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Zoamides A-D: New Marine Zoanthoxanthin Class Alkaloids from an Encrusting Philippine *Parazoanthus* sp.

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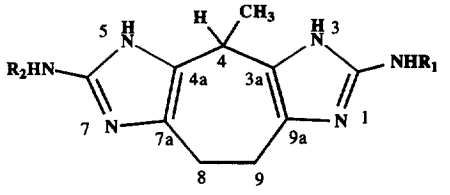
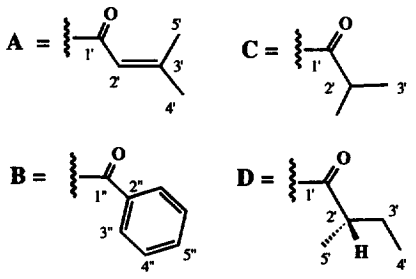
Abstract: Four 4,5,8,9-tetrahydro-zoanthoxanthin amides, zoamides A-D (**1-4**), have been isolated from an undescribed Philippine *Parazoanthus* sp. The structures of the zoamides were assigned primarily by 2-D NMR methods. © 1997, Published by Elsevier Science Ltd. All rights reserved.

The most common metabolites encountered from marine zoanthids are a group of nitrogenous, highly fluorescent aromatic alkaloids represented by two classes, the parazoanthoxanthins and the pseudozoanthoxanthins, both of which appear to be derived from arginine condensation.[2,3] Since the discovery of zoanthoxanthin, the first member of this class, in 1973,[4] approximately 20 related compounds have been reported.[2] As part of our ongoing program to investigate the natural products from diverse marine coelenterates, a dark black undescribed *Parazoanthus* sp. was collected in March, 1988 near Siquior Island, Philippines. The organism occurred as an encrusting epibiont on dead gorgonian corals, thus extraction required removing the zoanthids from the gorgonian endoskeleton. Extraction of the freeze-dried animal (86 g) with 1/1 chloroform/methanol, followed by removal of solvent under vacuum, and purification of the condensed extract (2.1 g) by reversed-phase (40-65 μm RP-18) vacuum flash chromatography (VFC) using a MeOH/H_2O gradient, led to isolation of the zoamides A-D (**1-4**) as mixtures in the medium polarity fractions. The mixtures were next purified by normal phase chromatography, first on silica gel (70-230 mesh, EtOAc/HOAc 99:1) and then on a cyanopropyl column (isooctane/EtOAc/HOAc, 70:30:0.2) yielding pure samples of zoamides A-D (**1**, 63 mg; **2**, 35 mg; **3**, 95 mg and **4**, 45 mg).

On spectroscopic analysis it was observed that the zoamides had been isolated as their acetate salts (^{13}C NMR δ: 21.6 CH_3; 175.0 CO), apparently produced during purification by acidification with HOAc. The zoamides could be readily reconverted to their free bases by partitioning the water-soluble salts between



1, zoamide A, $R_1 = R_2 = A$
2, zoamide B, $R_1 = B$, $R_2 = A$
3, zoamide C, $R_1 = B$, $R_2 = C$
4, zoamide D, $R_1 = B$, $R_2 = D$



saturated $NaHCO_3$ and EtOAc solutions. Although the free bases were stable, many of their NMR bands were broadened. Thus, NMR analyses were conducted using the acetate salts (see Table).

Zoamide A (**1**) was obtained as a gummy semi-solid which analysed for $C_{20}H_{27}N_6O_2$ $(M+H)^+$ by HRFABMS and ^{13}C NMR methods.[5] The IR spectrum of **1** showed absorption bands at 3280 and 1667 cm^{-1} characteristic of NH and amide carbonyl functionalities. UV absorption maxima at 200 and 290 nm (ε 20500, 18500) indicated considerable unsaturation. Although the molecular formula for **1** was secure, the NMR spectra showed only 12 of the requisite 20 carbons and approximately half of the protons present in the molecule. On this basis, zoamide A was concluded to possess a symmetry plane. The observation of 12 carbons indicated that the symmetry plane dissected two carbon atoms. Those carbons could easily be recognized as an methyl bearing methine carbon (-CH-CH_3) by virtue of characteristic NMR bands observed at half the intensity of the others [^{13}C NMR: δ 32.2 (CH), and δ 24.9 (CH_3); ^1H NMR: δ 4.10 (q, J = 7.2) and δ 1.52 (d, J = 7.2)]. Other prominent structural features, assigned by 2-D NMR methods, were an isolated ethylene linkage, C8 - C9 (bissected by the symmetry plane), and two amide functionalities derived from 3-methyl-2-butenoic acid.

Because of the source of the animal, and the spectral characteristics of **1**, the zoamides were concluded to be related to the zoanthoxanthins. Subsequent NMR analyses allowed all protons and carbons to be assigned resulting in a confident structure assignment for zoamide A (**1**). Proton - ^{13}C long range correlations allowed the cycloheptadiene ring to be constructed incorporating the -CH-CH_3 and -CH_2-CH_2- symmetry plane elements. What remained was the construction of the imidazole rings and locating the site of amide formation. The imidazole rings were confirmed on the basis of UV absorptions and the presence of the highly characteristic guanidinium carbons [C-2, -6 observed at δ 140.1 (C)] and two olefininic carbons [δ130.1, 126.1 (2C)]. Three feasible locations for the amides, acylation of the imidazole ring nitrogens at N-1(N-7) or N-3 (N-5), and acylation at the 2-amine location were considered. First, amide formation involving the ring nitrogens is known to generate N-acyl imidazoles with IR carbonyl absorptions at 1745 cm^{-1},[6] not the more typical amide frequencies (ca. 1667 cm^{-1}) observed here. An N-acyl imidazole can also be ruled out because they typically show UV absorptions at 245 nm rather than the 290 nm absorptions we observe.[7] In fact, 290 nm absorptions are typically of 2-aminoacyl imidazoles, examples of which are intermediates prepared during the syntheses of the parazoanthoxanthins.[8] Lastly, NOE studies failed to show any correlations between the α to carbonyl (C-2') protons and either the C-4 methine or methyl protons, or the C-8 (-9) methylene protons. Models show that the C-2' amide protons would indeed be within NOE proximity with these latter protons. On the basis of these observations, the amides were placed at the 2-amino imidazole positions.

In contrast to **1**, zoamide B (**2**) analysed for $C_{22}H_{25}N_6O_2$ $(M+H)^+$ by HRFABMS and combined spectral methods. In this case, the molecule lacked the symmetry plane and all carbons and protons were resolved. Zoamide B showed almost identical IR and UV absorptions as **1**, thus it was clear that the molecule was a mixed bis-amide. By NMR, one of the amides was also shown to be derived from 3-methyl-2-butenoic acid (A). NMR data also demonstrated the presence of a benzoic acid amide (B) by its characteristic ^1H and ^{13}C NMR bands (Table).

Zoamide C (**3**) analysed for $C_{21}H_{24}N_6O_2$ by HREIMS and combined spectral methods. All 21 carbons were readily observed, thus the symmetry plane was also lacking in this derivative. NMR data showed the presence of a benzoic acid amide and a C_4 amide which was assigned as isobutryl amide (C) by COSY NMR methods. Given that all other data were analogous to **1**, the compound was assigned as structure **3**.

Table. ^{1}H and ^{13}C NMR Assignments for Zoamides A-D (**1-4**)*.

	Zoamide A (1)		Zoamide B (2)		Zoamide C (3)		Zoamide D (4)	
C#	**$^{1}H^{a}$**	**$^{13}C^{a}$**	**$^{1}H^{b}$**	**$^{13}C^{b}$**	**$^{1}H^{b}$**	**$^{13}C^{b}$**	**$^{1}H^{b}$**	**$^{13}C^{b}$**
2		140.1		140.3		141.1		140.8
3a		130.1		130.6		129.7		129.8
4	4.10 (q, J = 7.2)	32.2	4.02 (q, J = 7.2)	32.6	4.03 (q, J = 7.2)	32.1	4.03 (q, J = 7.2)	32.2
4 (CH_3)	1.52 (d, J = 7.2)	24.9	1.50 (d, J = 7.2)	24.4	1.49 (d, J = 7.2)	24.5	1.49 (d, J = 7.2)	24.6
4a		130.1		130.7		129.6		129.8
6		140.1		141.2		140.3		139.9
7a		126.1		126.5		126.0		127.4
8	2.94 (m)	25.0	2.90 (bm)	25.4	2.88 (bm)	25.0	2.88 (bm)	24.9
9	2.94 (m)	25.0	2.90 (bm)	25.4	2.88 (bm)	25.0	2.88 (bm)	24.9
9a		126.1		126.7		126.0		127.4
1'		166.4		166.9		178.4		177.9
2'	5.95 (sep, J = 1.2)	118.4	5.88 (sep, J = 1.2)	118.2	2.66 (bd, J = 6.9)	36.6	2.45 (tq, J = 7.2, 7.2)	43.8
3'		157.7		156.6	1.20 (d, J = 6.9)	19.8	1.53 (ddd, J = 13.5, 7.2, 7.2) 1.71 (ddd, J = 13.5, 7.2, 7.2)	28.4
4'	1.98 (d, J = 1.2)	28.1	1.93 (d, J = 1.2)	27.5			0.92 (t, J = 7.2)	12.4
5'	2.28 (d, J = 1.2)	20.9	2.23 (d, J = 1.2	20.3			1.18 (d, J = 7.2)	17.9
1"				169.3		168.4		168.4
2"				135.3		134.5		134.6
3"			7.98 (dd, J = 7.5, 1.5)	128.9	7.99 (dd, J = 7.5, 1.5)	129.3	7.99 (dd, J = 7.5, 1.5)	129.3
4"			7.50 (bdd, J = 7.5, 7.5)	129.6	7.46 (bdd, J = 7.5, 7.5)	129.9	7.46 (bdd, J = 7.5, 7.5)	130.0
5"			7.56 (tt, J = 7.5, 1.5)	133.2	7.52 (tt, J = 7.5, 1.5)	133.8	7.52 (tt, J = 7.5, 1.5)	133.9

* Spectra were acquired on the acetic acid salts of the zoamides A-D (**1-4**). ^{1}H and ^{13}C spectra were recorded at 200 MHz and 50 MHz, respectively. Spectra were recorded in a) 3/1 MeOH-d_4/DMSO-d_6, b) MeOH-d_4. Assignments are by COSY analysis and by 1, 2 and 3-bond XHCORR methods.

Zoamide D (**4**) analysed for $C_{22}H_{26}N_6O_2$ by HREIMS and combined spectral methods. In this case, the familiar benzoic acid amide was present, as was a new C_5 carboxylic substituent identified by NMR methods as 2-methyl butrylamide (D). Since C-2' is chiral in 2-methyl butrylamide, it remained to establish the absolute stereochemistry at that center. Treatment of **4** with excess Na_2O_2 in H_2O, followed by HCOOH acidification,[9] gave benzoic acid and 2-methyl butyric acid, respectively. The rotation recorded for the latter, $\alpha_D = +0.04^\circ$, indicated the acid was the S-(+) enantiomer (lit.$[\alpha]_D = +18^\circ$, neat)[10]

Of the zoamides, only zoamide D showed a measurable optical rotation at the sodium D line. We presume this is due to the chiral 2-methyl butrylamide component. But, it remains that the unsymmetrical zoamides B and C could be chiral at C-4. Since we failed to observe a rotation, the zoamides are either racemic or possess such small rotations that they are unmeasurable. Although it is clear that the zoamides are related to other zoanthoxanthin alkaloids, they differ significantly in their degrees of unsaturation and heteroaromaticity.

Acknowledgments

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References and Notes

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5. Additional data for **1-4**: For zoamide A (**1**): a gummy solid; $[\alpha]_D = 0^\circ$; HRFABMS: $(M+H)^+$ observed 383.2197, calc. for $C_{20}H_{27}N_6O_2$ 383.2195.; IR (film on NaCl): 3280, 1667, 1641, 1595 cm^{-1}; UV(MeOH): λ_{max} 200 nm (ε 20500), 290 nm (ε 18500). For zoamide B (**2**): a gummy solid; $[\alpha]_D = 0^\circ$; HRFABMS: $(M+H)^+$ observed 405.2043, calc. for $C_{22}H_{25}N_6O_2$ 405.2039; IR (film on NaCl): 3320, 1665, 1650, 1590 cm^{-1}; UV(MeOH): λ_{max} 220 nm (ε 29000), 290 nm (ε 23500); For zoamide C (**3**): a gummy solid; $[\alpha]_D = 0^\circ$; HREIMS: $(M)^+$ observed 392.1962, calc. for $C_{21}H_{24}N_6O_2$ 392.1960; IR (film on NaCl): 3300, 1650, 1600 cm^{-1}; UV(MeOH): λ_{max} 225 nm (ε 19600), 270 nm (ε 13600); For zoamide D (**4**): a gummy solid; $[\alpha]_D = 15.1^\circ$; HREIMS: $(M)^+$ observed 406.2095, calc. for $C_{22}H_{26}N_6O_2$ 406.2117; IR (film on NaCl): 3280, 1650, 1590 cm^{-1}; UV(MeOH): λ_{max} 230 nm (ε 17000), 270 nm (ε 14700).

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