Isolation of Spongosine and 2'-Deoxyspongosine from a Western Australian Sponge of the Order Hadromerida (Tethyidae)

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ISOLATION OF SPONGOSINE AND 2′-DEOXYSPONGOSINE FROM A WESTERN AUSTRALIAN SPONGE OF THE ORDER HADROMERIDA (TETHYIDAE)

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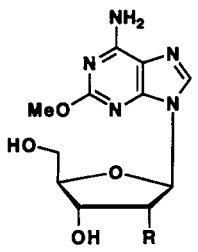
ABSTRACT.—The nucleoside, 9β-(2-deoxy-D-ribofuranosyl)-2-methoxyadenine [**2**], previously unknown as a natural product, has been isolated from a sponge in addition to the known nucleoside 9β-D-ribofuranosyl-2-methoxyadenine (spongosine) [**1**]. Thymine and 2-methoxyadenosine were also isolated from this sponge as a mixture.

Nucleosides with biological activity have been isolated from a variety of marine sources (1,2). The potent antifungal activity demonstrated by toyocamycin (3,4) prompted our investigation of nucleosides from sponges. We have examined a sponge collected from Western Australia, and have isolated two nucleosides, spongosine [**1**], originally reported by Bergmann from *Cryptotethya crypta* (Hadromerida) (5–7), and 2′-deoxyspongosine [**2**]. Compound **2** has previously been obtained as a synthetic product (8), but this is the first report of it as a natural product. We also present herein completely assigned nmr spectral data for both **1** and **2** for the first time.

The orange sponge (93-08-112; order Hadromerida, family Tethyidae) was collected in January 1993, at Exmouth Gulf, Western Australia. Freeze-dried animals were extracted with MeOH and the extract partitioned against organic solvents according to a modified Kupchan scheme (9). The $CHCl_3$ and *n*-BuOH fractions exhibited distinctive signals in their ^{1}H-nmr spectra suggesting nucleosides and tlc indicated only three polar, uv active spots. These were separated by flash chromatography to afford **1** (0.43% dry wt of animal), **2** (0.39%) and a third mixed fraction, all as white solids.



1 R=OH
2 R=H

Compound **2** was crystallized from H_2O as a microcystalline white solid with mp 174–175°, $[\alpha]_D$ −22.5° (c=1.0, DMSO), *m/z* 282.1178 MH^+, Δ mmu 2.4 for $C_{11}H_{16}N_5O_4$. Examination of the ^{1}H-nmr and COSY spectra in DMSO-d_6 (Table 1) revealed two substructures. A 2-deoxyribose was suggested by analysis of the COSY spectrum which revealed a contiguously coupled spin system from H-1′ through to H-5′, including most notably an upfield methylene group H-2′, δ 2.22 (1H, ddd, J=13.4, 7.8, and 2.7 Hz) and 2.73 (1H, ddd, J=13.2, 6.2, and 5.8 Hz). The remainder of the ^{1}H-nmr spectrum consisted of a methoxyl signal at δ 3.80 (3H, s), a sharp downfield one-proton signal at δ 8.12 (s, H-8), and a broad NH_2 signal at δ 7.30 (NH_2-6), suggestive of a 2-, methoxyadenosine. Full nmr assignments were made possible through 2D HETCOR and COLOC experiments (Table 1). In the COLOC spectrum both the NH_2 protons and H-8 showed long-range correlations to C-5, δ 115.7 (s). The methoxyl protons δ 3.80 (s) showed a three-bond correlation to C-2 at δ 161.7 (s) and thus the base is 2-methoxyadenine. A 2-methoxyadenine nucleoside was also supported by the fabms which showed the expected fragmentation pattern and the base peak at

m/z 166 (MH^+, 2-methoxyadenine). The structure of **2** was confirmed by comparison with an authentic sample of the synthetic material (8). In our hands the synthetic material exhibited mp 173–174° [lit. (8) mp 173–174.5°], $[\alpha]_D$ −22.0° (c=0.41, DMSO), and identical ^{1}H- and ^{13}C-nmr data to those of the natural product.

Compound **1** was crystallized from H_2O as an amorphous white solid: mp 190–191°, $[\alpha]_D$ −57.5° (c=1.0, DMSO), *m/z* 298.1158, MH^+, Δ mmu 0.7 for $C_{11}H_{16}N_5O_5$. The ^{1}H- and ^{13}C-nmr data for **1** (Table 1) were similar to those of **2**, however COSY revealed the sugar in **1** was ribose, rather than 2-deoxyribose. Complete nmr asignments were made using COSY and HETCOR experiments. As far as we are aware, complete nmr data for **1** have not been published. Comparison of physical data for compound **1** with the published data for spongosine [lit. (7) mp 192–193°; $[\alpha]_D$ −43.5°, c=0.46, 8% aqueous NaOH] confirmed the structure.

The third mixed fraction predominantly contained thymine, as shown by comparison with reported ^{1}H- and ^{13}C-nmr data (10). ^{1}H-Nmr indicated that a minor component of this fraction (ca. 10%), was 2-methoxyadenine, but further separation was not attempted. Despite the large amount of thymine present in the sponge, no thymine nucleosides were detected in the extract.

Compounds **1** and **2** were inactive against *Candida albicans, Saccharomyces carlsbergensis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* in the disk diffusion assay at 200 μg/disk.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a General Electric QE-300 spectrometer operating at 300 MHz for ^{1}H and 75.4 MHz for ^{13}C. ^{1}H nmr and ^{13}C nmr were referenced to $(CD_3)_2SO$ solvent signals at 2.49 ppm and 39.5 ppm, respectively. Multiplicities of ^{13}C-nmr spectra were assigned by DEPT experiments. Standard pulse sequences were employed for DEPT, magnitude COSY, HETCOR, and COLOC experiments. Mps were determined using an Electrothermal apparatus. Optical rotations

TABLE 1. Nmr Data for Nucleosides **1** and **2**.[a]

Position	Spongosine [1]		2′-Deoxyspongosine [2]		
	δ_C (mult.)	δ_H (integral, mult., J Hz)	δ_C (mult.)	δ_H (integral, mult., J Hz)	COLOC
2	161.7 (s)		161.7 (s)		
4	151.0 (s)		150.7 (s)		
5	115.7 (s)		115.7 (s)		
6	156.8 (s)		156.8 (s)		
6-NH_2		7.32 (2H, br s)		7.30 (2H, br s)	C-5
8	138.7 (d)	8.14 (1H, s)	138.3 (d)	8.12 (1H, s)	C-4, C-5
2-OMe	54.1 (q)	3.81 (3H, s)	54.0 (q)	3.80 (3H, s)	C-2
1′	87.5 (d)	5.78 (1H, d, 6.2)	83.6 (d)	6.25 (1H, dd, 7.8, 6.2)	
2′	73.1 (d)	4.62 (1H, ddd, 6.2, 6.2, 4.8)	39.1 (t)	2.22 (1H, ddd, 13.2, 7.8, 2.7) 2.73 (1H, ddd, 13.2, 6.2, 5.8)	
2′-OH		5.41 (1H, d, 6.2)			
3′	70.7 (d)	4.15 (1H, ddd, 4.8, 4.6, 3.2)	71.0 (d)	4.41 (1H, dddd, 5.8, 4.0, 2.7, 2.7)	
3′-OH		5.18 (1H, d, 4.6)		5.30 (1H, d, 4.0)	
4′	85.6 (d)	3.92 (1H, ddd, 4.1, 4.1, 3.2)	87.8 (d)	3.85 (1H, ddd, 4.6, 4.6, 2.7)	
5′	61.8 (t)	3.53 (1H, ddd, 12.0, 6.0, 4.1) 3.65 (1H, ddd, 12.0, 4.1, 3.9)	62.0 (t)	3.51 (1H, ddd, 11.6, 5.0, 4.6) 3.60 (1H, ddd, 11.6, 5.0, 4.6)	
5′-OH		5.16 (1H, dd, 6.0, 3.9)		5.04 (1H, t, 5.0)	

[a]300 MHz (^{1}H), 75.4 MHz (^{13}C), DMSO-d_6 referenced to solvent signals at 2.49 ppm and 39.5 ppm. ^{1}H-^{1}H coupling constants determined with the aid of single-frequency homonuclear decoupling experiments. COLOC experiment optimized for J_{C-H}=8 Hz.

were measured on a Jasco DIP-370 digital spectropolarimeter. Uv spectra were recorded on a Hewlett-Packard 8450A spectrophotometer. Ir spectra were recorded on Mattson Galaxy 3000 Fourier transform instrument at 4 cm^{-1} resolution. Mass spectra were provided by the University of Minnesota Chemistry Department Mass Spectrometry Service Laboratory. Tlc was carried out on 0.2 mm silica $60F_{254}$ plates (Merck 9735), and visualized with 1% vanillin/EtOH/H_2SO_4. All solvents were distilled in glass before use.

SPONGE COLLECTION AND IDENTIFICATION.—The orange sponge (93-08-112, order Hadromerida, family Tethyidae) was collected in January 1993 by hand using scuba at a depth of −10 m at Exmouth Gulf, Western Australia. The animals were immediately frozen at −20° until required. The body is massive, globular (approximately 15 cm) and has a bright orange exoderm (2–3 mm) with a thin superficial layer of sediment. The surface is covered with low rounded tubercules and the cortex is ill-defined. The megascleres are anisostrongyloxea (450–1700 μm) arranged in stout radiate bundles. The oxyspherasters are most unique and characteristic (60–70 μm), always with six rays terminally branched 4–5 times. Other megasters present are oxyasters (30–35 μm) and the micrasters are acanthotylasters (12–16 μm). From Sarà's recently published key (11), the sponge best fits the new genus *Tectitethya*. Voucher specimens are archived at the Department of Chemistry, University of California, Davis and the Benthic Invertebrate Collection, Scripps Institution of Oceanography, La Jolla (registry number P1148).

EXTRACTION AND ISOLATION.—Lyophilized animals (97.2 g) were extracted with MeOH (3×600 ml) and filtered. The extracts were combined, concentrated to approximately 200 ml, and successively extracted using a modified Kupchan partition (9) as follows. The water content (% v/v) of the MeOH extract was adjusted prior to sequential partitioning against *n*-hexane (10% v/v H_2O), CCl_4 (20%), and $CHCl_3$ (40%). The aqueous phase was concentrated to remove MeOH then extracted with *n*-BuOH. ^{1}H-Nmr spectroscopy indicated the presence of nucleosides in the $CHCl_3$ (246 mg) and *n*-BuOH (4.03 g) extracts. A portion (1.70 g) of the *n*-BuOH extract was pre-absorbed onto Si gel from MeOH and purified by flash chromatography (Si gel, $CHCl_3$-MeOH, 90:10, then $CHCl_3$-MeOH, 85:15) to afford three fractions, in order of elution as follows. The first fraction was identified by ^{1}H nmr as a 9:1 mixture of thymine and 2-methoxyadenine (327.1 mg). The next two fractions to elute were separately crystallized from H_2O to give pure 2′-deoxyspongosine (**2**, 161.1 mg) and spongosine (**1**, 176.5 mg).

9β-D-Ribofuranosyl-2-methoxyadenine (spongosine) [**1**].—$C_{11}H_{15}N_5O_5$; white amorphous solid; mp 190–191° (from H_2O); $[\alpha]_D$ −57.5° (*c*=1.0, DMSO); uv (MeOH) λ max 253 (sh), 267 (ε 14200) nm; ir (KBr disk) ν max 3490, 3455, 3326, 1645, 1600 cm^{-1}; ^{1}H and ^{13}C nmr, see Table 1; fabms *m/z* 298 (MH^+, 42), 279 (16), 220 (18), 205 (11), 178 (12), 166 (72); hrfabms found *m/z* 298.1158 (MH^+), $C_{11}H_{16}N_5O_5$ requires 298.1151.

9β-(2-Deoxy-D-ribofuranosyl)-2-methoxyadenine (2′-deoxyspongosine) [**2**].—$C_{11}H_{15}N_5O_4$; white amorphous solid; mp 174–175° (from H_2O); $[\alpha]_D$ −22.5° (*c*=1.0, DMSO); uv (MeOH) λ max 253 (sh), 267 (ε 13700) nm; ir (KBr disk) ν max 3450, 3390, 3355, 1650, 1600 cm^{-1}; ^{1}H and ^{13}C nmr, see Table 1; fabms *m/z* 282 (MH^+, 68), 220 (32), 205 (17), 192 (13), 178 (9), 166 (100); hrfabms found *m/z* 282.1178 (MH^+), $C_{11}H_{16}N_5O_4$ requires 282.1202.

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