Dragmacidins: New Protein Phosphatase Inhibitors from a Southern Australian Deep-Water Marine Sponge, *Spongosorites* sp.

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Received October 29, 1997

A *Spongosorites* sp. collected during trawling operations off the southern coast of Australia returned the new alkaloid dragmacidin E (**3**), the structure of which was secured by detailed spectroscopic analysis. Dragmacidin E (**3**), and its co-metabolite dragmacidin D (**1**) have been identified as potent inhibitors of serine−threonine protein phosphatases.

Marine sponges of the genera *Topsentia*,[1,2] *Spongosorites*,[3,4] *Hexadella*,[5] *Hamacantha*,[6] and *Dragmacidon* [7,8] have been reported to yield novel bisindole alkaloids. Continued interest in this class of compounds has no doubt been stimulated both by their novel molecular structures and by their wide range of biological properties (including cytotoxic,[3−5] antimicrobial,[1,2,6] antitumor,[3,4,7,8] antiviral,[3,4,8] and antifungal[4,8] activity). In this report we describe the bioassay-directed fractionation of a *Spongosorites* sp. collected off the southern coast of Australia to yield bisindole alkaloids that are potent inhibitors of protein phosphatases.

The aqueous ethanol extract of a *Spongosorites* sp. collected at a depth of 90 m along the coast of South Australia was examined and found to inhibit serine−threonine protein phosphatases, as well as to retard growth of the bacterium *Escherichia coli* and the fungus *Candida albicans* and to display a brilliant fluorescent yellow coloration. Initial chemical analysis of this extract resulted in isolation of the known deep-water Caribbean sponge metabolite dragmacidin D (**1**),[8] along with the new bisindole alkaloid isobromotopsentin (**2**),[9] and a new fluorescent yellow pigment designated as dragmacidin E (**3**).

The crude aqueous ethanol extract of the *Spongosorites* sp. was partitioned to return a butanol solution portion (2% dry wt of the sponge) that was subjected to gel chromatography (LH-20, MeOH) to yield two bioactive yellow pigments, dragmacidin D (**1**) and E (**3**). Dragmacidin D (**1**) was identified by conversion to the trifluoroacetate salt and comparison with literature spectroscopic data.[8] Mass spectrometry confirmed that the new metabolite dragmacidin E (**3**) was isomeric with dragmacidin D (**1**). The principle differences between the ^{1}H NMR data for **3** (Table 1) with that of **1** were the absence of a resonance for H-5, a downfield shift for H 2″ (δ 7.52 to δ 8.26), an upfield shift for H-6‴ (δ 4.33 to δ 3.46) and the C-6‴ methyl (δ 1.33 to δ 1.06), and the appearance of a deshielded ABq (δ 3.04, 3.17) in place of H-4‴. All these observations could be accommodated if dragmacidin E (**3**) is the cyclic analogue of dragmacidin D (**1**) as shown.

Considerable difficulty was encountered in acquiring complete ^{13}C NMR data for **3**, and, despite numerous attempts on various instruments with different pulse parameters, concentrations, and solvents, the resonances for C-3 and C-6 were eventually observed only after addition of DCl to the NMR solvent. Although dragmacidin D (**1**) is presented in the literature exclusively in the pyrazinone tautomeric form, in the case of dragmacidin E (**3**), a ROESY correlation between H-4′ and an exchangeable proton is only possible for the pyrazine tautomer (the exhangeable proton corresponding to the pyrazine OH). Whether dragmacidin E (**3**) exists exclusively in the pyrazine form or in tautomeric equilibrium with the pyrazinone form remains unresolved. It is speculated that the addition of acid to the NMR sample diminished the broadening effect of a pyrazinone/pyrazine tautomeric equilibrium (Figure 1), thereby facilitating measurement of the carbon resonances. That the ^{13}C NMR shift for C-2‴ did not vary on addition of acid suggests that dragmacidin E (**3**) occurs naturally as a guanidinium salt rather than the free amine. This conclusion was further supported by a hypsochromic shift in the UV spectrum (427−410 nm) on addition of NaOH.

A detailed 2D NMR analysis of **3** (COSY, ^{1}H−^{13}C gHMBC, ^{1}H−^{15}N gHMBC, ROESY; see Table 1) provided connectivity sequences that not only confirmed the gross structure, but also defined the relative stereochemistry about C-5‴ and C-6‴. A ROESY correlation between H-4_b‴ and H-6‴ required that both protons reside on the same face of dragmacidin E (**3**). Also informative were gHMBC correlations between H_a-4‴, H_b-4‴, and the C-6‴ methyl to C-5 on the pyrazinone ring, correlations that substantiated the C-5/C-5‴ ring closure as postulated.

Although earlier workers could not measure an $[\alpha]_D$ for the trifluoroacetate salt of dragmacidin D (**1**),**[8]** our reisolation yielded material (not the trifluoroacetate salt) with a measurable $[\alpha]_D$ +12°. Dragmacidin E (**3**) also yielded a measurable $[\alpha]_D$ −34°. Although we have confirmed that both dragmacidins D and E are not

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S0163-3864(97)00483-7 CCC: $15.00 © 1998 American Chemical Society and American Society of Pharmacognosy
Published on Web 05/01/1998

Table 1. NMR Data for Dragmacidin E (3) (DMSO-d_6, 600 MHz)

no.	^{1}H δ, m, J	^{13}C ppm	COSY	^{1}H−^{13}C gHMBC	^{1}H−^{15}N gHMBC	ROESY
1-NH[a]	9.31, br s					H 4′
2		154.9				
3		138.4[b]				
5		129.1				
6		131.0[b]				
1′-NH	11.78, br s		H 2′	C 2′,C 3′,C 3a′,C 7a′	1′−N	H 2′,H 7′
2′	8.74, d, 2.4	131.1	1′-NH	C 3′,C 3a′,C 7a′		
3′		112.1				
3a′		124.9				
4′	8.65, d, 2.4	125.5	H 5′	C 3′,C 5′,C 6′,C 7a″		1-NH,H 5′
5′	7.45, dd, 1.8, 8.4	123.5	H 4′, H 7′	C 3a′,C 7′		H 4′
6′		114.8				
7′	7.64, d, 1.8	114.0	H 5′	C 3a′,C 5′,C 6′		1′-NH
7a′		137.2				
1″-NH	12.07, br s		H 2″	C 3″	1″−N	H 2″
2″	8.26, d, 2.4	126.0	1″-NH	C 3″,C 3a″,C 7a″		
3″		106.6				
3a″		122.4				
4″		124.5				
5″	6.85, d, 7.8	121.4	H 6″	C 3a″,C 7″,C 6‴		H 6″, H 6‴
6″	6.62, d, 7.8	106.6	H 5″	C 4″,C 7″,C 7a″		H 5″
7″		143.2				
7a″		126.4				
7″-OH	9.95, br s					
2‴		158.7				
2‴-NH_2	7.91, br s					
4‴	3.02, d, 10.2	55.2		C 5,C 2‴,C 5‴,C 6‴	3‴−N	H 4_b‴
	3.17, d, 10.2			C 5,C 5‴,C 6‴	3‴−N	H 4_a‴,H 6‴
5‴		70.0				
6‴	3.46, q, 7.2	48.8	H 7‴	C 5,C 3a″,C 4″,C 5″, C 4‴,C 5‴		H 5″,H 4_b‴,H 7‴
7‴	1.06, d, 7.2	19.7	H 6‴	C 4″, C 5‴, C 6‴		H 6‴

[a] Or 2-OH. [b] Resonances only observed in CD_3OD + DCl, 400 MHz.

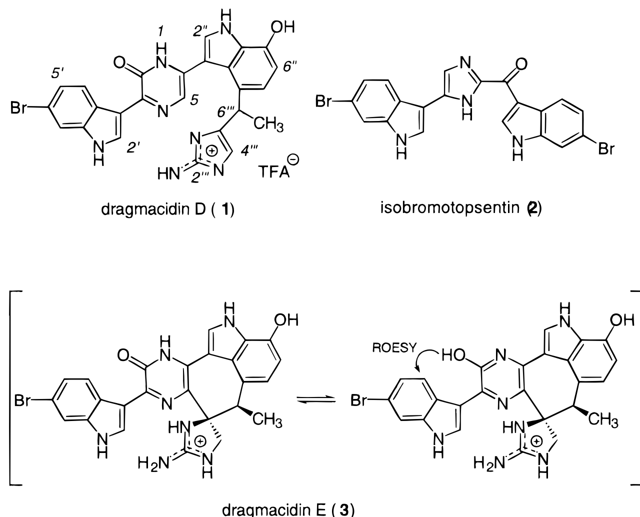

Figure 1.

racemic, at this time their absolute stereochemistry remains unresolved.

Dragmacidins D (**1**) and E (**3**) are potent inhibitors of serine−threonine protein phosphatases. Although dragmacidin E (**3**) inhibits both PP1 and PP2A, preliminary results suggest that dragmacidin D (**1**) is a selective inhibitor of PP1. Detailed investigations into the nature of these inhibitory properties is in progress and will be reported elsewhere. As an interesting side note, although dragmacidins D (**1**) and E (**3**) were both bright yellow pigments, the latter was especially brilliant and tenacious at staining glassware. Dragmacidin E (**3**) features an unprecedented carbon skeleton that is very likely derived from cyclization of a "dragmacidin D" precursor. The dragmacidins D (**1**) and E (**3**) were solely responsible for both the antibiotic character of the *Spongosorites* extract and the observed protein phosphatase inhibition.

Experimental Section

General Procedures. The general procedures were those described by Urban et al.[10] ESIMS were acquired on a Micromass Quattro II mass spectrometer at varying cone voltages using a 50% MeCN−H_2O matrix. HRESIMS were run on a Bruker BioApex 47E FT mass spectrometer using a 50% MeCN−H_2O matrix. Selected NMR spectra were acquired on a Varian Inova 600 MHz instrument.

Sponge Material. A *Spongosorites* sp. (432 g dry wt) was collected by epibenthic sled at a depth of 90 m off the coast of South Australia during a scientific cruise aboard the *R. V. Franklin* in May 1991 [Demospongiae, Halichondrida, Halichondriidae, growth form massive; texture very hard (stoney), areneceous; ectosomal skeleton with embedded detritus and protruding erect, slightly larger oxeas from ascending choanosomal tracts, surmounted by a paratangential felt-like network of slightly smaller oxeas; choanosomal with a crisscross halichondroid reticulation of both smaller and larger oxeas forming vaguely directionless tracts, eventually ascending to the surface, and with large sand grains and other detritus throughout the skeleton; oxeas moderately small, slender, sharply pointed, fusiform, some with centrangulate swellings, more or less divided into two size classes but with numerous intermediates (85−160 × 3−5 μm); no microscleres). After freezer transportation to the laboratory, the sponge was diced, steeped in EtOH, and stored at −18 °C. A voucher specimen was deposited with the Queensland Museum (registry no.: QMG301315).

Extraction and Isolation. The EtOH extract was decanted, filtered through a bed of Celite, and then partitioned into BuOH- and H_2O-soluble fractions. The BuOH-soluble material (1.95 g, 0.45%) was concentrated to a yellow oil that was further fractionated by gel permeation chromatography (elution with MeOH through Sephadex LH-20, 2 cm × 80 cm column, followed by elution with MeOH through Toyopearl HW 40S, 1.5 cm × 50 cm column, both equipped with an ISCO fraction collector and an ISCO UV/vis detector) to yield dragmacidin D (**1**) (1.45 g, 0.34%) and dragmacidin E (**3**) (0.043 g, 0.01%). Dragmacidins D (**1**) and E (**3**) displayed MICs against *E. coli* of 16 and 22 ppm and against *C. albicans* of 20 and 36 ppm, respectively.

Dragmacidin D (natural salt): yellow solid; $[\alpha]_D$ +12° (*c*, 0.95, EtOH); ^{13}C NMR (DMSO, 400 MHz) 155.1 (C 2), 148.5 (C 3), 147.5 (C 2‴), 143.1 (C 7″), 137.4 (C 7a), 131.4 (C 5‴), 131.5 (C 2′), 131.2 (C 6), 127.4 (C 2″), 126.9 (C 3a″), 125.9 (C 4″), 125.6 (C 7a″), 125.3 (C 3a), 124.6(C 4′), 123.3 (C 5), 123.3 (C 5′), 118.6 (C 5″), 115.1 (C 6′), 114.6 (C 7′), 112.2 (C 3′), 109.0 (C 4‴), 107.5 (C 3″), 106.5 (C 6″), 31.2 (C 6‴), 20.5 (C 7‴) ppm; ^{13}C NMR (CD_3OD, 400 MHz) 157.0 (C 2), 149.8 (C 3), 148.5 (C 2‴), 144.4 (C 7″), 138.8 (C 7a), 133.8 (C 5‴), 133.6 (C 2′), 132.2 (C 6), 128.5 (C 7a″), 127.8 (C 2″), 126.9 (C 3a″), 126.3 (C 3a′), 125.6 (C 4″), 125.4 (C 5), 125.3 (C 4′), 124.8 (C 5′), 120.0 (C 5″), 116.9 (C 6′), 115.3 (C 7′), 113.1 (C 3′), 109.9 (C 4‴), 108.5 (C 3″), 107.3 (C 6″), 33.0 (C 6‴), 20.6 (C 7‴) ppm.

Dragmacidin E (3): fluorescent yellow solid; $[\alpha]_D$ −34° (*c*, 0.9, EtOH); ESIMS *m/z* 532/530 (M^+ + H, 60); (+)-FAB MS *m/z* 532/530 (M^+ + H, 4); HREIMS 529.0862 ($C_{25}H_{20}BrN_7O_2$ requires 529.0862); IR (film) ν_{max} 3350, 1685, 1640 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6, 500 MHz) see Table 1; UV (EtOH) λ_{max} 211 nm (ϵ 46200); λ_{max} 281 nm (ϵ 7530); λ_{max} 427 nm (ϵ 27 700); UV (EtOH + HCl) λ_{max} 427 nm (ϵ 26 400); UV (EtOH + NaOH) λ_{max} 410 nm (ϵ 26 100); ^{13}C NMR (CD_3OD, 400 MHz) 160.6 (C 2‴), 157.1 (C 2), 144.6 (C 7″), 132.0 (C 2′), 138.7 (C 7a′), 128.3 (C 7a″), 126.3 (C 3a′), 126.2 (C 3a″), 126.1 (C 2″), 125.3 (C 4′), 124.7 (C 5′), 123.9 (C 4″), 123.0 (C 5″), 116.8 (C 6′), 115.2 (C 7′), 113.8 (C 3′) 108.2 (C 3″), 108.2 (C 6″), 72.1 (C 5‴), 56.3 (C 4‴), 51.1 (C 6″, 19.7 (C 7‴) ppm; ^{13}C NMR (CD_3OD + DCl, 400 MHz) 160.5 (C 2‴), 157.0 (C 2), 144.4 (C 7″), 138.8 (C 7a′) 138.37 (C 3), 132.2 (C 2′) 131.0 (C 6), 129.4 (C 5), 128.3 (C 7a″) 126.5 (C 3a″), 126.2 (C 3a′) 125.5 (C 2″), 125.5 (C 4′), 124.8 (C 5′), 124.0 (C 4″), 122.9 (C 5″), 116.8 (C 6′), 115.4 (C 7′), 113.8 (C 3′), 108.3 (C 3″), 108.3 (C 6″), 72.1 (C 5‴), 56.4 (C 4‴), 51.4 (C 6‴), 19.9 (C 7‴) ppm.

Acknowledgment. The assistance of Y. Bone of the CSIRO Division of Oceanography and the crew of the *O. R. V. Franklin* in the collection of sponge specimens is gratefully acknowledged. We also note taxonomic support from J. N. A. Hooper, technical support from L. Goudie and P. Niclasen, and financial assistance from the Australian Research Council.

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NP970483T