Corydendramines A and B, Defensive Natural Products of the Marine Hydroid *Corydendrium parasiticum*

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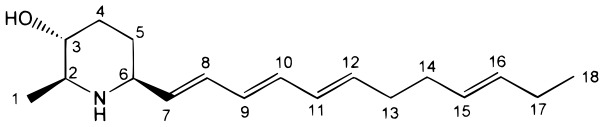
Two novel piperidinol metabolites, corydendramines A (**1**) and B (**2**), have been isolated from the marine hydroid *Corydendrium parasiticum*. The structures of these compounds, which deter feeding by a potential hydroid predator, were determined by interpretation of their spectral data.

Marine hydroids are commonly thought to be defended from predators by nematocysts that are capable of penetrating predators tissues, typically injecting proteinaceous venoms.[1] But not all hydroids possess penetrating nematocysts. Recently, an increasing number of bioactive secondary metabolites have been isolated from marine hydroids.[2] Results of a recent investigation[3] to test the hypothesis that penetrating nematocysts and lipophilic secondary metabolites represent alternative defensive strategies among hydroids found that four of six species collected from North Carolina (USA) yielded lipophilic extracts that significantly deterred feeding by the pinfish, *Lagodon rhomboides*. Three of these four species do not possess penetrating nematocysts, only entangling or adherent nematocysts thought to function in prey capture. The two species that did not yield deterrent extracts have penetrant nematocysts. *Corydendrium parasiticum* L. 1767 (Clavidae) was found to be unpalatable to pinfish and to lack defensive nematocysts.[3] In this paper, we report the structures of corydendramines A (**1**) and B (**2**), which were identified by bioassay-guided fractionation as potent fish feeding-deterrents.[3]



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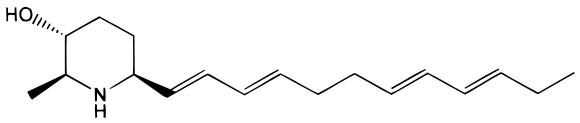

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Corydendramine A (**1**) analyzed for $C_{18}H_{29}NO$ by HRFABMS in conjunction with ^1H and ^{13}C NMR data (Table 1). Of the five units of unsaturation, four were accounted for as carbon−carbon double bonds. The UV spectrum of **1** indicated the presence of a conjugated triene system and an isolated C−C double bond. All four C−C double bonds of **1** were assigned a trans configuration based on observed olefinic proton couplings of at least 15 Hz. 1D ^1H and ^{13}C NMR data (Table 1) and COSY and HSQC results indicated that the final unsaturation was a 2-methyl-6-alkyl-3-piperidinol ring. The position of the triene (C-7 to C-12) was determined from the 7.8-Hz ^3J coupling between the C-7 olefinic proton and the C-6 ring proton. The isolated double bond was assigned to C-15 to C-16 based on the 6.2-Hz ^3J coupling between the C-16 olefinic proton and the C-17 methylene protons, which were also clearly coupled to the C-18 terminal methyl. A 10-Hz coupling constant between the C-2 and C-3 protons established their axial configuration, which, in conjunction with the positive NOE enhancement observed for the C-6 axial proton upon irradiation of the C-2 proton, confidently established the relative stereochemistry of the piperidinol ring.

Corydendramine B (**2**) was shown to be isomeric with **1** based on HRFABMS and ^1H and ^{13}C NMR data (Table 1). The UV spectrum and ^1H NMR data of **2**, however, revealed the presence of two conjugated dienes, one of which clearly coupled to the C-6 proton, while the other clearly coupled to the C-17 methylene. All double bonds were assigned a trans configuration based on observed olefinic coupling constants of at least 14.2 Hz (Table 1). The relative stereochemistry of the piperidinol ring of **2** was identical to that of **1**, based on the 9.5-Hz coupling constant between the C-2 and C-3 protons and NOE interactions (C-2 to C-6 axial protons).

Corydendramines A and B (**1** and **2**) are similar in structure to 2,6-dialkyl-3-piperidinols previously isolated from plants and fire-ant venom[4] and are only the second group of hydroid secondary metabolites shown to deter potential predators.[3] Stachowicz and Lindquist[5] previously demonstrated that the novel dithiocarbamate natural product, tridentatol A, isolated from the hydroid *Tridentata marginata*[2e] is also a potent fish feeding-deterrent and may, in addition, function as a sunscreen. Although the unpalatability of hydroids has often been attributed to nematocysts, four of the six species investigated by Stachowicz and Lindquist[3] yielded lipophilic extracts that significantly reduced feeding by a potential hydroid predator. These four species are not closely related [each is in a separate family spanning two suborders (athecate and thecate)], suggesting that chemical defenses may be common among taxonomically diverse hydroids.

Experimental Section

General Experimental Procedures. UV and IR spectra were obtained on a Beckman DU-640 and Perkin-Elmer 1600 FTIR spectrometer, respectively. 1D ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) and 2D NMR spectra were recorded on a

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Assignments for Corydendramines A (**1**) and B (**2**)[a]

carbon no.	**1**		**2**	
	δ^{13}C	δ^1H	δ^{13}C	δ^1H
1	16.8	1.30 (3H, d, J = 6.3 Hz)	17.4	1.20 (3H, d, J = 6.3 Hz)
2	58.9	2.79 (1H, dq, J = 10.0, 6.3 Hz)	59.2	2.56 (1H, dq, J = 9.5, 6.3 Hz)
3	71.6	3.27 (1H, ddd, J = 10.2, 10.2, 4.3 Hz)	73.0	3.12 (1H, ddd, J = 4.5, 9.5, 9.5 Hz)
4	34.0	1.54 (1H, ax, m)	34.0	1.41 (1H, ax, m)
		2.08 (1H, eq, m)		2.01 (1H, eq, m)
5	30.3	1.54 (1H, ax, m)	32.0	1.41 (1H, ax, m)
		1.91 (1H, eq, m)		1.78 (1H, eq, m)
6	59.6	3.54 (1H, bddd, J = 11, 8, 3 Hz)	59.5	3.25 (1H, m)
7	129.4	5.56 (1H, dd, J = 15.3, 7.8 Hz)	132.0	5.50 (1H, dd, J = 15.1, 7.3 Hz)
8	130.2	6.37 (1H, dd, J = 15.3, 10.5 Hz)	133.4	6.21 (1H, dd, J = 15.1, 10.4 Hz)
9	136.3	6.12 (1H, dd, J = 15.2, 10.5 Hz)	131.3	6.03 (1H, dd, J = 15.0, 10.2 Hz)
10	137.1	6.28 (1H, dd, J = 15.0, 10.5 Hz)	135.7	5.71 (1H, dt, J = 14.8, 6.8 Hz)
11	131.5	6.09 (1H, dd, J = 15.2, 10.5 Hz)	33.7	2.15 (2H, m)
12	137.1	5.77 (1H, dt, J = 15.2, 7.0 Hz)	33.7	2.15 (2H, m)
13	33.2	2.16 (2H, dt, J = 7.0, 7.0 Hz)	132.0	5.52 (1H, dt, J = 14.7, 6.8 Hz)
14	33.3	2.08 (2H, m)	132.3	5.98 (1H, m)
15	129.5	5.47 (1H, dt, J = 15.2, 6.2 Hz)	132.7	5.96 (1H, m)
16	133.7	5.40 (1H, dt, J = 15.2, 6.2 Hz)	134.9	5.58 (1H, dt J = 14.2, 6.8 Hz)
17	26.5	1.99 (2H, dq, J = 6.2, 7.4 Hz)	26.5	2.06 (2H, dq, J = 6.8, 7.3 Hz)
18	14.3	0.95 (3H t, J = 7.4 Hz)	14.3	0.98 (3H, t, J = 7.3 Hz)

[a] Measured in MeOH-d_4 with TMS as internal standard.

Varian INOVA 500 spectrometer, with TMS as internal standard. MS data were recorded on a JEOL SX102 mass spectrometer.

Animal Material. *C. parasiticum* was collected from floating docks in the Intracoastal Waterway near Wrightsville Beach, NC. A voucher (5M91-H1) of this hydroid is deposited at the Institute of Marine Sciences. Dale Calder of the Royal Ontario Museum is gratefully acknowledged for identifying the hydroid.

Extraction and Isolation. The CH_2Cl_2/MeOH extract of the frozen hydroid (56 g wet mass) was evaporated by rotary evaporation, combined with the water extract of the hydroid, and then partitioned between water and CH_2Cl_2. The water-soluble material was further partitioned between butyl alcohol and water. The CH_2Cl_2-soluble and butyl alcohol-soluble compounds were combined and then partitioned between hexanes and MeOH. Size-exclusion chromatography over a 1 × 30 cm bed of Sephadex LH-20 with MeOH as the eluting solvent was then used to fractionate the MeOH-soluble material, which contained the feeding deterrent.[3] Of the 25 fractions collected (ca. 5 mL each), three groups of sequentially eluted fractions were recombined based on their TLC characteristics. The second grouping was the only one that deterred fish feeding.[3] This active group of compounds was further fractionated by thick layer chromatography using a 20 × 20 cm glass plate having a 1000 μm thick layer of Si gel (Whatman) and sequential development with 0, 10, 25, and 40% MeOH in diethyl ether. This procedure distributed the compounds into four discrete bands based on UV fluorescence and acid charring visualization. The feeding deterrents were finally purified from the active thick-plate bands using C_{18} reversed-phase HPLC (4.6 × 250 mm column, 1 mL/min, and gradient elution with 60% MeOH/0.1 M $AcONH_4$ as the initial condition ramping to 70% MeOH/0.1 M $AcONH_4$ in 20 min) to yield **1** (1.0 mg) and **2** (1.6 mg).

Corydendramine A (1): pale yellow oil; $[\alpha]_D$ −24.3° (*c* 0.17, MeOH); IR (NaCl) 2950 (br), 2800 (br) cm^{-1}; UV (MeOH) λ_{max} 258 (ϵ 24 200), 268 (ϵ 32 700), 278 (ϵ 25 600); HRFABMS *m/z* obsd $[M + H]^+$ 276.2341 (calcd for $C_{18}H_{30}NO$, 276.2327); ^{1}H and ^{13}C NMR, see Table 1.

Corydendramine B (2): amorphous white powder; $[\alpha]_D$ +83.7° (*c* 0.083, MeOH); IR (NaCl) 2950 (br), 2850 (br) cm^{-1}; UV (MeOH) λ_{max} 236 (ϵ 40 400); HRFABMS *m/z* obsd $[M + H]^+$ 276.2332 (calcd for $C_{18}H_{30}NO$, 276.2327); ^{1}H NMR and ^{13}C NMR, see Table 1.

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