Ainigmaptilones, Sesquiterpenes from the Antarctic Gorgonian Coral *Ainigmaptilon antarcticus*

Katrin B. Iken[†] and Bill J. Baker*[,‡]

Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, and Department of Chemistry, University of South Florida, 4202 E. Fowler Avenue SCA 400, Tampa, Florida 33620

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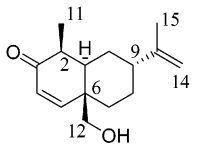
Antarctic octocorals from the Weddell Sea of Western Antarctica appear to select either physical or chemical means of defense. Fractionation of the bioactive extract from the chemically defended Antarctic gorgonian coral *Ainigmaptilon antarcticus* yielded two sesquiterpenes, ainigmaptilones A (**1**) and B (**2**). Ainigmaptilone A has broad spectrum bioactivity toward sympatric predatory and fouling organisms, including antibiotic activity.

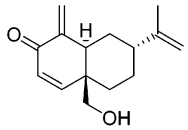
Gorgonian corals are known as "horny corals" due to their flexible, hornlike, endoskeleton, made of the proteinaceous material gorgonin,[1] from which individual animals branch. The collective group of individual animals, or polyps, arrayed on the endoskeleton constitute the coral colony. While the colony lacks the calcareous exoskeleton that protects the reef-building scleractinian corals, polyps of a gorgonian colony share the common Cnidarian protective nematocysts, or stinging cells, and the soft outer tissues may additionally be protected from predation by the presence of calcareous spicules or chemical toxins. Among seven deep-water Antarctic octocorals, two morphologies are distinguishable: four of these gorgonians are replete with spicules and yield chemical extracts that are palatable to the common Antarctic predaceous sea star *Odontaster validus*, while three are devoid of spicules but produce extracts that deter predation by *O. validus*.[2] Thus it appears these deep-water Antarctic horny corals select either physical (spicules) or chemical means of defense. We have subjected one of the chemically defended Antarctic horny corals, *Ainigmaptilon antarcticus*, to chemical analysis and report herein bioactive sesquiterpenes based on the eudesmane carbon skeleton. Eudesmane sesquiterpenes are uncommon in corals, having been reported only from two other (Alcyonacean) corals.[3−5]

Ainigmaptilon antarcticus was collected by trawling during a 1998 cruise of the RV Polarstern (Germany) in the Eastern Weddell Sea of Western Antarctica. Frozen tissues were extracted with acetone, and the concentrated slurry was partitioned with ethyl ether. Fractionation of the ether partition fraction was carried out using step gradient flash chromatography on silica gel. The fraction eluting with 25% ethyl acetate in hexane was further purified by HPLC to yield two compounds, ainigmaptilones A (**1**) and B (**2**), displaying ^{1}H NMR signals characteristics of terpenes.

Ainigmaptilone A (**1**) was obtained as a yellow oil. Analysis of the ^{1}H NMR spectrum revealed one vinyl and one aliphatic methyl group, three olefinic, and two downfield *H*-C−O signals. A fourth olefinic signal could be observed at 300 K; the water signal in CD_3OD moved upfield to δ 4.73, revealing the previously coincident olefinic resonance at δ 4.84. Fifteen signals were evident in the ^{13}C NMR spectrum, including one ketone, one exomethylene, two CH, and one quaternary olefin carbon, an oxygen-bearing methylene, one aliphatic quaternary carbon, and eight aliphatic carbons, of which three each were methine and methylene and two were methyl groups. Assignments of carbon and proton count were corroborated by high-resolution electron impact mass spectrometry, which provided a molecular formula of $C_{15}H_{22}O_2$ (Δ 0.1 mmu).



Ainigmaptilone A (**1**)



Ainigmaptilone B (**2**)

The COSY spectrum of ainigmaptilone A (**1**) yielded four spin systems. Two mutually coupled olefinic doublets appeared conjugated to the ketone on the basis of the low-field (δ 6.97) shift of the β-proton. Two other olefinic protons were mutually coupled and located on the same carbon on the basis of analysis of the HMQC spectrum; these two olefinic protons also coupled to a vinyl methyl group at δ 1.74, describing an isopropylidene group. Besides two, coupled, oxygen-bearing methylene protons, the remaining protons were part of a contiguous spin system which was unambiguously connected to the other three spin systems by analysis of the HMBC spectrum (Table 1).

The mass spectrum of ainigmaptilone A (**1**) suggests a propensity for aromatization of the enone-bearing ring. The major fragmentation pattern appears to occur on the rearrangement product **3**, resulting in a series of homologous fragmentations (Figure 1).

Ainigmaptilone B (**2**) was two mass units less than ainigmaptilone A. The ^{1}H NMR spectrum displayed one fewer methyl group and two additional, mutually coupled, olefinic protons, relative to ainigmaptilone A. The HMBC (Table 1) demonstrated the new olefinic signals to correlate with C-1, -2, and -3 and H-1 moved downfield 0.5 ppm, suggesting the C-2 methyl group of ainigmaptilone A was an exomethylene in ainigmaptilone B. Further COSY, HSQC, and HMBC data supported the assignment.

Stereochemical analysis of ainigmaptilones A (**1**) and B (**2**) could be accomplished by NOE studies. Defining relationships were observed by 1D difference NOE in ainigmaptilone A (Figure 2). Irradiation of the vinyl proton

* To whom correspondence should be addressed. Tel: (813) 974-1967. Fax: (813) 974-1733. E-mail: bjbaker@chuma1.cas.usf.edu.
[†] Alfred Wegener Institute. Current address: Institute of Marine Science, School of Fisheries and Ocean Sciences, P.O. Box 757220, University of Alaska Fairbanks, Fairbanks, AK 99775-7220.
[‡] University of South Florida.

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Table 1. NMR Data for Ainigmaptilones A (**1**)[a] and B (**2**)[b]

	1			**2**		
position	δ_H (m, *J* (Hz))	δ_C[c]	HMBC[e]	δ_H (m, *J* (Hz))	δ_C[d]	HMBC[e]
1	2.28 (dt, 2.7, 6.6)	40.8 (CH)	2, 10, 12	2.83 (ddd, 2.6, 2.8, 12.6)	42.1 (CH)	2, 6, 12
2	2.33 (br q, 7.2)	45.9 (CH)	1, 3, 6, 11		146.0 (C)	
3		205.8 (C)			188.9 (C)	
4	5.91 (d, 10.3)	128.1 (CH)	2, 6	6.20 (d, 9.9)	129.2 (CH)	2, 6
5	6.97 (d, 10.1)	162.9 (CH)	1, 3, 6, 7, 12	6.88 (d, 10.0)	158.9 (CH)	1, 3, 7, 12
6		43.5 (C)			42.8 (C)	
7	eq 2.02 (dt, 5.4, 13.3)	29.6 (CH_2)	6, 9	1.88 (ddd, 3.7, 3.7, 7.0)	27.6 (CH_2)	1, 5, 8, 9, 12
	ax 1.33 (ddd, 8.2, 12.2, 13.1)		5, 6, 8, 12	1.62 (dt, 4.1, 13.4)		5, 6, 8, 9, 12
8	eq 1.96 (m)	24.5 (CH_2)	6, 7, 9, 10	2.03 (dd, 2.6, 11.9)	23.1 (CH_2)	9, 10, 13
	ax 1.96 (m)		6, 7, 9, 10	1.85 (m)		7, 13
9	2.47 (br s)	39.9 (CH)		2.55 (br s)	37.6 (CH)	1, 7, 8, 10, 13, 14
10	eq 1.65 (dd, 0.9, 13.6)	28.0 (CH_2)	2, 6, 8, 9, 13	2.13 (dd, 2.3, 11.9)	25.1 (CH_2)	1, 8, 9, 13
	ax 1.90 (m)		1, 6, 13	1.76 (m)		1, 9, 13
11	1.10 (d, 7.6)	14.6 (CH_3)	1, 2, 3	*E* 6.12 (dd, 1.0, 1.2)	117.5 (CH_2)	1, 2, 3
				Z 5.25 (dd, 1.0, 1.2)		1, 3
12	α 4.12 (d, 10.8)	65.0 (CH_2)	1, 5, 6, 7	3.88 (d, 10.9)	64.1 (CH_2)	1, 5, 7
	β 3.72 (d, 10.8)		1, 5, 6, 7	3.78 (10.9)		1, 5, 7
13		147.9 (C)			145.7 (C)	
14	*E* 4.93 (q, 1.5)	111.8 (CH_2)	9, 13, 15	4.97 (d, 1.1)	111.5 (CH_2)	9, 13, 15
	Z 4.84 (s)		9, 13, 15	4.83 (s)		9, 13, 15
15	1.74 (s)	23.1 (CH_3)	9, 13, 14	1.76 (s)	22.7 (CH_3)	9, 13, 14

[a] Recorded at 400/125 MHz in CD_3OD. [b]Recorded at 600/150 MHz in $CDCl_3$. [c] HMQC and DEPT. [d] HSQC. [e]Carbon position.

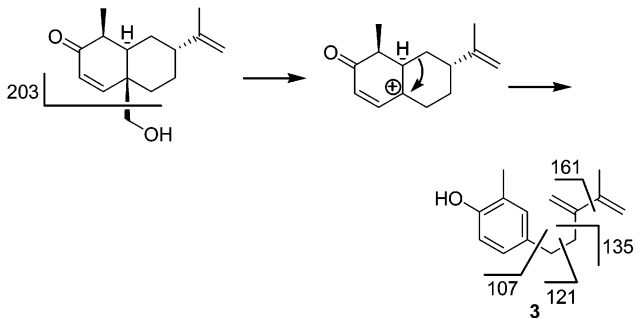

Figure 1. EIMS fragmentation pathway in ainigmaptilone A (**1**).

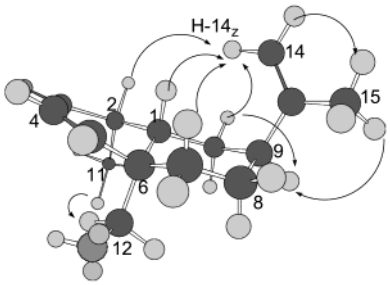

Figure 2. Key NOE relationships observed in ainigmaptilone A (**1**) superimposed on Chem3D energy-minimized model.

H-14_Z (δ 4.84) resulted in enhancements of axial protons on C-1 and C-7, the pseudoequatorial methine at C-2, and equitorial methylene protons on C-8 and -10. This relationship places the isopropylidene and C-2 methyl groups on the opposite face of the decalin. Irridiation of either H_3-11 (δ 1.10) or H_α-12 (δ 4.12) resulted in enhancement of the other, placing the C-2 methyl and the hydroxymethylene on the same face of a *trans*-decalin. Ainigmaptilone B was subjected to 2D NOESY analysis, which showed similar relationships observed in ainigmaptilone A by difference NOE. Thus, H-14_Z displayed correlations with C-1 and -7 axial hydrogens and C-8 and -10 equatorial protons.

Ainigmaptilone A (**1**) was evaluated in antipredator assays and found to significantly inhibit predation by *O. validus*, the details of which will be reported elsewhere.[2] It was also examined for bioactivity in two laboratory-based assays. It showed antibiotic activity toward three of four sympatric Antarctic bacteria[6] at levels as low as 5 mg/mL and was lethal to sympatric diatoms[7] at natural tissue-level concentration (0.275 mg/mL). Ainigmaptilone A is clearly a defensive metabolite elaborated by *A. antarcticus* for a wide variety of protective purposes. An isomer of ainigmaptilone A reported[8] from an unidentified Southern Ocean sponge may originate from *A. antarcticus*.

Experimental Section

Ainigmaptilon antarcticus was collected at a depth of ca. 400 m by trawling during a Sept−Nov 1998 cruise of the RV Polarstern, German expedition ANTXV/3 (Eastern Weddell Sea, Western Antarctica). Fresh tissues were either prepared for bioassay or extracted in acetone; details of the bioassay will be reported elsewhere.[2] The coral (123.2 g wet; 15.17 g dry after extraction) was extracted with acetone (3×), and the combined acetone extracts were concentrated to a slurry. The slurry was partitioned with diethyl ether (3×), and the combined ether extracts were concentrated to yield 1.56 g of lipophilic extract. This lipophilic extract was bioassayed and found to deter predation by the common antarctic predator *Odontaster validus*.[3] Fractionation of 433.6 mg of the lipophilic extract was carried out by step gradient flash chromatography (20 cm × 1.5 cm; 100 mL column volume) using 200 mL each hexane, then 10%, 25%, 50%, and 100% ethyl acetate in hexane, followed by 10% methanol in ethyl acetate. The 50% ethyl acetate in hexane fraction (62.8 mg) was further purified by HPLC (Phenomonex 5 μ silica, 10 mm × 25 cm, 25% ethyl acetate in hexane) to yield 10.8 mg (0.23% dry weight) of ainigmaptilone A (**1**) and 5.5 mg of ainigmaptilone B (**2**).

Ainigmaptilone A (1): yellow oil; $[\alpha]_D^{24}$ 26.8° (*c* 0.23, $CHCl_3$); IR (thin film) 3400 (br), 2925, 1700, 1650, 1035 cm^{-1}; UV (MeOH) λ_{max} 244 (ϵ 8000) nm; ^1H and ^{13}C NMR, see Table 1; 70 eV EIMS (%) 234 (20), 216 (15), 204 (90), 203 (75), 161 (90), 147 (85), 135 (70), 121 (70), 107 (65), 91 (100), 77 (70), 67 (50); HREIMS, 234.1621 *m*/*z* (calcd for $C_{15}H_{22}O_2$, 234.1620).

Ainigmaptilone B (2): colorless oil; $[\alpha]_D^{24}$ −63.1° (*c* 0.23, $CHCl_3$); IR (thin film) 3400 (br), 2929, 2856, 1665, 1620, 1038 cm^{-1}; UV (MeOH) λ_{max} 241 (ϵ 750), 275 (sh) (ϵ 650) nm; ^1H and ^{13}C NMR, see Table 1; FABMS (%) 233 (2), 195 (20), 155 (42), 135 (52), 119 (100) *m*/*z*.

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Supporting Information Available: ^{1}H, COSY, HMQC, HMBC (CD_3OD, 400 MHz), and ^{13}C (CD_3OD, 125 MHz) NMR spectra for ainigmaptilone A (**1**); ^{1}H, COSY, HSQC, HMBC, NOESY ($CDCl_3$, 600 MHz), and ^{13}C ($CDCl_3$, 150 MHz) NMR spectra for ainigmaptilone B (**2**). Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

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