Pergamon

Tetrahedron 54 (1998) 7891–7896

TETRAHEDRON

Epioxyzoanthamine, a New Zoanthamine-type Alkaloid and the Unusual Deuterium Exchange in this Series.

Antonio H. Daranas, José J. Fernández , José A. Gavín and Manuel Norte*

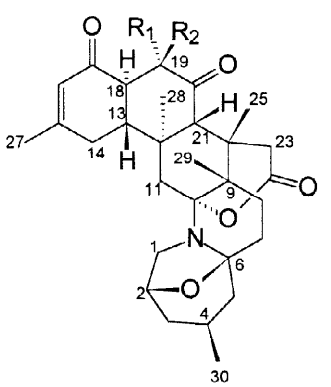
Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain

Received 23 March 1998; revised 28 April 1998; accepted 30 April 1998

Abstract: A new alkaloid, epioxyzoanthamine **4**, has been isolated from a marine zoanthid. Its structure and relative stereochemistry were determined through the interpretation of NMR spectral data. This compound as well as norzoanthamine, showed an unusual deuterium exchange at the methylene C-11 after treatment with D_2O.

As part of our ongoing program to investigate natural products from diverse marine organisms, a colonial zoanthid of the genus *Zoanthus* has been studied. These curious and, at times, beautiful creatures are polyps, which are constructed on the radial system. They have a cylindrical body with a broad base and rows of hollow tentacles around the upper disk; and a central sack with a slit like mouth at the top of the disk. These animals grow as small colonies on intertidal rocks along the North coast of Tenerife and were collected in March, 1996.

Recently, it has been suggested that zoanthamine-type alkaloids, which suppress the decrease in bone weight and strength in ovariectomized mice, could be good candidates for an osteoporotic drug.[1] These zoanthamine alkaloids **1-4** belong to a class of unknown biosynthetic origin. Faulkner et al.[2] suggest a triterpenoid origin, and recently Uemura et al.[1] proposed a polyketide biogenetic pathway for zoanthamines, but as far as we know, neither proposal has been confirmed.

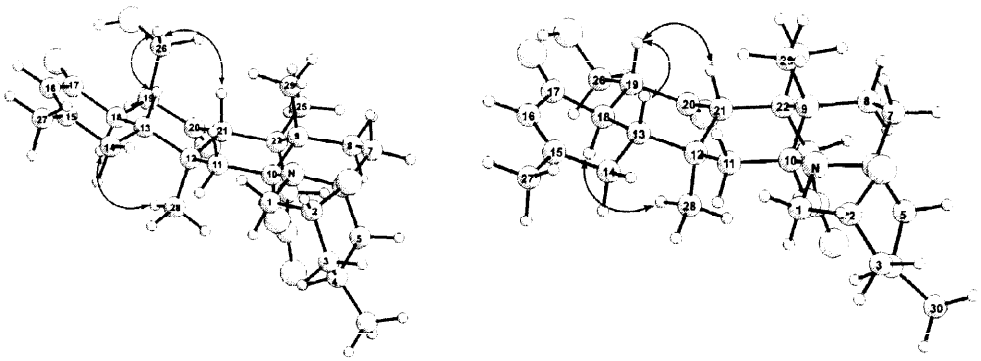


1 Zoanthamine R_1=H; R_2=CH_3
2 Norzoanthamine R_1=H; R_2=H
3 Oxyzoanthamine R_1=H; R_2=CH_2OH
4 Epioxyzoanthamine R_1=CH_2OH; R_2=H

Extraction of the fresh animal (520 g) with 1:1 acetone:methanol, followed by removal of solvent under vacuum and purification of the condensed extract (20 g) by Sephadex LH-20 size-exclusion chromatography (CH_2Cl_2:MeOH:n-Hex/1:1:2); silica gel flash chromatography and then reverse-phase HPLC in CH_3CN:MeOH:H_2O mixtures, yielded the previously known alkaloids zoanthamine **1**,[2-5] norzoanthamine **2**[1] and oxyzoanthamine **3**[1] along with the new compound epioxyzoanthamine **4**.

0040-4020/98/$19.00
PII: S0040-4020(98)00423-2

Epioxyzoanthamine **4** (2.6 mg) proved to be an isomer of oxyzoanthamine **3** on the basis of its mass spectrum and its NMR spectroscopical data showed that the chemical structure was closely related to that of oxyzoanthamine. Once the COSY, HMQC and HMBC experiments of compound **4** were accomplished and compared with those of compound **3**, the most remarkable differences were observed both in the chemical shifts and in the chemical coupling constants of the proton signals H-18, H-19 and H-26 which shifted from δ 2.79, 3.07 and 3.91/3.99 in **3** to δ 2.91, 2.80 and 3.63/ 4.11 in **4**. Moreover, the coupling constant value $J_{H\text{-}18/H\text{-}19}$ = 6.4 Hz in compound **3** changed to 10 Hz in compound **4**. Taking these data into account, we arrived at the conclusion that these compounds must be differentiated in their stereochemistry around C-19. This was confirmed by the observed ROE correlations of the methine protons H-13 and H-21 with the proton signal H-19 in compound **4**, instead with the proton signals H-26 as observed in compound **3**. These ROE correlations establish that the relative stereochemistry at C-19 in compound **4** must be R^* (**Figure 1**). On the other hand, and in accordance with the observed ROE data in the ROESY experiment, the relative stereochemistry at carbons C-2, C-4, C-6, C-9, C-10, C-12, C-13, C-18, C-21 and C-22 in both compounds remains identical with that observed for zoanthamine **1** and norzoanthamine **2**.



Oxyzoanthamine **3** Epioxyzoanthamine **4**

Figure 1

A remarkable fact in the study of both compounds, together with the observed diastereotopicity of H-26 methylene protons, was that these protons showed different coupling constant values with H-19 as well as with the proton of the -OH group (**Table 1**). This could be due to the presence of a restricted rotation around the C-19/C-26 bond as a consequence of a H-bond in these molecules, and we decided to carry out a deuterium exchange experiment. After addition of D_2O the exchange of the hydroxyl proton occurs rapidly, although we still observe the diastereotopicity of H-26, thus establishing that the rotation in the C-19/C-26 bond is still restricted. On the other hand, there were observed a surprisingly deuterium exchange of H-11 protons, that centred at δ 1.91 in one hour and the other centred at δ 2.14 over a period of two days. The first one was identified as H-11β by its intense ROE correlation with the methyl group Me-29, while that centred at δ 2.14 showed correlation with the methylene protons H-1. Moreover, the fully protonated compound was obtained after treatment of the deuterated sample with $MeOH/H_2O$. A similar experiment was carried out with norzoanthamine and a similar exchange at C-11 was observed (**Figure 2**).

	Oxyzoanthamine 3				Epioxyzoanthamine 4			
n° C	δ ^{13}C	δ ^{1}H	Mult.	J (Hz)	δ ^{13}C	δ ^{1}H	Mult.	J (Hz)
1	47.2	(α) 3.29 (β) 3.24	br d dd	6.4 6.4 ; 6.3	47.1	(α) 3.27 (β) 3.22	br d dd	6.5 6.5 ; 6.5
2	73.7	4.54	m	-	74.2	4.55	m	-
3	38.8	(α) 1.55 (β) 1.47	ddd ddd	3.4 ; 4.5 ;12 3 ; 11.3 ; 12	38.8	(α) 1.57 (β) 1.47	ddd ddd	4.5 ; 3 ;12 3 ; 12 ;12
4	22.9	2.28	m		22.9	2.26	m	
5	44.4	(α) 2.10 (β) 1.09	dd dd	5.1 ; 12.8 12 ; 12.8	44.2	(α) 2.08 (β) 1.09	dd dd	5 ; 12.9 11.8 ; 12.9
6	89.8	-	-	-	89.9	-	-	-
7	29.9	(α) 1.76 (β) 1.90	ddd ddd	4 ; 4 ;13 4.5 ;13 ;13	29.9	(α) 1.78 (β) 1.89	ddd ddd	3 ; 4 ; 13 4 ; 13 ; 13
8	23.7	(α) 1.70 (β) 1.58	ddd ddd	4 ;13 ;13 4 ; 4.5 ; 13	23.6	(α) 1.71 (β) 1.58	ddd ddd	4 ; 13 ; 13 3 ; 4 ; 13
9	39.9	-	-	-	39.9	-	-	-
10	101.6	-	-	-	101.4	-	-	-
11	42.2	(β) 1.92 (α) 2.17	d d	13.8 13.8	42.4	(β) 1.91 (α) 2.14	d d	14.0 14.0
12	39.1	-	-	-	39.8	-	-	-
13	49.9	2.57	ddd	5.3 ; 11 ; 13	53.3	2.23	ddd	5.4 ; 11 ; 13
14	31.2	(α) 2.26 (β) 2.23	dd dd	13 ; 11 5.3 ; 13	32.9	(α) 2.27 (β) 2.20	dd dd	13 ; 11 5.4 ; 13
15	160.9	-	-	-	159.2	-	-	-
16	126.4	5.91	s	-	125.8	5.93	s	-
17	198.5	-	-	-	198.9	-	-	-
18	47.5	2.79	dd	6.4 ; 13	48.3	2.91	dd	10 ; 13
19	53.9	3.07	ddd	4.2 ; 6.4 ; 6.5	51.6	2.80	ddd	2.6 ; 3.5 ; 10
20	210.0	-	-	-	211.1	-	-	-
21	56.4	3.23	s	-	58.8	2.78	s	-
22	36.3	-	-	-	36.6	-	-	-
23	36.1	(α) 3.70 (β) 2.39	d d	20.3 20.3	35.8	(α) 3.75 (β) 2.39	d d	20.3 20.3
24	172.5	-	-	-	172.2	-	-	-
25	20.7	1.00	s		21.1	1.00	-	-
26	61.0	3.91 3.99	ddd ddd	4.2 ; 4.2 ; 11.1 6.5 ; 7.5 ; 11.1	60.6	4.11 3.63	ddd ddd	2.6 ; 5.4 ;11.8 3.5 ; 9 ;11.8
27	24.4	2.02	s	-	23.9	2.01	s	-
28	18.7	1.00	s	-	18.7	0.99	s	-
29	18.4	1.18	s	-	18.4	1.15	s	-
30	21.7	0.90	d	6.5	21.8	0.92	d	6.5
-OH		2.33	dd	4.2 ; 7.5		2.31	dd	5.4 ; 9

Table 1.- NMR data for compounds **3**, and **4** in $CDCl_3$

On the other hand, we have carried a NMR study of the deuterium incorporation by using a ^{13}C-enriched sample of norzoanthamine obtained from [1,2-$^{13}C_2$] sodium acetate feeding experiments. Thus, it was treated with D_2O and **Figure 2** shows the proton and carbon NMR spectra for the monodeuterated and bideuterated ^{13}C-enriched norzoanthamine, **Figure 2B** and **2C**, respectively. In these figures, the carbon signals C-9 and C-12 were accompanied by γ ($^{3}\Delta\delta$ -0.03) and β ($^{2}\Delta\delta$ -0.09) deuterium shifted signals, respectively.[6]

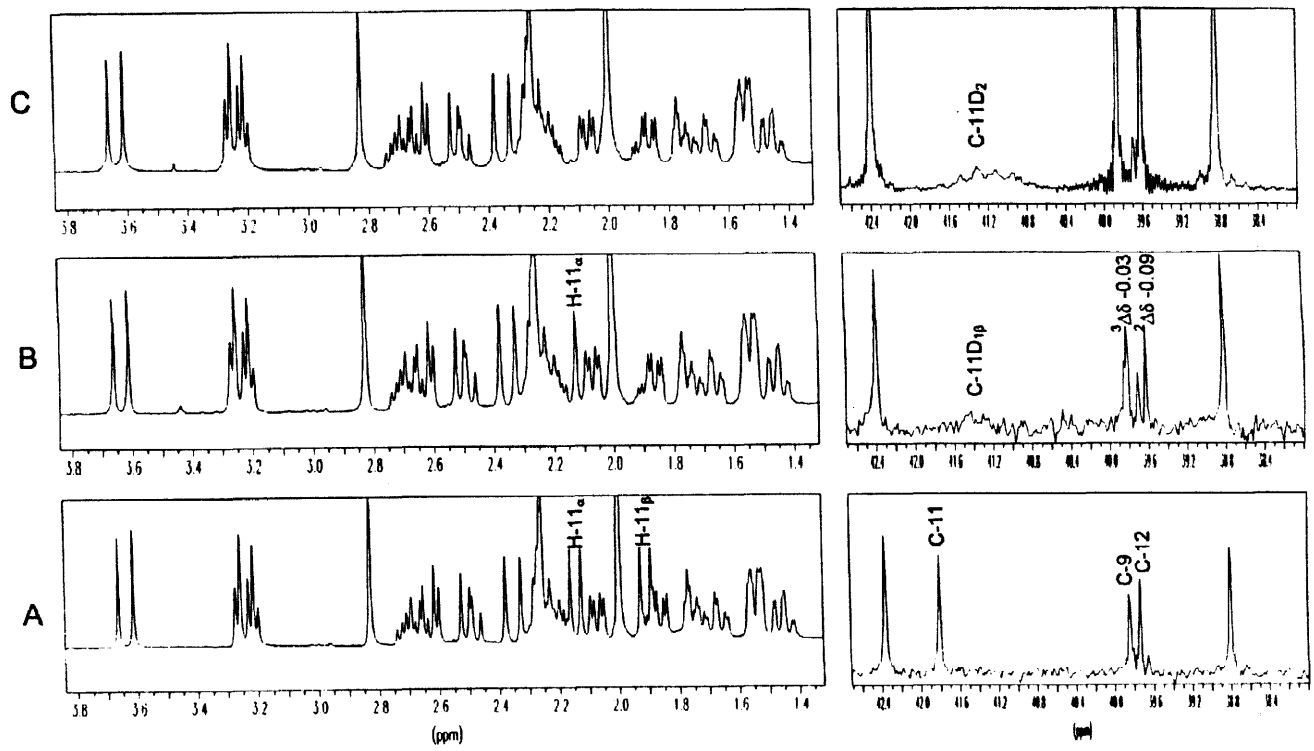
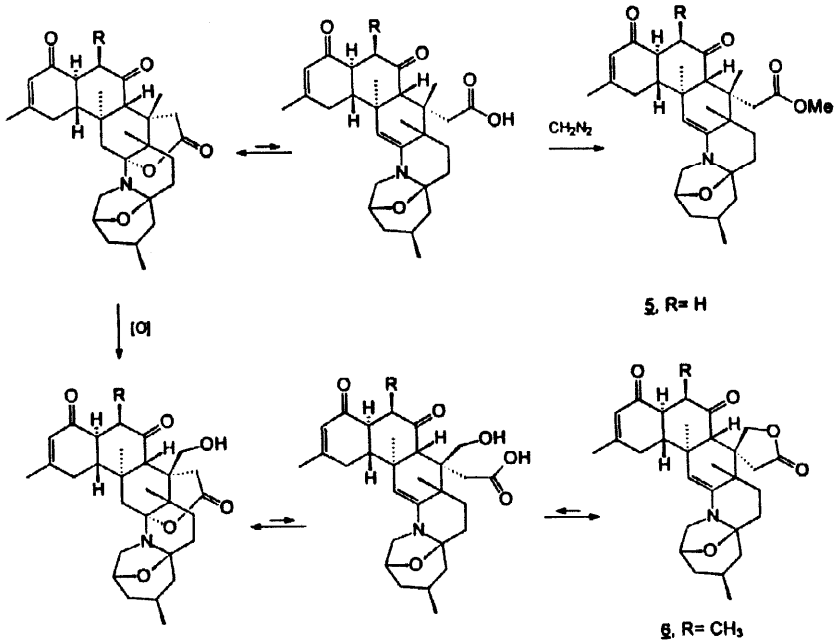


Figure 2.- NMR spectra of norzoanthamine (A), norzoanthamine C-11$D_{1\beta}$ (B), norzoanthamine C-11D_2 (C)

These exchanges could be explained by the presence of an equilibrium in solution between the lactone and the enamine forms which is, as far as we know, an unusual phenomenon for this type of compounds. In order to ensure the existence of this equilibrium in solution, an ethereal solution of norzoanthamine was treated with diazomethane and, after a few minutes, a clean sample of the methyl ester **5** was obtained (**Scheme 1**).



Scheme 1

All the published compounds in this series possess a lactone moiety observed in norzoanthamine except those in which the methyl group Me-25 was oxidised to hydroxy-methyl. In those cases, a δ-lactone between the carboxyl and the hydroxy-methyl groups was observed. This fact could be explained on the basis of the previously described equilibrium, the 28-deoxyzoanthamide **6**[4] being one of these examples (**Scheme 1**). On the other hand, there are two hypotheses regarding the biosynthetic origin of these compounds: either triterpenoid or polyketide. In our opinion, the isolation of the epimers **3** and **4**, reinforce the polyketide pathway for the formation of these kinds of compounds.

Acknowledgements

A.H. Daranas is grateful to the MEC (Spain) for a research fellowship. We acknowledge financial support from the Programa Nacional de Tecnología de Alimentos, Ref. ALI-95-1012-C05-02 and Ciencia y Tecnología Marina MAR-95-1981-C04-C02.

EXPERIMENTAL

General methods. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. IR spectra were measured on a Bruker IFS55 spectrometer. The NMR spectra were obtained with a Bruker AMX-400 instrument. Chemical shifts are reported relative to TMS and coupling constants are given in Hz. HRMS were performed on a Kratos MS-80RFA spectrometer. HPLC was carried out with a LKB 2248 system equipped with a differential diffractometer detector. Silica gel CC and TLC were performed on Silica gel Merck 60 G. TLC plates were visualised by spraying with Dragendorff's reagent and heating. All solvents were purified by standard techniques.

Animal material: Samples of *Zoanthus sp.* were collected in March 1996 in the intertidal zone at Punta Hidalgo, La Laguna (Tenerife, Canary Islands), and a few polyps were deposited at the Departamento de Biología Marina, Universidad de La Laguna, Tenerife.

Extraction. The fresh material (0.52 kg) was extracted with 1:1 acetone:methanol for 24 h each at room temperature. The combined extracts were evaporated *in vacuo* to leave a dark-green viscous oil (20 g, 3.8 % dry weight).

Chromatographic separation. The crude extract was chromatographed on a Sephadex LH-20 (600x70 mm∅) column, using *n*-hexane:$CHCl_3$:MeOH (2:1:1) as eluent, and a medium pressure silica gel chromatography using $CHCl_3$:MeOH (95:5) as eluent, collecting 15 ml. fractions. Fractions exhibiting similar tlc profiles with Dragendroff's reagent were selected and each one was rechromatographed on a medium pressure reverse-phase Lobar LiChropred RP-8 (310x25 mm∅), with MeOH:H_2O (85:15) as eluent and later on a μ-Bondapack C-18 (150x19 mm∅) column HPLC reverse phase chromatography using as eluent CH_3CN:MeOH:H_2O in different proportions. This final purification carried out by HPLC yielding pure zoanthamine **1** (7.3 mg) and norzoanthamine **2** (25 mg), along with the new products oxyzoanthamine **3** (3.8 mg) and epioxyzoanthamine (2.6 mg).

Compound 3, Oxyzoanthamine: amorphous white solid, $[\alpha]^{25}_D$ = -0.66 (*c*, 0.91); IR υ_{max} ($CHCl_3$): 3684, 3020, 1711, 1663, 1521 and 1214 cm^{-1}; HRMS: 511.29193 (calc. 511.29338, $C_{30}H_{41}NO_6$), 496.26631 (calc. 496.26991, $C_{29}H_{38}NO_6$); MS m/z: 511, 496, 468, 403, 330, 272 and 207; ^1H and ^{13}C-NMR (Table 1).

Compound 4, Epioxyzoanthamine: amorphous white solid, $[\alpha]^{25}_D$ = -17.5 (*c*, 0.24); IR υ_{max} ($CHCl_3$): 3694, 3010, 2956,1705, 1675 and 1248 cm^{-1}; HRMS: 511.29222 (calc. 511.29338, $C_{30}H_{41}NO_6$), 496.27402 (calc. 496.26991, $C_{29}H_{38}NO_6$); MS m/z: 511, 496, 468, 454, 403, 358, 330, 272 and 207; ^1H and ^{13}C-NMR (Table 1).

Compound 5: To a stirred solution of 10 mg (0.02 mmol) of norzoanthamine in 1 mL of ether was added dropwise 0.1 mmol of CH_2N_2 in ether. The reaction mixture was stirred at rt for 5 min, and concentrated under reduced pressure. Flash chromatography (1:1 n-hexane to ethyl acetate) yielded the compound **5** as a amorphous white solid, ^1H -NMR ($CDCl_3$) δ: 3.22 (H-1α, br d, J= 6.5 Hz), 3.13 (H-1β, dd, J=6.5, 7 Hz), 4.60 (H-2, m), 1.56 (H-3α, ddd, J= 3, 4, 12 Hz), 1.42 (H-3β, ddd, 3, 11, 12 Hz), 1.77 (H-4, m), 1.95 (H-5α, dd, J= 5, 12.3 Hz), 1.12 (H-5β, dd, J=12, 12.3 Hz), 1.50 (H-7α, ddd, J= 3, 4, 13), 1.86 (H-7β, ddd, J= 4, 13, 13 Hz), 1.88 (H-8, 2H, m), 4.10 (H-11, s), 2.12 (H-13, ddd, J= 4, 12, 12 Hz), 2.30 (H-14α, dd, J= 12, 14 Hz); 2.42 (H-14β, dd, J= 4 , 14 Hz), 5.90 (H-16, s), 2.63 (H-18, ddd, J= 6.4, 12, 12 Hz), 2.61 (H-19α, dd, J= 6.4, 14.9 Hz), 2.56 (H-19b, dd, J= 12, 14.9 Hz), 2.88 (H-21, s), 2.52 (H-23, d, J= 14 Hz), 3.22 (H-23, d, J= 14 Hz), 1.14 (H-25, s), 2.00 (H-27, s), 1.00 (H-28, s), 1.22 (H-29, s), 0.88 (H-30, d, J= 6.3 Hz), 3.67 (OMe, s). ^{13}C-NMR ($CDCl_3$) δ: 50.6 (C-1), 73.8 (C-2), 38.5 (C-3), 23.3 (C-4), 42.6 (C-5), 90.1 (C-6), 30.1 (C-7), 26.0 (C-8), 42.6 (C-9), 143.9 (C-10), 98.4 (C-11), 42.0 (C-12), 52.3 (C-13), 32.7 (C-14), 160.5 (C-15), 125.6 (C-16), 199.3 (C-17), 47.3 (C-18), 42.0 (C-19), 210.0 (C-20), 60.6 (C-21), 39.9 (C-22), 37.9 (C-23), 173.6 (C-24), 25.1 (C-25), 24.3 (C-27), 19.7 (C-28), 18.2 (C-29), 21,7 (C-30), 51.3 (OMe).

References and notes

1. a) Fukuzawa, S.; Hayashi, Y.; Uemura, D.; Nagastu, A.; Yamada, K.; Ijyum, Y., *Heterocycl. Commun.*, **1995**, 1, 207. b) Kuramoto, M.; Hayashi, K.; Fujitani, Y.; Yamaguchi, K.; Tsuji, T.; Yamada, K.; Ijuin, Y. and Uemura, D., *Tetrahedron Lett.*, **1997**, 38, 5683.
2. Rao, C. B.; Anjaneyula, A. S. R.; Sarma, N. S.; Venkatateswarlu, Y.; Rosser, R. M.; Faulkner, D. J.; Chen M. H. and Clardy, J., *J. Am. Chem. Soc.*, **1984** ,106, 7983.
3. Rao, C.B.; Anjaneyulu, A. S. R.; Sarma, N. S.; Venkatateswarlu, Y.; Rosser R. M. and Faulkner, D. J., *J. Org. Chem.*, **1985**, 50, 3757.
4. Rao, C. B.; Rao, D. V.; Raju, V. S. N.; Raju, B. W.; Sullivan, B. W. and Faulkner, D. J., *Heterocycles*, **1989**, 28, 103.
5. Atta-ur-Rahman; Ahmed Alvi, K.; Ali Abbas, S.; Iqbal Choudhary, M. and Clardy, J., *Tetrahedron Lett.*, **1989**, 30, 6825.
6. The F.I.D. was recorded over an acquisition time of 0.33 s using a pulse angle of 30° (4 μs) and a relaxation delay 3 s to optimise the intensity of deuterated carbons.