Pyraxinine, a Novel Nitrogenous Compound from the Marine Sponge *Cymbastela cantharella*

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Four minor polar nitrogen compounds have been isolated from the New Caledonian marine sponge, *Cymbastela cantharella*. Three are the known products allantoin, homarine, and trigonelline and the fourth is novel. The structure of the new compound, pyraxinine (**1**), was shown to be 3-pyridylguanidine by spectroscopic analysis and synthesis.

Several compounds have been isolated from the New Caledonian marine sponge *Cymbastela cantharella*[1] (also originally called *Pseudaxinyssa cantharella* Lévi); these include sterols and linear and cyclic imidazole and/or pyrrole derivatives: A-norsterols,[2] (+)-dibromophakelline,[3] dibromocantharelline,[3] odiline[3] [syn. stevensine[4]], oroidine,[3] aldisine and its monobrominated derivative,[3,5] and girolline.[6,7] Girolline was responsible for the cytotoxicity and the antitumor activity of the crude aqueous extract of the sponge.

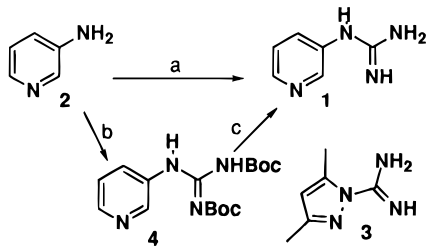
To facilitate extensive pharmacological, toxicological, and chemical studies on girolline, 220 kg of sponge was collected and extracted. Purification of the most polar fractions gave three nitrogen compounds identified as allantoin, homarine, and trigonelline, all long known and of diverse origins including marine. A fourth compound isolated, which we named pyraxinine (**1**), is novel. A total of 5 mg of this compound as an amorphous solid was obtained after a series of chromatographic separations on Si gel.

The FABMS of **1** revealed an ion $(M + H)^+$ at 137. The ^{1}H-NMR spectrum exhibited three signals for four protons at 8.55 (br d, 2H), 7.86 (dd, 1H), and 7.58 (dd, 1H) ppm. The ^{13}C NMR exhibited six signals: four methines (148.7, 146.9, 135.6, 125.3 ppm) and two quaternary carbons (weak signals 157.5, 132.75 ppm). These data led to a pyridine structure, substituted in position 3 by the $-NH-C(NH)-NH_2$ group.

Compound **1** was synthesized in one step by condensing 3-aminopyridine (**2**) and 1-(carboxyamidino)-3,5-dimethylpyrazole nitrate (**3**).[8] It was also prepared by condensing 3-aminopyridine (**2**) and *N*,*N*′-(bis-*tert*-butoxycarbonyl)thiourea in the presence of mercuric chloride and deprotecting intermediate **4** with trifluoroacetic acid (Scheme 1).[9,10] In both procedures, the synthetic product was identical with the natural product **1**.

In addition to homarine and trigonelline, several pyridine compounds, often substituted in position 3 by a fatty chain as in the niphatynes,[11] have been isolated from marine organisms. Such 3-alkylpyridines can be "associated", as in the niphatoxins,[12] and they can even form macrocyclic 3-alkylpyridinium salts, as in the cyclostellettamines.[13] The free guanidine group is present in several marine molecules, such as tubastrine,[14] agelasidines,[15,16] crambines,[17−19] nazumamide A,[20] onnamides,[21] aplysinamisine II,[22] phloeodictines,[23,24] and, recently, 3′-deshydroxytubastrine from a sponge in which homarine is also present.[25]

Scheme 1[a]



[a] Key: (a) **3**, Et_3N, DMF; (b) BocNH-CS-NHBoc, $HgCl_2$, Et_3N, DMF; (c) CF_3COOH, CH_2Cl_2; NaOH, H_2O.

Many of the compounds described above are biologically active in various ways. At a 100 μM concentration, pyraxinine (**1**) inhibited macrophagic NO synthase induced by lipopolysaccharide (LPS)[26] up to 50% without significant cytotoxicity.

Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM300 spectrometer. ^{1}H and ^{13}C chemical shifts were referenced to solvent peaks: δ_H 4.88 for D_2O and δ_C 67.4 for dioxane added to D_2O (as internal reference).

Extraction and Isolation. The lyophilized sponge was extracted as already described:[3] 35.98 kg (corresponding to 220 kg of fresh sponge, *Cymbastela cantharella*) provided 125 g of girolline[6,7] and 23 g of polar fractions during the last partitioning. A part (10 g) of these fractions was subjected to two successive chromatographies (400 g and 90 g SiO_2 6−35 μ) with AcOEt−butanone−H_2O−HCO_2H (from 5.5:3.2:0.5:0.5 to 2:3:2:2) to afford 250 mg of mixture; this mixture was finally purified by TLC [SiO_2: AcOEt−butanone−H_2O−HCOOH (5.5:3.2:2:2)] to yield pyraxinine (**1**) (5 mg).

Pyraxinine (1): colorless amorphous solid; $C_6H_8N_4$; IR (KBr) ν_{max} 3600−2800, 1689, 1629, 1576, 1390, 1350 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 203 (2300), 231 (1900), 260 (sh, 1100) nm; (EtOH + NaOH 10%) 215 (2800), 243 (2000), 277 (900) nm; no change in acidic medium; HRFABMS (thioglycerol) *m*/*z* $[M + H]^+$ 137.0824 (0.3, $C_6H_9N_4$, req. 137.0827)]; ^{1}H and ^{13}C NMR, see Table 1.

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Table 1. NMR Data of Compounds **1** and **2** (in D_2O)

	1		2	
	δ^{13}C	δ^1H (mult, *J* in Hz)	δ^{13}C	δ^1 (mult, *J* in Hz)
2	148.7	8.55 (br d)	148.4	8.88 (br s)
3	132.75		129.75	
4	135.6	7.86 (dd, 8, 2)	137.05	8.16 (br d, 8)
5	125.3	7.58 (dd, 8, 5)	125.0	7.52 (dd, 8, 5)
6	146.9	8.55 (br d, 5)	152.0	8.66 (br d, 5)
3a	157.5			

Synthesis of 3-Pyridylguanidine (1) (Pyraxinine). Direct Method. To a solution of 940 mg (10 mmol) of 3-aminopyridine **2** in 30 mL of DMF were added 2.02 g (14.63 mmol) of 1-(carboxyamidino)-3,5-dimethylpyrazole nitrate (**3**) and 2.8 mL of triethylamine. The mixture was left overnight at room temperature and then heated at 55 °C for 4 h, and the DMF was distilled. The residue was solubilized in CH_2Cl_2. The solution was filtered and the solvent evaporated to yield 335 mg of **1** (24.6%); HRFABMS (thioglycerol) *m/z* $[M + H]^+$ 137.0822 (0.5, $C_6H_9N_4$, req. 137.0827)].

Synthesis of 3-Pyridylguanidine (1) (Pyraxinine). Indirect Method. To a solution of 94 mg (1 mmol) of 3-aminopyridine (**2**) in 2 mL of DMF kept at 0 °C were added 276.4 mg (1.0 mmol) of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (prepared by the literature procedure[29]), 0.46 mL of triethylamine, and 298.6 mg (1.09 mmol) of $HgCl_2$. The mixture was stirred for 1 h at 0 °C and 1 h at room temperature; 20 mL of EtOAc were added; after filtration, the solution was filtered over Celite, washed with H_2O (3 times), dried over $MgSO_4$, and evaporated. Purification by SiO_2 column chromatography (heptane−AcOEt, 60:40) yielded 136 mg of **4** (40%).

Compound 4: amorphous solid; $C_{16}H_{24}N_4O_4$; IR ($CHCl_3$) ν_{max} 3240, 2987, 1630, 1426, 1328, 1145 cm^{-1}; EIMS *m/z* $[M^+]$ 336, 280, 263, 235, 208, 181, 135; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.62 and 11.41 (2H, 2 br s, 2 NH), 8.66 (1H, d, $J = 2$ Hz, H-2), 8.35 (1H, d, $J = 5$ Hz, H-6), 8.23 (1H, dd, $J = 8$, 2 Hz, H-4), 7.28 (1H, dd, $J = 8$, 5 Hz, H-5), 1.48 (18H, s, t-Bu); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.0, 153.2, 152.0 (3 CO), 134.0 (C-3), 145.6, 143.35, 129.7, 123.6 (C-2, C-4, C-5, C-6), 28.2 (t-Bu).

Deprotection of **4** was accomplished according to the literature procedure.[9] Compound **4** (55 mg, 0.16 mmol) was treated with $CF_3COOH-CH_2Cl_2$ (1:1) at room temperature for 2 h. The solvent was evaporated, and then NaOH (1N) was added to the reaction residue. Pure **1** (20 mg, 89%) was obtained after preparative TLC (CH_2Cl_2−MeOH, 4:1).

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