THE ISOLATION AND STRUCTURES OF FIVE NEW ALKALOIDS, NORZOANTHAMINE, OXYZOANTHAMINE, NORZOANTHAMINONE, CYCLOZOANTHAMINE AND EPINORZOANTHAMINE

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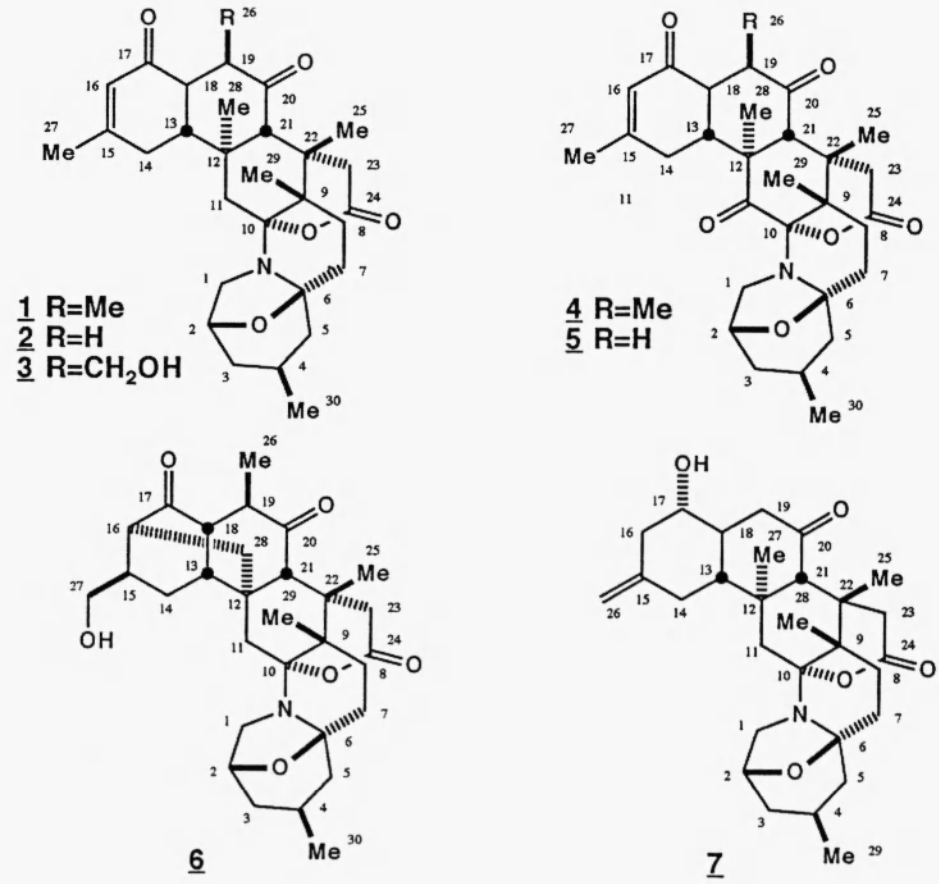
Abstract: Norzoanthamine, oxyzoanthamine, norzoanthaminone, cyclozoanthamine and epinorzoanthamine have been isolated from a colonial zoanthid *Zoanthus* sp. collected in the Amami Islands of Japan and their structures have been elucidated by detailed spectroscopic analysis and X-ray crystallographic analysis.

In our continuing search for physiologically active substances from marine organisms (1), we found five novel cytotoxic alkaloids, norzoanthamine, oxyzoanthamine, norzoanthaminone, cyclozoanthamine and epinorzoanthamine from the genus *Zoanthus* on the Ayamaru coast of the Amami Islands. They inhibited growth of P388 murine leukemia cells with IC_{50} values of 24, 7.0, 1.0, 24 and 2.6 μg/ml, respectively. Although a couple of zoanthamine derivatives possessing the unique heterocycles has been already reported (2), we wish to report herein the isolation and structures of newly isolated compounds of this series.

The wet specimens (5.0 kg) which occurred as dense mat were minced by a Waring blender and extracted with ethanol. The ethanolic extract was filtered and concentrated *in vacuo*. The aqueous residue was partitioned between ethyl acetate and water, and the water layer was subsequently extracted twice with ethyl acetate. The lipid-soluble extracts were chromatographed on silica gel (eluted with chloroform containing methanol), followed to be separated by preparative TLC on SiO_2 with acetonitrile, diethyl ether or ethyl acetate as solvent, giving zoanthamine **1** (6.3×10^{-3} %), norzoanthamine **2** as a colorless crystal [21 mg, 4.2×10^{-6} %, mp. 282~285°C; $[\alpha]_D$ 1.6° (c, 1.0, $CHCl_3$)], oxyzoanthamine **3** [9.1 mg, 1.8×10^{-6} %, $[\alpha]_D$ +5.3° (c 0.38, $CHCl_3$)], zoanthaminone **4** (2.5×10^{-6} %), norzoanthaminone **5** (8.2 mg, 1.6×10^{-6}%), cyclozoanthamine **6** [7.9 mg, 1.5×10^{-6} %, $[\alpha]_D$ -14.8° (c 0.42, $CHCl_3$)], and epinorzoanthamine **7** [3.1 mg, 0.6×10^{-6} %, $[\alpha]_D$ +67.4° (c 0.19, $CHCl_3$)], as colorless oily materials. Zoanthamine **1** and zoanthaminone **4** are the well-known, unique molecules (2).

The molecular formula of norzoanthaminone **2** was determined to be $C_{29}H_{39}NO_5$ from the HR-EIMS data. The ^1H, ^{13}C NMR data of **2** are shown in Table 1 and Table 2, respectively. Fortunately, recrystallization of norzoanthamine from methanol gave a well-formed, orthorhombic crystal, which had the space group $P2_12_12_1$. X-ray crystallographic analysis of norzoanthamine has been done and the structure of norzoanthamine has been unambiguously determined to be **2**. The computer-generated perspective drawing of a molecule of norzoanthamine is shown in Fig.1.

Oxyzoanthamine **3** showed the M^+ ion peak at *m/z* 511.2912 in the HR-EIMS, indicating the molecular formula of $C_{30}H_{41}NO_6$. In order to elucidate the structure of oxyzoanthamine **3**, it was necessary to assign all signals completely in both ^1H (Table 1) and ^{13}C NMR (Table 2). Assignment of all signals was performed by means of ^1H-^1H COSY and ^1H-^{13}C COSY spectra (3). As a result, oxyzoanthamine **3** might be biogenetically one of oxidation products of zoanthamine **1**. The presence of the $-CH_2OH$ moiety was deduced from the characteristic



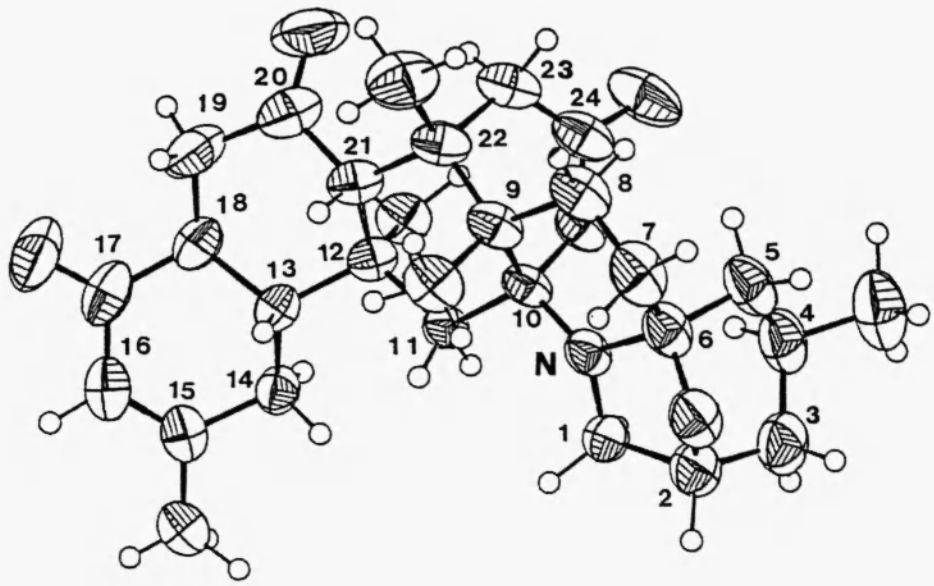


Fig. 1: The ORTEP drawing of norzoanthamine 2

signals in the ^{13}C NMR spectrum [δ 62.6 (t)] and ^{1}H NMR spectrum [δ 3.90, 1H, *J*= 11.3, 4.7 Hz and 3.99, 1H, *J*= 11.3, 6.5 Hz]. The stereochemistry was mainly determined by NOE experiments and the values of coupling constants (Fig.2). Oxyzoanthamine **3** was biogenetically considered to be the precursor of norzoanthamine **2** as described later.

Table 1: ^{1}H NMR data of norzoanthamine **2**, oxyzoanthamine **3** and norzoanthaminone **5**

C	**2**	**3**	**5**
1α	3.26 1H d 6.6	3.28 1H d 6.6	3.03 1H d 8.5
1β	3.21 1H dd 6.6, 4.5	3.23 1H dd 6.6, 5.9	3.99 1H dd 8.5, 7.3
2	4.57 1H m	4.54 1H m	4.53 1H m
3	1.45 1H br. t 11.9	1.26 1H m	1.44 1H dt 2.9, 11.9
3	1.54 1H m	1.58 1H m	1.54 1H m
4	2.20 1H	2.23 1H dd 6.6, 4.8	2.28 1H m
5	2.07 1H dd 12.7, 4.8	2.09 1H dd 13.2, 4.8	1.97 1H dd 13.3, 4.9
5	1.07 1H dd 12.7, 11.5	1.09 1H br. t 13.2	1.09 1H dd 13.3, 12.1
7	1.75 1H ddd 13.3, 4.6, 3.5	1.88 1H m	1.91 1H m
7	1.67 1H ddd 13.3, 9.4, 3.8	1.77 1H m	1.72 1H m
8	1.87 1H ddd 13.5, 9.4, 4.6	1.79 1H m	1.88 1H m
8	1.55 1H ddd 13.5, 3.8, 3.5	1.56 1H m	1.60 1H m
11	2.14 1H d 13.9	2.17 1H d 13.9	
11	1.89 1H d 13.9	1.92 1H d 13.9	
13	2.20 1H m	2.57 1H m	2.61 1H ddd 14.3, 3.7 12.4
14	1.32 1H m	2.25 2H m	2.27 1H dd 18.3, 12.4
14	2.28 1H m		3.36 1H dd 18.3, 3.6
16	5.90 1H br. s	5.92 1H s	5.91 1H br. s
18	2.70 1H ddd 11.8, 11.4, 6.6	2.78 1H dd 13.6, 5.9	2.71 1H ddd 14.3, 6.2 12.1
19	2.65 1H dd 14.4, 6.2	3.06 1H ddd 4.7, 5.9, 6.5	2.52 1H m
19	2.50 1H dd 14.4, 11.4		2.57 1H dd 18.3, 12.1
21	2.83 1H br. s	3.24 1H br. s	2.96 1H br. s
23	3.63 1H d 20.2	3.69 1H d 20.2	4.13 1H d 20.6
23	2.35 1H d 20.2	2.38 1H d 20.2	2.54 1H d 20.6
25	0.98 3H s	1.00 3H s	1.01 3H s
26	2.00 3H s	3.90 1H dd 11.3, 4.7	2.03 3H s
26		3.99 1H dd 11.3, 6.5	
27	0.99 3H s	2.02 3H s	1.28 3H s
28	1.15 3H s	1.00 3H s	1.06 3H s
29	0.89 3H d 6.6	1.18 3H s	0.89 3H d 6.6
30		0.91 3H d 6.6	

Spectra recorded on a JEOL JMN GSX-400 spectrometer in $CDCl_3$

Norzoanthaminone showed the M^+ ion peak at *m/z* 495.2635 in the HR-EIMS, indicating the molecular formulas of $C_{29}H_{37}NO_6$. A detailed comparison of the NMR data (Table 1) with those of **4** has reasonably suggested that the planar structure of norzoanthaminone are **5**. The stereochemistry of norzoanthaminone **5** was mainly determined by NOE experiments and from the values of coupling constants.

Cyclozoanthamine showed the M^+ ion peak at *m/z* 511.2922 in the HR-EIMS, indicating the molecular formula of $C_{30}H_{41}NO_6$. Complete assignment of proton, carbon signals (Tables 3 and 4) has been done by the aid of the ^{1}H-^{1}H DQF-COSY, ^{1}H-^{13}C COSY and HMBC (4) spectra, and those data have eventually proposed cyclozoanthamine has the planar structure of **6**. The stereochemistry of **6** was mainly determined by NOE experiments and from the values of coupling constants (Fig.3). Since the coupling constant between H18 and H19

Table 2: ^{13}C NMR data of norzoanthamine **2**, oxyzoanthamine **3** and norzoanthaminone **5**

C	**2**		**3**		**5**	
1	47.1	t	47.7	t	48.4	t
2	74.2	d	74.2	d	74.6	d
3	38.8	t	39.4	t	38.6	t
4	22.9	d	23.5	d	22.7	d
5	44.3	t	44.9	t	44.0	t
6	90.0	s	90.0	s	90.6	s
7	29.9	t	30.5	t	29.8	t
8	23.6	t	24.3	t	24.2	t
9	36.4	s	40.5	s	43.0	s
10	101.5	s	101.8	s	103.2	s
11	41.8	t	42.8	t	202.7	s
12	39.7	s	36.9	s	53.3	s
13	53.0	d	50.6	d	42.6	d
14	31.9	t	31.8	t	34.3	t
15	159.9	s	160.9	s	162.1	s
16	125.5	d	126.4	d	124.6	d
17	198.4	s	198.5	s	198.0	s
18	46.3	d	48.1	d	48.2	d
19	42.4	t	54.5	d	46.8	t
20	209.0	s	210.0	s	207.8	s
21	59.0	d	57.0	d	59.5	d
22	39.8	s	39.7	s	37.3	s
23	35.8	t	36.7	t	35.0	t
24	172.4	s	172.5	s	170.9	s
25	21.0	q	19.3	q	21.3	q
26	24.3	q	62.6	t	24.3	q
27	18.3	d	25.0	q	16.4	q
28	18.4	t	21.2	q	17.3	q
29	21.8	q	19.0	q	21.7	q
30			22.3	q		

is 1.1 Hz, it has been suggested that both protons are equatorial protons and C26 corresponds to axial methyl group. The NOE observation between H13 and H21 means that they are axial protons.

Epinorzoanthamine **7** showed the M^+ ion peak at *m/z* 483.2980 in the HR-EIMS, indicating the molecular formula of $C_{29}H_{41}NO_5$. The ^1H-NMR spectrum (Table 3) indicated the presence of one exo methylene [δ 4.82, 2H, br. s] and oxymethine proton [δ 3.41, H17]. The ^{13}C-NMR spectrum (Table 4) indicated the presence of only one ketonic carbonyl [δ 209.2, C20] and one ester carbonyl [δ 172.7, C24] in comparison with zoanthamine **1**. Detailed analysis of the ^1H-^1H COSY, ^1H-^{13}C COSY spectra proposed the planar structure of **7**. The relative stereochemistry of **7** was mainly determined by NOE experiments and from the values of coupling constant (Fig.4) The coupling constant between H17 and H16a is 12.0 Hz, suggesting that they are axial protons and the hydroxyl at C17 is, therefore, equatorial.

The series of zoanthamines belongs to ultraordinary peculiar compounds of marine natural products (5). Interests of researchers of this field focused on biosynthesis of these molecules. Zoanthamines may be suspected to be triterpenoids because the carbon skeleton is composed of thirty carbon atoms. It is, however, impossible to understand by general isoprene rule. Considering their biogenesis, oxyzoanthamine **3** described here is one of very important metabolites. Now, we propose two possible pathways: i. **3** is seemed to be the precursor of norzoanthamine **2**; ii. the hydroxymethyl group of oxyzoanthamine **3** corresponds to one extra carbon atom and therefore, norzoanthamine (C29) may be the genuine precursor of zoanthamine (C30). The former means that

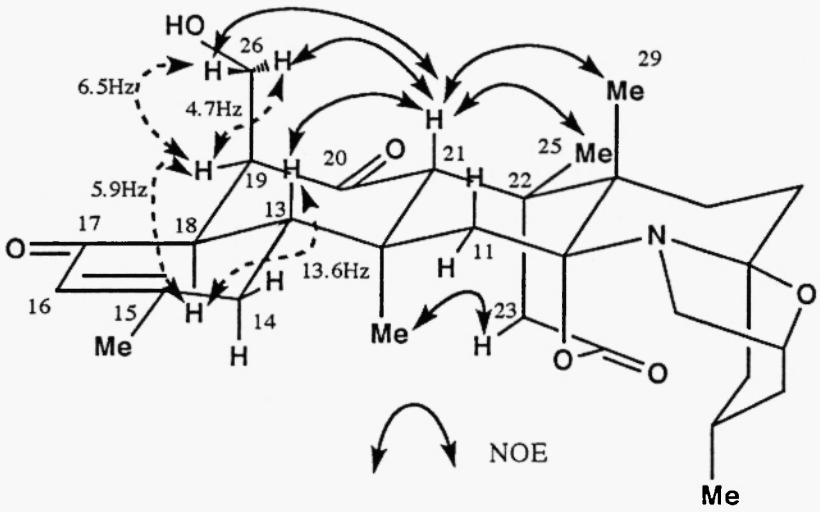


Fig. 2: Stereochemistry of oxyzoanthamine 3

Table 3: ^{1}H NMR data of cyclozoanthamine 6 and epinorzoanthamine 7

C	6	7
1α	3.33 1H d 6.6	3.28 1H d 6.6
1β	3.25 1H dd 6.6, 6.2	3.24 1H dd 6.6, 6.2
2	4.57 1H m	4.54 1H m
3	1.47 1H dd 11.7, 2.9	1.47 1H dd 11.7, 2.9
3	1.56 1H dd 11.7, 4.0	1.56 1H dd 11.7, 4.0
4	2.27 1H m	2.26 1H m
5	1.09 1H br. t 13.2	1.09 1H br. t 12.1
5	2.08 1H dd 13.2, 4.4	2.11 1H br. d 12.1
7	1.81 1H m	1.77 1H m
7	1.91 1H m	1.87 1H m
8	1.56 1H m	1.49 1H m
8	1.74 1H m	1.69 1H m
11	2.14 1H d 14.4	1.89 1H d 13.9
11	2.34 1H d 14.4	2.14 1H d 13.9
13	2.43 1H m	1.85 1H m
14α	2.28 1H m	1.82 1H m
14β	1.32 1H m	2.26 1H dd 12.8, 4.3
15	2.17 1H m	
16α	2.25 1H m	2.07 1H dd 12.5, 12.0
16β		2.66 1H dd 12.5, 4.8
17		3.41 1H m
18	2.26 1H m	1.50 1H m
19α	2.88 1H dq 1.1, 7.7	2.80 1H dd 11.1, 4.8
19β		2.12 1H m
21	3.16 1H br. s	2.90 1H br. s
23	2.37 1H d 20.2	2.33 1H d 20.2
23	3.49 1H d 20.2	3.64 1H d 20.2
25	0.99 3H s	1.18 3H s
26	1.35 3H s	4.82 2H br. s
27	3.39 1H dd 11.0, 5.9	0.95 3H s
27	3.49 1H dd 11.0, 5.9	
28	1.57 1H d 14.7	1.00 3H s
28	2.33 1H d 14.7	
29	1.20 3H s	0.91 3H d 6.2
30	0.91 3H d 6.6	

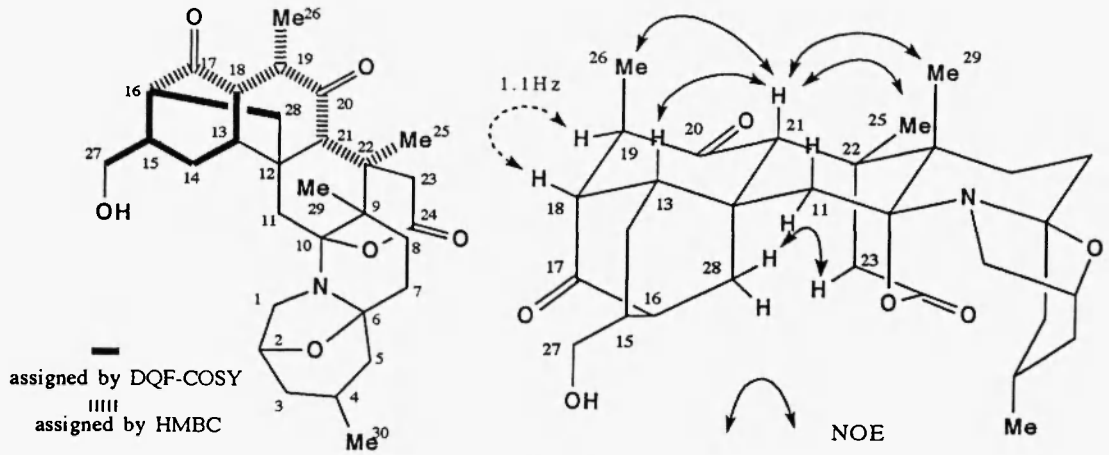


Fig. 3: Stereochemistry of cyclozoantrhamine **6**.

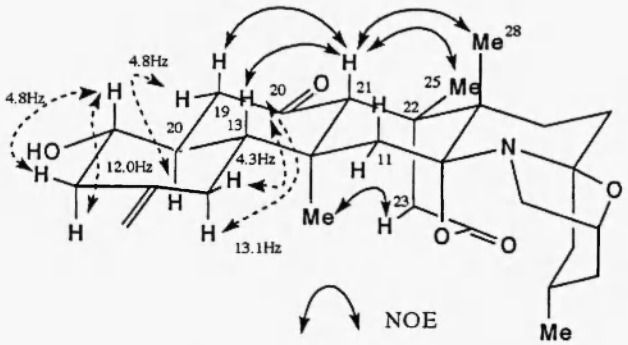


Fig. 4 : Stereochemistry of epinorzoanthamine **7**.

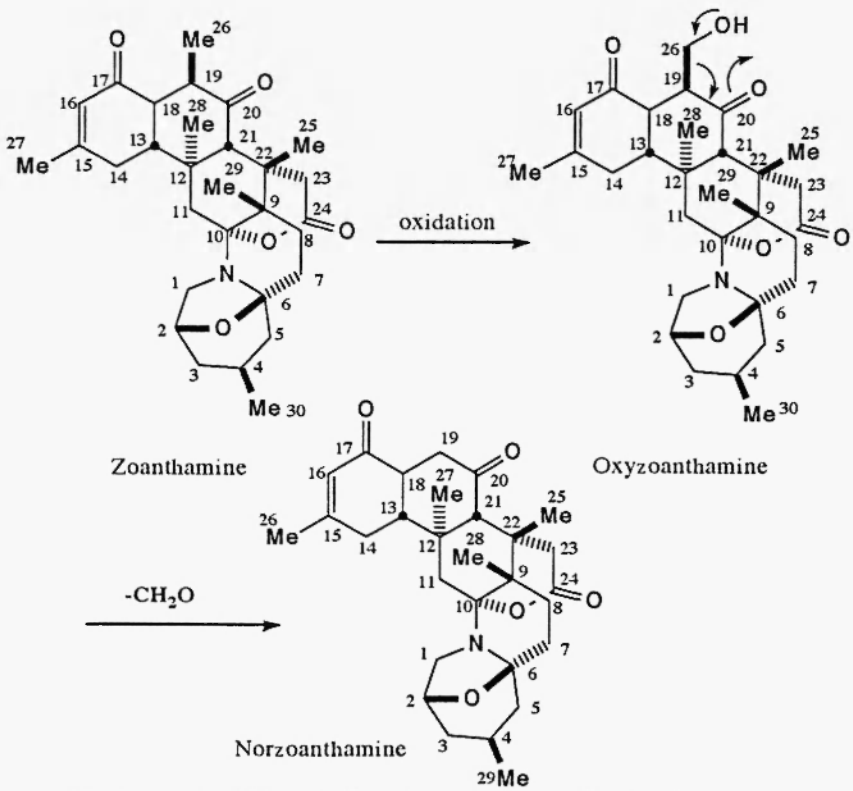


Fig. 5: Proposed biosynthetic pathway on norzoanthamine **2**

oxidation of zoanthamine gives oxyzoanthamine, which generates norzoanthamine by retro-aldol type reaction (Fig.5). Most interesting problem, however, must be biosynthsis of unusual carbon skeleton of this series. If the latter pathway does occur, it is expected that one of new concepts about unusual marine metabolites may be discovered. In addition, the carbon framework of cyclozoanthamine **7** is also characteristic. Since this point of view is extremely important and interesting, further investigation toward understanding of biosynthesis is necessary and now, in progress. Unless to say, the significant bioactivity (6) of this series must depend on the presence of unique heterocyclic rings.

Table 4: ^{13}C NMR data of cyclozoanthamine **6** and epinorzoanthamine **7**

C	**6**		**7**	
1	47.3	t	47.1	t
2	74.2	d	74.2	d
3	38.8	t	38.9	t
4	23.0	d	22.9	d
5	44.2	t	44.5	t
6	89.9	s	90.0	s
7	29.9	t	30.0	t
8	23.6	t	23.6	t
9	39.9	s	39.9	s
10	102.3	s	101.9	s
11	43.4	t	42.3	t
12	36.5	s	36.4	s
13	41.3	d	45.9	d
14	24.3	t	33.9	t
15	37.1	d	143.6	s
16	45.6	d	44.2	t
17	214.9	s	75.6	d
18	57.5	d	54.4	d
19	49.5	d	46.4	t
20	211.5	s	209.2	s
21	54.4	d	59.7	d
22	38.4	s	38.8	s
23	34.8	t	35.9	t
24	171.8	s	172.7	s
25	21.0	q	18.4	q
26	17.7	q	111.3	t
27	65.7	d	18.1	q
28	33.5	t	21.1	q
29	18.7	q	21.8	q
30	21.7	q		

Experimental Section

Field Collection and Extraction: *Zoanthus* sp. (5.0 kg) was collected in February 1992 on the Ayamaru coast of the Amami Islands Kagoshima Pref., Japan. This grey-colored invertebrate was freshly stored frozen. Upon workup, the marine zoantid was crushed and extracted by Waring blender with ethanol. The extract was filtered and their residue was rinced with methanol. The alcoholic extract was concentrated and the resulting residue was dissolved in water. This solution was extracted with ethyl acetate. The organic layer was concentrated

in vacuo to give us an oily material. Thus obtained extracts were then chromatographed on silica gel. Each fraction was eluted with mixtures of chloroform and methanol, guided by Dragendorff test.

Norzoanthamine 2: Purification by PTLC (CH_3CN) gave 21 mg (4.2 X 10^{-6} %) as a glassy material. Norzoanthamine showed $[\alpha]_D$ +1.6° (c 0.42, $CHCl_3$) and exhibited the following spectral properties: IR ($CHCl_3$) 3010, 2950, 1720, 1680, 1580, 1360, 1240 cm^{-1}; UV (MeOH) 234 nm; HR-EIMS, obsd *m/z* 481.2796, $C_{29}H_{39}NO_5$ requiring 481.2828.

Oxyzoanthamine 3: Purification by PTLC (EtOAc) gave 9.1 mg (1.8 X 10^{-6} %) as an oily material. Oxyzoanthamine showed the following physicochemical data: $[\alpha]_D$ +5.3° (c 0.38, $CHCl_3$); IR (KBr) 3410, 2950, 2930, 2870, 1710, 1660, 1240, 750 cm^{-1}; UV (MeOH) 236 nm; HR-EIMS, obsd *m/z* 511.2912, $C_{30}H_{41}NO_6$ requiring 511.2934.

Norzoanthaminone 5: Purification by PTLC (Et_2O) gave 8.2 mg (1.6 X 10^{-6} %) as a glassy material. Norzoanthaminone showed $[\alpha]_D$ -14.8° (c 0.42, $CHCl_3$) and exhibited the following spectral properties: IR ($CHCl_3$) 3010, 2950, 1720, 1680, 1580, 1360, 1240 cm^{-1}; UV (MeOH) 234 nm; HR-EIMS(20 eV), obsd *m/z* 495.2635, $C_{29}H_{37}NO_6$ requiring 495.2621.

Cyclozoanthamine 6: Purification by PTLC (EtOAc) gave 7.9 mg (1.5 X 10^{-6} %) as an oily material. Cyclozoanthamine showed $[\alpha]_D$ -14.8° (c 0.42, $CHCl_3$) and its spectral properties were as follows: IR (KBr) 3440, 2950, 2920, 2860, 1710, 1680, 1450, 1360, 1240, 950, 750 cm^{-1}; UV (MeOH) 210 nm; HR-EIMS, obsd *m/z* 511.2922, $C_{30}H_{41}NO_6$ requiring 511.2934.

Epinorzoanthamine 7: Purification by PTLC (EtOAc) give 3.1 mg (6.2 X 10^{-7}%) as an oily material. Epinorzoanthamine showed $[\alpha]_D$ +67.4° (c 0.19, $CHCl_3$) and exhibited the following spectral properties: IR (KBr): 3420, 2945, 2810, 1710, 1650, 1240, 750 cm^{-1}; UV (MeOH) 214 nm; HR-EIMS, obsd *m/z* 483.2980, $C_{29}H_{41}NO_5$ requiring 483.2985.

References

(1) D. Uemura, in: "Bioorganic Marine Chemistry," Vol. 4, P. J. Scheuer (Ed.), Springer-Verlag, Berlin Heidelberg, pp1-31 (1991)

(2) a) C. B. Rao, A. S. R. Anjaneyula, N. S. Sarma, Y. Venkatateswarlu, R. M. Rosser, D. J. Faulkner, M. H. M. Chen, and J. Clardy, *J. Am. Chem. Soc.* **106**, 7983 (1984)

b) C. B. Rao, A. S. R. Anjaneyula, N. S. Sarma, Y. Venkatateswarlu, R. M. Rosser, D. J. Faulkner, M. H. M. Chen, and J.Clardy, *J. Org. Chem.* **50**, 3757 (1985)

c) C. B. Rao, D. V. Rao, V. S. N. Raju, B. W. Sullivan, and D. J. Faulkner, *Heterocycles* **28**, 103 (1989)

d) Atta-ur-Rahman, K. A. Alvi, S. A. Abbas, M. I. Choudhary, and J. Clardy, *Tetrahedron Lett.* **30**, 6825 (1989)

(3) D. G. Davis and A. Bax, *J. Am. Chem. Soc.* **107**, 2820 (1985)

(4) M. F. Summers, L. G. Marzilli, and A Bax, *J. Am. Chem Soc.* **108**, 4285 (1986)

(5) For a recent review on the marine natural products, see: D. J. Faulkner, *Nat. Prod. Rep.* **9**, 323 (1992) and references cited therein

(6) The biological data except for cytotoxicity mentioned here will be reported very soon elsewhere