$/MeOH, 90:10) to give 4 bands which were identified in decreasing R_f order as 11-deoxyfistularin (**1**, 12 mg), aerothionin (**3**,[7] 112 mg), fistularin-3 (**2**,[6] 22 mg), and 11-oxoaerothionin (**4**,[11] 60 mg). Comparison of reported ^{1}H and ^{13}C NMR data for the known compounds matched with the obtained data.

11-Deoxyfistularin-3 (1): white powder; mp 128−130 °C $[\alpha]^{25}_D$ +194.17° (c = 4.12 g/100 mL, MeOH); UV λ_{max} (MeOH) 209 nm (ϵ 42 000), 235 (ϵ 21 600), 283 (ϵ 11 200); IR (film) 3650−3000, 1656, 1315, 1275 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 1; HRFABMS (nitrobenzyl alcohol) m/z 1120.6906 $[M + Na]^+$ (calcd for $C_{31}H_{30}N_4O_{10}Br_6Na$, 1120.6898).

Reduction of 11-Ketofistularin-3 (5) To Form 11-Deoxyfistularin-3 (1).[12] To a solution of 11-ketofistularin-3 (**5**, 9.5 mg, 0.008 mmol) in MeOH (3 mL) was added tosylhydrazine (2 mg, 0.01 mmol) and *p*-toluensulfonic acid (2 mg). This solution was stirred at room temperature for 24 h. After the formation of the tosylhydrazone, cyanoborohydride (2.3 mg, 0.03 mmol) was added and the reaction was followed by TLC. After 48 h the solvent was evaporated and EtOAc (5 mL) was added. This solution was extracted with water (2 × 5 mL), and the organic phase was concentrated in a vacuum and separated by HPLC using a C_{18} column (5 μm) with MeOH/H_2O. Both the retention time of the product and its ^{1}H and ^{13}C spectra matched those of **1**.

Bioassay. MCF-7 (human breast carcinoma) cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 100 units/mL penicillin, and 2 μg/mL of amphotericin B in a 5% CO_2 atmosphere. The cells were harvested from a 25 cm^2 culture flask after trypsinization and seeded into 96-microwell plates. After 24−48 h of incubation at 37 °C the cells were observed to determine semiconfluence. The old medium was then removed and fresh medium containing the metabolite was added. After 96 h of exposure at different concentrations of the metabolite (2.5−25 μg/mL) these cells were used for cytotoxicity evaluation with the MTT method using tetrazolium salt[13] at a final concentration of 0.4 μg/mL in culture medium. After an incubation time of 3 h with MTT the formazan product was dissolved in DMSO and the plate was read in an automatic plate reader at 570 nm.

Acknowledgment. The assistance of the Armada de Venezuela (Venezuelan Navy) and Grupo BIOSUB of Facultad de Ciencias, UCV in the collection of specimens is gratefully appreciated. We thank CONICIT (Grant No. S1 95000569) for financial support in this study. We also thank Dr. José D. Medina of IVIC for assistance and helpful comments on this work.

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NP9901938