Purine and Nucleoside Metabolites from the Antarctic Sponge *Isodictya erinacea*[1]

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The bright yellow sponge *Isodictya erinacea* is one of several chemically defended sponges found on the benthos of McMurdo Sound, Antarctica. An investigation of the metabolites from this sponge has resulted in the isolation of purine and nucleoside metabolites, including the previously unreported erinacean (**1**) and *p*-hydroxybenzaldehyde. The latter metabolite has been demonstrated to cause a feeding deterrence behavior in *Perknaster fuscus*, the major predator of antarctic sponges.

Sponges from McMurdo Sound, Antarctica, have been the subject of several recent chemical investigations.[2−12] Our own studies[9−11] are motivated by an interest in the functional roles[13] played by natural products in the ecological interactions of sponges with other members of the benthic community. Predation, in particular, is a functional role with the potential to influence and is influenced by the presence of secondary metabolites. While fish predation on sponges is thought to drive the development of deterrent natural products in temperate and tropical climates,[14] in McMurdo Sound, predation on sponges by sea stars is the dominant ecological factor driving the production of protective compounds.[15] We report here the results of our investigation of the McMurdo Sound sponge *Isodictya erinacea*, one of several conspicuous sponges on the antarctic benthos. *I. erinacea* lacks physical protection such as spicules and mucus, leading to the suggestion that the sponge is chemically defended.[15] No chemical investigations of *I. erinacea* have been reported to date, though a report on the chemical ecology of some Antarctic sponges demonstrated extracts from this sponge elicit a defensive behavioral response from the major antarctic sponge predator, the sea star *Perknaster fuscus*.[16]

I. erinacea (Topsent, 1916; Family Esperiopsidae) was collected from the shallow waters of Ross Island in McMurdo Sound, Antarctica, during the austral summers of 1992 and 1993 at depths ranging from 110 to 125 ft. The freeze-dried sponge was extracted sequentially in hexane, chloroform, and methanol and the sea star deterrent[16] methanol extract fractionated on Sephadex LH-20 using methanol as eluent. The majority of LH-20 fractions displayed ^{1}H NMR signals indicative of nucleosides. Separation and purification of these fractions was achieved by reversed phase chromatography, resulting in the isolation of inosine, uridine, 2′-deoxycytidine, 1,9-dimethylguanine, 7-methyladenine, and a previously undescribed purine erinacean (**1**). While purine and nucleoside metabolites are not uncommon in sponges, having been first reported by Bergman in the 1950's,[17] both the variety of purines and nucleosides and the presence of uncommon purines in *I. erinacea* are noteworthy. Besides the previously unreported erinacean, 7-methyladenine has been reported from nature only once, in an unrelated Antarctic sponge,[9] while 1,9-dimethylguanine is known only as a synthetic compound.[18] The structures of the nucleosides were confirmed by comparison with authentic samples (Aldrich) and the methyl purines by comparison of their spectra with those previously published.[9,18]

Table 1. ^{1}H and ^{13}C NMR Data of Erinacean (**1**) (360 MHz, DMSO-*d*6)

position	^{13}C δ, m	^{1}H δ, m, *J* (Hz)	HMBC
2	150.8, d	8.04 s	C4, C6
4	147.3, s		
5	104.2, s		
6	145.4, s		
6-NH		6.45, t, 5.6	C6
7		11.29, s	C5, C8
8	152.6, s		
9		9.93, s	C4, C5, C8
10	36.0, t	3.60, q, 6.1	C11, C6, C12
11	34.1, t	2.54, t, 6.3	C10, C12
12	173.1, s		
12-OH		12.34, s	

The ^{1}H NMR spectrum of erinacean (**1**) (Table 1) in DMSO-d_6 revealed seven signals, three of which displayed coupling. The ^{13}C and DEPT NMR spectra (Table 1) showed the presence of eight carbons, including two aliphatic methylene signals at 36.0 and 34.1 ppm, one methine signal at 150.8 ppm, and five nonprotonated carbon signals ranging from 104.2 to 173.1 ppm. The high-resolution mass spectrum of erinacean supported a molecular formula of $C_8H_9N_5O_3$, in agreement with both the ^{1}H and ^{13}C NMR spectra described above.

Proton−proton and proton−carbon connectivity was determined by 2D NMR techniques. The COSY experiment supported a coupling relationship between the 2.54 ppm triplet (H_2-11) and the quartet at 3.60 ppm (H_2-10); the 3.60 ppm signal showed further coupling to an exchangeable proton at 6.54 ppm, resulting in an isolated spin system $XCH_2CH_2X'H$. The heteroaromatic nature of the remaining carbons was supported by their

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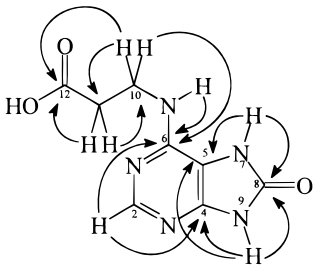

Figure 1. HMBC correlations observed in erinacean (**1**).

chemical shifts and warranted by consideration of the molecular formula. Correlations of the heteroaromatic carbons to the few nearby protons via HMBC spectroscopy allowed assignment of an 8-oxoadenine ring system (Table 1, Figure 1). Attachment of the isolated proton system to the adenine ring at C-6 was supported by observation of HMBC correlation from the 3.60 ppm (H_2-10) and 6.54 ppm signals (C_6NH) to C-6. The remaining connection, placing the C-12 carboxyl group at the terminus of the isolated proton spin system, was supported by HMBC correlations of both H_2-10 and H_2-11 to C-12. These assignments established the structure of erinacean (**1**) as N_6-(*β*-alanyl)-8-oxoadinine.

Erinacean (**1**) was evaluated for bioactivity. In addition to weak antibiotic activity (approximately 1 mm at 120 *μ*g/disk) against *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Escherichia coli*, erinacean displayed cytotoxicity (LD_{50} 50 *μ*g/mL) against L5178Y mouse lymphoblastoid cells. Neither erinacean, the other isolated purines, nor the nucleosides were active in the sea star deterrent assay;[16] the nature of the sea star deterrent substance in the sequential methanol extract of *I. erinacea* is currently under investigation.

Extracts from the sequential chloroform extract from *I. erinacea* also displayed sea star deterrent activity. Using the sea star deterrence bioassay to guide the fractionation of this extract, on site in Antarctica, an active component was isolated. Analysis of the active component by NMR spectroscopy on return to our home institutions revealed the sea star deterrent to be *p*-hydroxybenzaldehyde. Subsequent reevaluation in the field demonstrated an authentic sample (Aldrich) of *p*-hydroxybenzaldehyde caused significant sustained tube-foot retraction in *P. fuscus*, indicative of a feeding deterrent.[16]

Experimental Section

General Experimental Procedures. Details of NMR spectroscopy and isolation instrumentation have been reported.[19] The IR spectrum was recorded on a Nicolet Magna-IR 550 spectrometer. The UV spectrum was recorded on a Hewlett-Packard 8452A diode-array spectrometer. Electron impact mass spectra were recorded on a VG 70SE at the University of Florida.

Animal Material. Sponges were collected at a depth of 110−125 ft from several locations on the coast of Ross Island in McMurdo Sound, Antarctica, in the austral summers of 1992 and 1993. A voucher specimen (SC 1620) is on hand at Florida Institute of Technology.

Isolation of Metabolites. The freeze-dried sponge (99.6 g) was extracted sequentially in hexane, chloroform, methanol, and 7:3 methanol/water. The sea star deterrent methanol extract (10.1 g) was fractionated by gel filtration with methanol as eluent. Fractions were collected every 100 mL. Fractions 15 and 16 were combined and then purified by reversed-phase HPLC using 90:10 0.5% aqueous TFA/methanol to give erinacean (**1**) (38 mg, 0.038% of dry weight, t_R 48 min). The other fractions were combined and chromatographed using reversed-phase vacuum chromatography in 85:15 H_2O/methanol; 10 fractions of one column volume each were collected. Additional erinacean was obtained from fraction 3−5. Fraction 9 was purified by reversed-phase HPLC with 95:5 H_2O/methanol to yield three nucleosides, inosine (5 mg, 0.05% of dry weight, t_R 42 min), uridine (2 mg, 0.02% of dry weight, t_R 47 min), and 2′-deoxycytidine (2 mg, 0.02% of dry weight, t_R 25 min). Fraction 10 was subjected to reversed-phase HPLC with 95:5 H_2O/methanol to yield two purine derivatives, 1,9-dimethylguanine (3 mg, 0.03% of dry weight, t_R 48 min) and 7-methyladenine (4 mg, 0.04% of dry weight, t_R 45 min).

The sea star deterrent chloroform extract (1.7 g) was fractionated by flash column chromatography with a step-gradient elution scheme from pure hexane to pure ethyl acetate, two column volumes of eluent per fraction. Fraction 8 (the 40:60 hexane/EtOAc fraction) was purified on normal-phase column chromatography with 70:30 hexane/EtOAc as eluent, yielding 120 mg *of p*-hydroxybenzaldehyde (0.12% of dry weight, t_R 25 min). The structure of *p*-hydroxybenzaldehyde was confirmed by comparison with an authentic (Aldrich) sample.

Erinacean (1): white amorphous powder; IR (KBr) ν_{max} 3312, 1747, 1698, 1649, 1382, 1207, 996 cm^{-1}; UV (MeOH) λ_{max} 272 (ϵ 8444) nm; HREIMS *m/z* 224.0782 $[M + H]^+$ (calcd for $C_8H_{10}N_5O_3$, 224.0784); ^1H NMR and ^{13}C NMR data shown in Table 1.

Antimicrobial Assays. Column fractions or purified compounds (erinacean (**1**), inosine, 2′-deoxycytidine, and 7-methyladenine at 120 *μ*g/disk) were applied to 5 mm filter paper disks and dried. Disks containing the same volume of the appropriate solvent were used as a control. Disks were then placed on LB agar (bacteria) or tryptic soy agar (yeast) plates that had been spread with 0.1 mL of a fresh stationary culture of tester strains. Test microorganisms used in these assays were four Gram-positive microbes (*S. epidermidis*, *S. aureus*, *Bacillus cereus*, *Bacillus subtilis*), three Gram-negative microbes (*Pseudomonas aeruginosa*, *E. coli*, *Proteus mirabilis*), and one yeast (*Saccharomyces cerevisiae*). Plates were incubated for 2 days at 25 °C to allow tester strain growth. Zones of inhibition were measured in mm as excess diameter.

Cytotoxicity Assay. A trypan blue exclusion assay was performed on erinacean (**1**) to evaluate its cytotoxicity. Mouse lymphoblastoid cells (L5178Y) were grown in RPMI 1640 media with 10% horse serum at 37 °C and incubated in 5% CO_2 atmosphere. Erinacean (50 *μ*g) was added to 0.5 mL of lymphoblastoid cells and incubated at 37 °C for 24 h. Following incubation, 0.5 mL of 0.4% trypan blue and 0.3 mL of phosphate buffered saline were added to 0.2 mL of cells, which were then incubated for 15 min and the resultant cells counted using a hemocytometer. Nonviable cells were identified by their blue color. Viable cells were identified as those excluding trypan blue stain.

Sea Star Deterrence Assay. Using protocols developed previously,[16] *p*-hydroxybenzaldehyde was applied in a silicone grease (1:1) matrix to a glass rod and the rod placed in the proximity of a tube-foot from an upturned *P. fuscus*; control rods were used to correct for the effects of the silicone grease and the mechanical stimulation of the rod, and a feeding attractant control was employed to observe the effects of a desirable prey item. On contact with the experimental (*p*-hydroxybenzaldehyde) rods ($n = 10$), sustained retractions of *P. fuscus* tube-feet of 55 s, compared to 5 s retractions of the controls ($n = 10$ for each control; silicone grease, mechanical and feeding stimulant), demonstrate the significant ($p = 0.0051$) deterrent nature of *p*-hydroxybenzaldehyde.

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