Fistularin 3 and 11-Ketofistularin 3. Feline Leukemia Virus Active Bromotyrosine Metabolites from the Marine Sponge Aplysina archeri

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FISTULARIN 3 AND 11-KETOFISTULARIN 3. FELINE LEUKEMIA VIRUS ACTIVE BROMOTYROSINE METABOLITES FROM THE MARINE SPONGE *APLYSINA ARCHERI*

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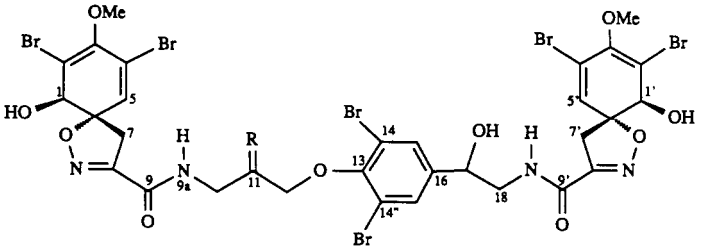
ABSTRACT.—Two brominated tyrosine metabolites, fistularin 3 [**1**] and a new compound 11-ketofistularin 3 [**2**] have been isolated from a marine sponge, *Aplysina archeri*. Their structures were determined on the basis of spectral data. Both compounds inhibited the growth of feline leukemia virus.

Bromotyrosine-derived metabolites are distinct markers for the marine sponges belonging to the order Verongida (1). Examples of these brominated metabolites are fistularins 1–3 (2), 11,19-dideoxyfistularin 3 (3), aerothionins (4–6), psammaplysin A (7,8), bastadin 1 (9), anamonian A (10), ceratinins A and B (11), aplysamines 1 and 2 (12), and a series of *Aplysina thiona* compounds (13). We now report another addition to the bromotyrosine family of compounds.

The MeOH/toluene extract of *Aplysina archeri* (Higgin) of the family Aplysinidae showed antiviral activity against feline leukemia virus. Bioassay-guided Si gel chromatography of EtOAc-soluble material from an MeOH/toluene extract followed by reversed-phase hplc furnished the antiviral constituents fistularin 3 [**1**] and 11-ketofistularin 3 [**2**]. Fistularin 3 was identified by analysis of spectral data, and its structure was confirmed by nmr (^{1}H and ^{13}C) and by tlc comparison with an authentic sample.

Hrfabms suggested the molecular formula $C_{31}H_{28}Br_6N_4O_{11}$ for metabolite **2**. The uv spectrum (λ max 283, 242, 225 nm; ε 9900, 14000, 27000) showed absorptions similar to those for fistularin 3 [**1**] (10). The ir spectrum has bands characteristic of NH and OH (3600–3000 cm^{-1}), keto group (1720 cm^{-1}) and α-iminoamide (1660, 1590, 1525 cm^{-1}) (2). Comparison of ^{1}H- and ^{13}C-nmr spectra of **2** with those of fistularin 3 (Table 1) indicated a close similarity. The ^{1}H-nmr spectrum of **2** indicated an absence of signals corresponding to the 11-CHOH group in fistularin 3. Further, the ^{1}H-nmr spectrum of **2** indicated an ABX pattern (X = N-H) for the 10-CH_2 group and a 2H singlet for the 13-CH_2 group. Oxidation of the 11-CHOH group in **1** accounts for these changes in **2**. Similarly, the ^{13}C-nmr spectrum of **2** showed a carbonyl signal at δ 201.34 instead of the 11-CHOH signal at δ 69.47 which is observed in **1** (Table 2). Combination of these data established the structure of **2** as 11-ketofistularin 3.

Fistularin 3 and 11-ketofistularin 3 gave feline leukemia virus activities of



1 R=H, OH
2 R=O

TABLE 1. ^{1}H nmr Data[a] for Fistularin 3 [**1**] and 11-Ketofistularin 3 [**2**] in Pyridine-d_5.

Proton	Compound	
	1	**2**
11′-OH	8.62 (1H, d, J = 7.9)	8.60 (2H, br s)
	8.61 (1H, d, J = 7.9)	
H-1, -1′	4.61 (1H, d, J = 7.9)	4.65 (s)
	4.58 (1H, d, J = 7.9)	4.64 (s)
H-5, -5′	6.62 (s)	6.62 (s)
	6.63 (s)	6.58 (s)
H-7, -7′	4.43, 3.47 (2H, ABq, J = 18.2)	4.45, 3.47 (2H, ABq, J = 18.2)
	4.40, 3.44 (2H, ABq, J = 18.2)	4.44, 3.40 (2H, ABq, J = 18.2)
H-9a	9.34 (1H, t, J = 5, 8)	9.80 (1H, t, J = 5.8)
H-9′a	9.71 (1H, t, J = 5.8)	9.71 (1H, t, J = 5.8)
H-10	4.25 (1H, m)	4.97 (2H, ABX, J = 18.0, 5.8)
	3.98 (1H, m)	
H-11	4.76 (1H, m)	
11-OH	7.23 (1H, d, J = 5.2)	
H-12	4.34 (1H, dd, J = 8.8, 5.5)	4.82 (2H, s)
	4.26 (1H, m)	
H-15, -15″	7.93 (2H, s)	7.91 (2H, s)
H-17	5.29 (1H, dt, J = 4.3, 5.8)	5.30 (1H, dd, J = 7.5, 4.6)
17-OH	7.77 (1H, d, J = 4.3)	
H-18	3.97 (1H, m)	3.97 (1H, ddd, J = 5.8, 13.3, 4.6)
	3.81 (1H, ddd, J = 15.3, 5.8, 4.3)	3.81 (1H, ddd, J = 5.8, 13.3, 7.5)
OMe	3.63 (6H, s)	3.63 (6H, s)

[a]Table entries are chemical shift, ppm from solvent (multiplicity, J in Hz).

ED_{50} 22 μM (4.8 μg/200 μl) and 42 μM (9.3 μg/200 μl), respectively. The highest concentrations tested for cytotoxicity against FeLV were 100 μg/200 μl, and neither compound was toxic at this dosage. The compounds were less active than 3′-azido-3′-deoxythymidine (AZT) but were comparable to 2′,3′-dideoxycytidine (ddCyd) in these assays (Table 3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—A uv spectrum was taken with a Perkin-Elmer Lambda 3 B uv/visible spectrophotometer. An ir spectrum was obtained on a Perkin-Elmer 1310 spectrophotometer. Nmr spectra were obtained on a Bruker instrument operating at 360 MHz for ^{1}H and 90.5 MHz for ^{13}C. The high resolution mass spectrum was obtained on a Kratos MS80-RFA mass spectrometer. Optical rotation was measured with a Jasco DIP 360 digital polarimeter.

EXTRACTION AND ISOLATION.—The marine sponge *A. archeri* was collected by scuba on a fore reef escarpment in Fernandez Bay, San Salvador Island, Bahamas at a depth of 30 m. A voucher specimen is deposited in the Harbor Branch Oceanographic museum (catalog no. 003:00174).

The freshly thawed sponge (50 g, wet wt) was extracted three times with MeOH-toluene (3:1). The concentrated extract was then partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (280 mg) was chromatographed on Si gel (Kiesel gel 60 H) using hexane/CH_2Cl_2 step gradient followed by CH_2Cl_2/MeOH step gradient. The fraction that eluted with 5% MeOH/CH_2Cl_2 (87 mg) on reversed-phase hplc (C-18, 5μ, 250 × 10 mm) with 30% H_2O/MeOH gave fistularin 3 [**1**] and 11-ketofistularin 3 [**2**].

Fistularin 3 [**1**].—Pale yellow solid (4.0 mg, 0.008% from frozen sponge); $[\alpha]^{26}D$ 102° (c = 0.1, MeOH) [lit. (2) $[\alpha]$ = 104.2°]; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

11-Ketofistularin 3 [**2**].—Pale yellow gummy solid (8.3 mg, 0.017%); $[\alpha]^{26}D$ 130° (c = 0.1, MeOH); ir (KBr) 3600–3200, 1720, 1660, 1590, 1525 cm^{-1}; uv λ max (MeOH) 283 nm (ε 9900), 242 (14,000), 225 (27,000); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2. Hrfabms (nitrobenzyl alcohol) m/z (most intense ion within a cluster of ions $[M+H]^+$) 1112.6897, Δ 0.6 mmu for $C_{31}H_{29}{}^{79}Br_3{}^{81}Br_3N_4O_{11}$.

IN VITRO TESTING.—The in vitro replication

TABLE 2. ^{13}C nmr Data[a] for Fistularin 3 [1] and 11-Ketofistularin 3 [2] in Pyridine-d_5.

Carbon	Compound	
	1	2
C-1, -1′	74.73 (d)	74.67 (d)
C-2, -2′	121.80 (s)	121.85 (s)
C-3, -3′	148.06 (s)	148.06 (s)
C-4, -4′	115.20 (s)	115.20 (s)
C-5, -5′	132.36 (d)	132.30 (d)
C-6, -6′	91.87 (s)	91.87 (s)
	91.93 (s)	92.13 (s)
C-7, -7′	40.30 (t)	40.30 (t)
		40.16 (t)
C-8, -8′	155.17 (s)	155.18 (s)
	155.25 (s)	154.77 (s)
C-9, -9′	160.52 (s)	160.46 (s)
		160.56 (s)
C-10	43.95 (t)	47.49 (t)
C-11	69.47 (d)	201.34 (s)
C-12	76.13 (t)	76.32 (t)
C-13	152.29 (s)	151.22 (s)
C-14, -14″	118.42 (s)	118.06 (s)
C-15, -15″	131.09 (d)	131.10 (d)
C-16	143.52 (s)	144.23 (s)
C-17	69.47 (d)	70.70 (d)
C-18	48.15 (t)	48.01 (t)
OMe	59.86 (q)	59.86 (q)

[a]Assignments based on APT, DEPT, and XHCORR experiments.

of feline leukemia virus (FeLV) was performed by adapting FeLV from the feline lymphoma cell line FL74 to Crandell feline kidney (CRFK) cells. CRFK cells (30,000/well in 96-well tissue culture plate) were allowed to adhere to plastic for 24 h. After treating CRFK cells with diethylamino ethyldextran (25 μg/ml), FeLV and test compounds were added and plates were incubated for 24 h. Fluids were replaced with new medium and compounds to remove any virus in supernatant fluids. After a 5-day incubation period cytotoxicity was determined by treating controls with MTT, and after 7 days supernatant fluids were tested for the viral protein p27 by an ELISA assay utilizing a monoclonal antibody to p27. The 50% inhibitory dose (ID_{50}) for toxicity of the compounds was defined as that concentration which caused approximately a 50% decrease in optical density compared to normal cells. Antiviral activity was measured by the failure of infected CRFK cells to produce p27 at non-toxic concentrations, indicating suppression or inhibition of viral replication. The antiviral activity was expressed as the 50% effective dose which was the drug concentration (CRFK cells + virus + drug) required to reduce the level of p27 by 50% compared to viral control (CRFK cells + virus).

TABLE 3. Comparison of Feline Leukemia Virus Activity of Compounds **1** and **2** to AZT and ddCyd.

Compound	$ED_{50}\mu M$[a]	$ID_{50}\mu M$[b]	Ratio[c]
Fistularin 3 [1]	22	>449	>20
11-Ketofistularin 3 [2]	42	>449	>11
AZT[d]	0.10	1517	15,170
ddCyd[e]	15	1990	133

[a]Effective dose required to reduce viral proliferation by 50%.
[b]Inhibitory dose required to reduce cell viability by 50%.
[c]Ratio of ID_{50} to ED_{50}, therapeutic index.
[d]3′-Azido-3′-deoxythymidine, Burroughs Wellcome Co.
[e]2′,3′-Dideoxycytidine, Hoffmann-LaRoche, Inc.

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ERRATUM

For the paper by Ando *et al.* entitled "Studies on the Synthesis of Sesquiterpene Lactones, 12. Synthesis of (+)-Colartin, (+)-Arubusclin A, and Their C-4 Epimers and Their Biological Activities," *J. Nat. Prod.*, **54**, 1017 (1991), the concentration of g/ml in Table 1 should be corrected to μg/ml for all entries.