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Petrosamine B, an Inhibitor of the *Helicobacter pylori* Enzyme Aspartyl Semialdehyde Dehydrogenase from the Australian Sponge *Oceanapia* sp.

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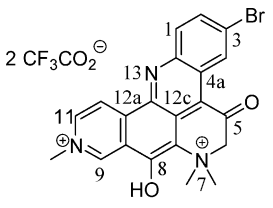
Bioassay-guided fractionation of the MeOH extract of the sponge *Oceanapia* sp. using the *Helicobacter pylori* enzyme, aspartyl semialdehyde dehydrogenase, ASD, to detect antibacterial activity, led to the isolation of a new pyridoacridine alkaloid, petrosamine B (**1**). Petrosamine B is a bright blue compound that is sparingly soluble in many organic solvents. The structure of **1** was determined from detailed NMR studies performed in TFA/D_2O. Petrosamine B was found to be a weak inhibitor of ASD with an IC_{50} of 306 μM.

Helicobacter pylori is a bacteria implicated in the development of gastric ulcers. Aspartate semialdehyde dehydrogenase (ASD) is an enzyme at the first branch point in the biosynthesis of lysine, isoleucine, methionine, and threonine from aspartic acid.[1] In bacteria 25% of all amino acid residues that are required for protein synthesis are produced via this enzyme. Furthermore cell wall synthesis in bacteria is reliant on the component diaminopimelic acid, which is produced via the aspartic acid pathway. Inhibition of ASD is thus considered to be an important target for the development of antibacterial agents.[2] As part of our search for novel natural products as useful leads for the discovery of therapeutic agents to treat gastric disorders 40 000 extracts from Australian plants and marine organisms were screened against ASD.[3] The MeOH extract of *Oceanapia* sp. was targeted for further investigation since it showed inhibition of ASD with an approximate IC_{50} of 35 μg/mL (dry weight). Bioassay-guided fractionation of the active extract led to the isolation of petrosamine B (**1**) (0.4%) as the active constituent.

The methanol extract of the sponge was fractionated on Sephadex LH-20, eluting with MeOH. The combined active fractions were fractionated on C_{18} with a stepped gradient from water to MeOH, followed by 5% TFA/95% MeOH. Three fractions showed activity. Two active early eluting fractions were highly fluorescent and were shown to be interfering with the screen. A third active band eluted with TFA was partitioned between water and CH_2Cl_2. The water fraction retained activity, and its contents were shown to be pure petrosamine B (**1**). Petrosamine B produced bright blue solutions when dissolved in MeOH, purple solutions in water and TFA, and green solutions in DMSO.



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Petrosamine B (**1**) was obtained as an optically inactive blue solid. The (+) ESIMS of petrosamine B (**1**) displayed two equally intense molecular ion peaks at *m/z* 422 and 424 $[M]^+$, which indicated that the molecule contained one bromine. A molecular ion in the positive HRESIMS at *m/z* 422.0492 (Δ 2.8 ppm) allowed the molecular formula $C_{21}H_{17}N_3O_2Br$ to be assigned to **1**. A strong absorption band at 1675 cm^{-1} in the IR spectrum suggested that **1** contained an unsaturated ketone functionality. Petrosamine B showed limited solubility in most common NMR solvents. The compound was dissolved in a mixture of TFA and D_2O (with ~1% DMSO-d_6) so that reasonable signal-to-noise could be obtained in heteronuclear NMR experiments. Analysis of the ^1H and COSY NMR spectra (Table 1) for petrosamine B indicated that the molecule contained a 1,3,4-trisubstituted aromatic spin system, a 3,4-disubstituted pyridine spin system, a methylene singlet, an *N*-methyl singlet, and an *N,N*-dimethyl singlet. Analysis of the ^{13}C and DEPT spectra indicated that the molecule contained six aromatic methine carbons, three *N*-methyl carbons, one *N*-methylene carbon, 10 aromatic quaternary carbons, and an aromatic ketone carbonyl carbon. A gHSQC spectrum allowed all of the protonated carbons to be assigned. A large number of correlations were observed in the gHMBC spectrum, and this allowed the gross structure to be assigned. The *N,N*-dimethyl proton singlet 1-N(CH_3)$_2$, δ 3.63, showed correlations to the methyl carbon, 57.3 ppm, the methylene carbon C-6, 73.1 ppm, and an aromatic quaternary carbon, C-7a, 121.1 ppm. The methylene protons H-6, δ 4.33, also showed correlations to the *N*-methyl carbon at 57.3 ppm and the quaternary aromatic carbon C-7a, 121.1 ppm, as well as to the ketone carbonyl carbon C-5, 188.2 ppm, and another quaternary aromatic carbon C-4b, 121.0 ppm. A three-bond correlation from H-4 to C-4b indicated the close proximity of the trisubstituted aromatic moiety to the ring containing the ketone. The bromine could be placed at C-3 from analysis of the chemical shifts of the carbons C-4, C-3, C-2, and C-1 (128.5, 131.7, 135.8, and 133.8 ppm, respectively). All of the expected three-bond correlations were observed from H-4, H-2, and H-1. The 10-NCH_3 proton showed correlations to C-11 and C-9, and both H-11 and H-9 showed correlations to 10-NCH_3, indicating an *N*-methylpyridyl group was present in the molecule. H-9 also showed correlations to an oxygenated aromatic quaternary carbon, C-8, 155.8 ppm, the carbon *para* to the nitrogen in the pyridine moiety, C-12a, 144.6 ppm, the quaternary β

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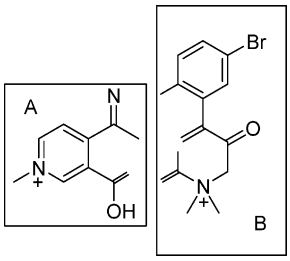
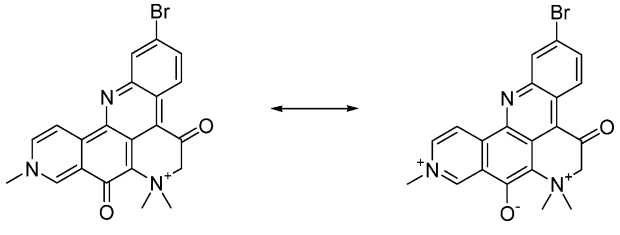
Table 1. ^{1}H (600 MHz), ^{13}C (150 MHz), HMBC, and COSY NMR Data for Petrosamine B (**1**) in TFA/D_2O/1% DMSO-d_6

atom	^{13}C (mult)	^{1}H (mult, J Hz)	$^{2,3}J_{CH}$ correlations	COSY
1	133.8 (d)	7.87 (d, 8.4 Hz, 1H)	C-3, C-4a,	H-2
2	135.8 (d)	7.62 (dd, 8.4, 1.8 Hz, 1H)	C-4, C-13a	H-1, H-4
3	131.7 (s)			
4	128.5 (d)	9.11 (d, 1.8 Hz, 1H)	C-2, C-3, C-4b, C-13a	H-2
4a	125.6 (s)			
4b	121.0 (s)			
5	188.2 (s)			
6	73.1 (t)	4.33 (bs, 2H)	C-4b, C-5, C-7a, 7-NCH_3	
7-$N(CH_3)_2$	57.3 (q)	3.63 (bs, 6H)	C-6, 7-NCH_3, C-7a	
7a	121.1 (s)			
8	155.8 (s)			
8a	127.4 (s)			
9	146.5 (d)	9.60 (s, 1H)	C-8, C-8a, C-11, C-12a	
10-NCH_3	50.3 (q)	4.25 (s, 3H)	C-9, C-11	
11	143.0 (d)	8.46 (d, 5.6 Hz, 1H)	C-9, 10-NCH_3, C-12, C-12a,	H-12
12	125.4 (d)	9.38 (d, 5.6 Hz, 1H)	C8a, C-11, C-12b	H-11
12a	144.6 (s)			
12b	140.3 (s)			
12c	130.5 (s)			
13a	146.2 (s)			

pyridine carbon C-8a, 127.4 ppm, and the α pyridine carbon C-11, 143.0 ppm. H-11, δ 8.46, showed correlations to C-9, C-12, and C-12a. Finally, H-12 showed correlations to C-11, C-8a, and a nitrogen-substituted aromatic quaternary carbon, C-12b, 140.3 ppm. Two partial structures, A and B, could be assigned from these correlations. The chemical shift of C-13a (146.2 ppm) dictated that it was attached to a nitrogen. The only remaining unsubstituted nitrogen was N-13, and thus a bond was assumed between C-13a and N-13 and between N-13 and C-12b. This left only one way of joining the remaining carbons, leading to structure **1**.



Petrosamine B (**1**) is isomeric with petrosamine (**2**) isolated from the sponge *Petrosia* sp.[4] The only difference between the two compounds is the position of the bromine atom. Petrosamine (**2**) was reported to exist in the solid state as a diketone and in solution as the C-8 ketone, C-5/C-6 enol, with C-5 being reported to resonate at 161 ppm in the ^{13}C NMR spectrum. For petrosamine B (**1**), however, there was no evidence for this keto−enol isomerism. HMBC correlations indicated that C-5 quite clearly resonated at 188.2 ppm, making it more indicative of an unsaturated ketone, and in contrast C-8 resonated at 155.8 ppm, supporting it as a phenolic resonance. It was therefore more likely that petrosamine also exists in the C-8 enol form and C-8 was the carbon at 161 ppm.



2

Petrosamine B (**1**) inhibited the phosphorylation of asparte semialdehyde to L-β-aspartyl phosphate by the enzyme ASD with an IC_{50} of 306 μM. The reference compound *S*-sulfo-L-cysteine inhibited the enzyme with an IC_{50} of 0.18 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. FTIR and UV spectra were recorded on a Perkin-Elmer 1725X spectrophotometer and a GBC UV/vis 916 spectrophotometer, respectively. NMR spectra were recorded at 30 °C on a Varian 600 MHz Unity INOVA at 599.926 MHz for ^{1}H and 149.98 MHz for ^{13}C. The ^{1}H and ^{13}C chemical shifts were referenced to the proto-deutero solvent peak (d_6-DMSO) at δ 2.49 and 39.5 ppm, respectively. Standard parameters were used for the 2D NMR spectra obtained, which included gradient COSY, HSQC ($^1J_{CH}$ = 140 Hz), and HMBC ($^nJ_{CH}$ = 8.3 Hz). HRESIMS was recorded on a Bruker BioAPEX 47e mass spectrometer equipped with a Bradford CT 06405 electrospray ion source. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC separations. An Alltech Davisil 30−40 μm 60 Å C_{18} column was used for MPLC work. Pharmacia Sephadex LH-20 was used for gel permeation chromatography. All solvents used for HPLC, UV, $[\alpha]_D$, and MS were Merck Omnisolv grade, and the H_2O used was Millipore Milli-Q PF filtered.

Animal Material. The sponge *Oceanapia* sp. (1313) (possibly a new species) is one of over 80 species of *Oceanapia* in the Queensland Museum collection that cannot yet be assigned to a known taxon. A major taxonomic revision of the group, which contains several hundred species worldwide, is required before it would be possible to allocate this specimen to a species. It was collected by dredge (−58 m) southwest of Port Musgrave in the Gulf of Carpentaria, Far North Queensland, Australia, in November 1991 and kept frozen prior to freeze-drying and extraction. A voucher specimen, QM G301014, has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

Description: The sponge was firm, friable, and crumbly and had a massive, lobate, subspherical growth form. It was glossy black with large oscules scattered over its surface. The ectosomal skeleton had irregular tangential isodictylal reticulations of the larger megascleres forming pauci to multispicular tracts that were associated with heavily pigmented collagen. Between the major tracts were fine unispicular tracts of smaller oxeas in a subisodictyal mesh. The ectosome had a distinct detachable peel with a prominent subectosomal cavity supported by multispicular ascending choanosomal tracts. The

choanosomal skeleton was irregular with open-meshed reticulation of large multispicular tracts, diverging and rejoining throughout the skeleton. The meshes were vaguely oval to elliptical in shape with a diameter up to 550 μm. Megascleres were either oxeas or strongyloxeas. Larger megascleres of the major tracts were slightly curved at the center and occasionally completely straight with points slightly tapering or less commonly completely evenly rounded (265−320 μm × 12−16 μm). Smaller oxeas that lay between the major tracts were relatively long, slightly curved at the center, and occasionally flexuous with long tapering points (184−275 μm × 3−6 μm). Very fine oxeas were also present. These ranged from centrally curved with straight points to flexuous, toxa-like with recurved points (90−120 μm × 1.5−2.5 μm).

Extraction and Isolation. The ground sponge sample (50 g) was extracted exhaustively with CH_2Cl_2 (5 × 150 mL), followed by MeOH (5 × 200 mL), followed by water (5 × 200 mL). The combined CH_2Cl_2 and MeOH extracts were fractionated on Sephadex LH-20, eluting with MeOH. Thirteen fractions were collected. Fractions three and four retained activity. The combined active fractions were fractionated on C_{18} with a stepped gradient from water to MeOH, followed by 5% TFA/95% MeOH. Three fractions showed activity. Two active early eluting fractions were highly fluorescent, and an active fraction eluting with TFA was highly colored. The late eluting fraction was partitioned between water and CH_2Cl_2. The water fraction retained activity and contained pure petrosamine B (**1**). Petrosamine B produced bright blue solutions when dissolved in MeOH, purple solutions in water and TFA, and green solutions in DMSO.

Petrosamine B (1): blue solid (200 mg, 0.4%); UV (MeOH) λ_{max} (ϵ) 205 nm (34 610), 288 (30 460), 378 (15 845), 609 (4385); IR ν_{max} (film) 3410, 3217, 1675, 1586, 1530 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); positive ESIMS *m/z* 422 (M^+ 50%), 424 (M^+ 50%); (+)-HRESIMS *m/z* 422.0492 $[M]^+$ (calcd for $C_{21}H_{17}N_3O_2{}^{79}Br$, 422.0504).

ASD Enzyme Assay. Assay buffer (150 μL, 200 mM HEPES, 30 mM Na_2HPO_4, 150 mM $NADP^+$, pH 8.0) and 4 μL of compound, extract, or fraction dissolved in DMSO were added to each well of a microtiter plate. The initial fluorescence was read at Ex_{355}/Em_{460}. *H. pylori* His-tagged ASD (20 μL, 800 nM) was added to each well, and the contents of each well were mixed by rotary shaking of the plate for 15 s, followed by incubation at ambient temperature (23 °C) for 15 min. The reaction was started with the addition of 20 μL of aspartyl semialdehyde (1.0 mM) to each well, and contents of the wells were mixed by rotary shaking of the plate for 15 s. After 18 min incubation at ambient temperature the reaction was stopped with the addition of 20 mL of 1.0 N HCl. The final fluorescence was read at Ex_{355}/Em_{460}. Percent activity was calculated as 100 − % controls.

$$\%\ \text{control} = \frac{Ex_{355}/Em_{460}(\text{compound}) - Ex_{355}/Em_{460}(\text{no substrate})}{Ex_{355}/Em_{460}(\text{control}) - Ex_{355}/Em_{460}(\text{no substrate})} \times 100$$

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Supporting Information Available: Color photo of the sponge *Oceanapia* 1313. This material is available free of charge via the Internet at http://pubs.acs.org.

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