Pergamon

Tetrahedron 55 (1999) 5539–5546

TETRAHEDRON

New Alkaloids from a Marine Zoanthid

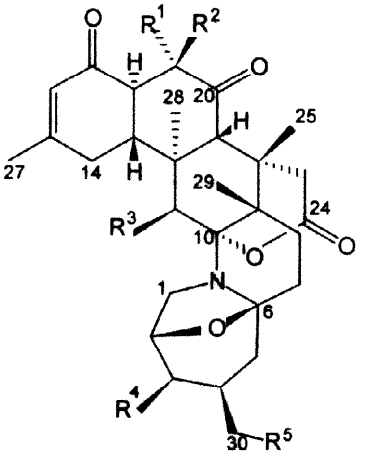
Antonio H. Daranas, José J. Fernández , José A. Gavín and Manuel Norte*

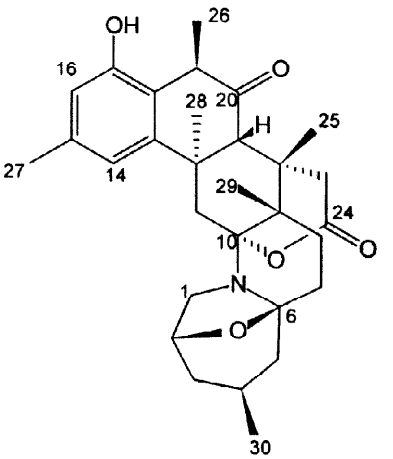
Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Astrofísico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Spain

Received 24 December 1998; revised 9 February 1999; accepted 25 February 1999

Abstract: Five new alkaloids zoanthenol 7, 3-hydroxynorzoanthamine 8, 30-hydroxynorzoanthamine 9, 11-hydroxynorzoanthamine 10 and 11-hydroxyzoanthamine 11 have been isolated from a marine zoanthid. Their structures were determined through the interpretation of 2D-NMR spectral data. Their relative stereochemistries were proposed on the basis of ROESY data.

Recently the chemical constituents of a colonial zoanthid *Zoanthus sp.* were investigated as a part of a programme to study new compounds with biological activity. This study yielded the previously known alkaloids zoanthamine 1[1-3] and norzoanthamine 2,[4-5] oxyzoanthamine 3,[4] epioxyzoanthamine 4,[6] zoanthaminone 5[7] and norzoanthaminone 6 [4] along with the new products zoanthenol 7, 3-hydroxynorzoanthamine 8, 30-hydroxynorzoanthamine 9, 11-hydroxynorzoanthamine 10 and 11-hydroxyzoanthamine 11.





7 Zoanthenol

1 Zoanthamine R 1= H; R2=CH_3; R3=H; R4=H; R5=H
2 Norzoanthamine R 1=H; R2=H; R3=H; R4=H; R5=H
3 Oxyzoathamine R1=H; R2=CH_2OH; R3=H; R4=H; R5=H
4 Epyoxyzoanthamine R1=CH_2OH; R2=H; R3=H; R4=H; R5=H
5 Zoanthaminone R 1=H; R2=CH_3; R3= O; R4=H; R5=H
6 Norzoanthaminone R 1=H; R2=H; R3= O; R4=H; R5=H
8 3-Hydroxynorzoanthamine R 1=H; R2=H; R3=H; R4=OH; R5=H
9 30-Hydroxynorzoanthamine R 1=H; R2=H; R3=H; R4=H; R5=OH
10 11-Hydroxynorzoanthamine R 1=H; R2=H; R3=OH; R4=H, R5=H
11 11-Hydroxyzoanthamine R 1=H; R2=CH_3; R3=OH; R4=H; R5=H

0040-4020/99/$ - see front matter
PII: S0040-4020(99)00198-2

Zoanthenol 7 was isolated as an amorphous white solid, $[\alpha]^{25}_D = 7.1$ (*c* 0.24, $CHCl_3$), and the molecular formula $C_{30}H_{39}NO_5$ was established by HRMS. The absorptions at 204, 276 and 282 in the UV spectrum and the comparison of its proton and carbon NMR spectra with those of zoanthamine 1, suggested the presence in the molecule of an aromatic system instead of the cyclohexenone moiety. Thus, in the proton NMR spectrum of 7 the disappearance was observed of proton signals H-13 and H-18; the substitution of the methylene signal H-14 by a new methine signal centred at δ_H 6.56, and that the signals of proton H-16 and the vinylic methyl group Me-27 were shifted to δ_H 6.49 and 2.30, respectively. In the carbon NMR spectrum, four new vinylic carbon signals centred at δ_C 154.1, 150.5, 120.5 and 115.8 substituted those of C-13, C-14, C-17 and C-18.

Table 1. NMR data for compound 7.

	Zoanthenol 7								
n° C	δ ^{13}C	δ ^{1}H		J (Hz)	**n° C**	δ ^{13}C	δ ^{1}H		J (Hz)
1	47.1	(α) 3.43 (β) 3.38	br d dd	6.8 6.8; 6.5	**16**	114.8	6.49	s	-
2	74.2	4.60	m	-	**17**	154.1	-	-	-
3	38.8	(α) 1.60 (β) 1.50	ddd ddd	3; 4; 11.8 3; 10.3; 11.8	**18**	120.5	-	-	-
4	22.8	2.33	m	-	**19**	46.3	3.56	ddd	6.4; 6.4; 6.4
5	44.3	(α) 2.15 (β) 1.12	dd dd	4.8 ; 13 12 ; 13	**20**	212.0	-	-	-
6	90.0	-	-	-	**21**	52.3	3.57	s	-
7	29.9	(α) 1.77 (β) 1.93	ddd ddd	3; 4 ; 13 4; 13 ; 13	**22**	36.1	-	-	-
8	23.6	(α) 1.80 (β) 1.59	ddd ddd	3; 4; 13 4; 13; 13	**23**	35.8	(α) 3.91 (β) 2.44	d d	20.2 20.2
9	39.8	-	-	-	**24**	172.7	-	-	-
10	102.0	-	-	-	**25**	20.9	1.07	s	-
11	41.5	(α) 2.51 (β) 2.65	d d	14.1 14.1	**26**	19.0	1.55	d	6.5
12	40.4	-	-	-	**27**	21.2	2.30	s	-
13	150.5	-	-	-	**28**	30.3	1.11	s	-
14	115.8	6.56	s	-	**29**	18.2	1.17	s	-
15	138.0	-	-	-	**30**	21.7	0.94	d	6.4

The correlations observed in HMBC (Figure 1) between a quaternary carbon signal centred at δ_C 120.5 with the protons at δ_H 3.56 (H-19) and 1.55 (Me-26) identified this signal as C-18, and their correlations with the proton signals at δ_H 6.56 and 6.49 established that they belong to H-14 and H-16, respectively. The carbon signal C-13 (δ_C 150.5) was identified by its correlations with the proton signals centred at δ_H 6.56 (H-14), 3.56 (H-19), 3.57 (H-21), 2.51 (H-11β) and 1.11 (Me-28). The correlations of the aromatic methyl group (Me-27; δ_H 2.30) with the carbon signals at δ_C 114.8 (C-16), 115.8 (C-14) and 138.0 (C-15), established that the remaining carbon signal at δ_C 154.1 must be assigned to C-17. On the other hand, the remainder of the structure was established unequivocally from its 2D-NMR

data as zoanthenol 7. The relative stereochemistry for 7 was determined by means of the ROESY spectrum to be the same as that of zoanthamine 1.

Compounds 8, 9 and 10 were isolated as amorphous solids. All of them proved to be isomers, as their shown by identical molecular formula $C_{29}H_{39}NO_6$, which was established by HREIMS and that indicate that they belong to the norzoanthamine series. For that reason, the COSY, HSQC, HMQC-TOCSY and HMBC experiments of these compounds were accomplished and compared with those of norzoanthamine 2. It was immediately clear that in compounds 8 and 10 a methylene group was substituted by a methine α to a hydroxyl group. For compound 8 (Table 2), it was located at C-3, through the COSY and TOCSY connectivities. Thus, proton signal H-3 (δ_H 3.34), was shown to be connected with the characteristic proton signals of methines H-2 (δ_H 4.51) and H-4 (δ_H 2.21), which in turn were connected with the protons of methylenes H-1 (δ_H 3.25 and 3.17) and H-5 (δ_H 1.93 and 1.23), respectively. The relative stereochemistry at C-3 was established in accordance with the correlations observed in the ROESY experiment. Thus, the correlation of H-11α (δ_H 1.94) with the proton signal at δ_H 3.17 identified H-1α, which in turn showed ROE correlation with H-3 (Figure 2). This ROE correlation together with that observed between H-3 and H-4 only could be explained if the relative configuration at C-3 is R*.

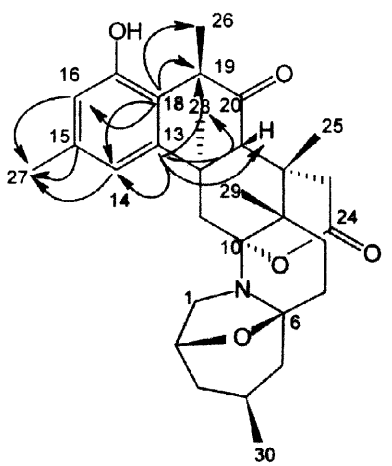


Fig. 1. Significant HMBC correlations for 7

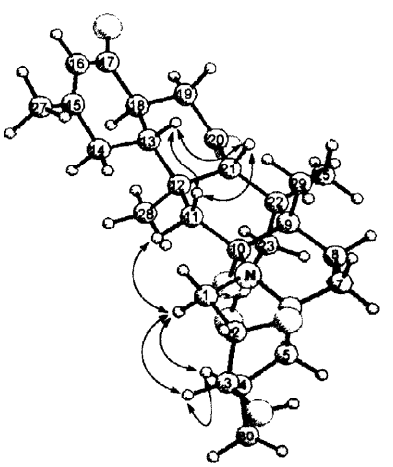


Fig. 2. Selected ROESY correlations for 8

Compound 10 showed the disappearance of the characteristic proton signals due to the isolated methylene group C-11 in norzoanthamine 2, which were substituted by a new methine group (δ_H 4.27, δ_C 67.4). Taking into account that this proton signal did not show COSY connectivities, its localisation at C-11 was confirmed by the HMBC correlations. Thus, the proton signal centred at δ_H 4.27 was correlated with carbon signals centred at δ_C 40.4 (C-9); 107.8 (C-10); 20.1 (C-28), 45.1 (C-12) and 55.0 (C-21), while its carbon signal, (δ_C 67.4), was correlated with the proton signals at 1.18 (C-28). The relative stereochemistry

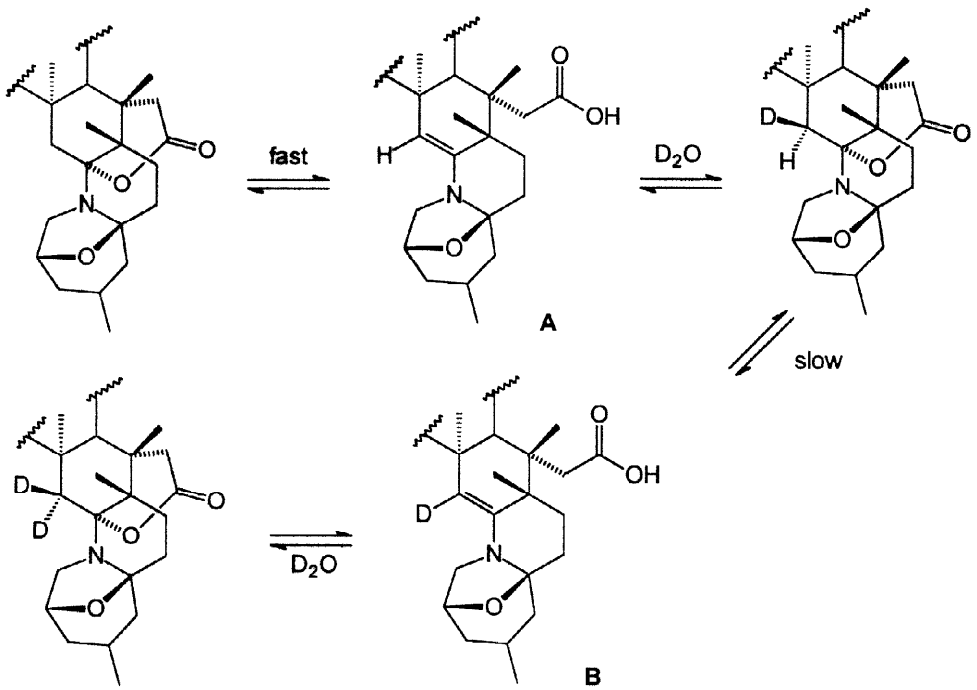
at C-11 was established as S in accord with the ROE correlations of H-11 with protons H-14$_\beta$ (δ_H 2.53), H-1$_\alpha$ (δ_H 2.23), H-1$_\beta$ (δ_H 3.83) and Me-28 (δ_H 1.19), while all of the other chiral centres were established as being identical with those observed for norzoanthamine.

Compound 9 showed the presence of a CH_2-OH moiety instead of the usual methyl group Me-30. It was established by the presence of an AB system centred at δ_H 3.50 and 3.44, which was connected with H-4 in the COSY experiment. The relative stereochemistry was determined by ROESY experiments as identical with that observed for norzoanthamine 2, thus establishing the structure of compound 9 as 30-hydroxynorzoanthamine.

Table 2. ^{13}C NMR data for compound 1 and NMR data for compounds 8 and 9.

	1	3-Hydroxynorzoanthamine 8				30-Hydroxynorzoanthamine 9			
n° C	δ ^{13}C	δ ^{13}C	δ ^{1}H		J (Hz)	δ ^{13}C	δ ^{1}H		J (Hz)
1	47.2	44.98	(α) 3.17 (β) 3.25	br d dd	7.4 7 ; 7.4	47.6	(α) 3.27 (β) 3.28	br d dd	6.5 6.5 ; 6.5
2	74.2	78.4	4.51	br dd	3.3 ; 7	74.3	4.62	m	-
3	38.8	70.9	3.34	ddd	3.3 ; 3.3 ;10	33.8	(α) 1.60 (β) 1.58	ddd ddd	3; 4; 12 3; 12; 12
4	24.5	27.19	2.21	m	-	31.6	2.46	m	-
5	44.4	39.7	(α) 1.93 (β) 1.23	dd dd	5.1 ; 13.5 12.6 ; 13.5	39.1	(α) 2.16 (β) 1.19	dd dd	4.6 ; 13 12 ; 13
6	89.9	90.2	-	-	-	90.4	-	-	-
7	29.9	29.6	(α) 1.78 (β) 1.94	ddd ddd	3 ; 3.4 ; 12.4 5 ; 12.4 ; 12.4	30.6	(α) 1.79 (β) 1.89	ddd ddd	4 ; 4 ; 13 4.5 ; 13 ; 13
8	23.7	23.6	(α) 1.68 (β) 1.58	ddd ddd	3.4 ; 12.4 ; 13.5 3 ; 5 ; 13.5	24.2	(α) 1.68 (β) 1.58	ddd ddd	4 ; 13 ; 13 4 ; 4.5 ; 13
9	40.1	40.1	-	-	-	40.3	-	-	-
10	101.6	100.8	-	-	-	102.0	-	-	-
11	41.9	41.8	(α) 1.94 (β) 2.16	d d	13.8 13.8	42.3	(α) 1.94 (β) 2.16	d d	14.1 14.1
12	34.9	36.7	-	-	-	40.2	-	-	-
13	48.0	53.3	2.26	ddd	6 ; 11 ; 12	53.5	2.24	ddd	*6 ; 11 ; 12*
14	30.6	31.9	(α) 2.25 (β) 2.23	dd dd	13 ; 11 6 ; 13	32.3	2.27 (2H)	m	-
15	159.9	159.8	-	-	-	160.0	-	-	-
16	126.8	125.6	5.93	s	-	126.0	5.93	s	-
17	197.2	198.4	-	-	-	198.7	-	-	-
18	45.8	46.4	2.72	ddd	6.4 ; 11.8 ; 12	46.8	2.71	ddd	6.3 ; 11.3 ; 12
19	48.0	42.3	(α)2.66 (β)2.52	dd dd	6.4; 14.2 11.8;14.2	42.8	(α)2.65 (β)2.51	dd dd	6.3; 14 11.3;14
20	212.0	208.9	-	-	-	209.2	-	-	-
21	53.8	59.1	2.84	s	-	59.5	2.84	s	-
22	39.5	40.3	-	-	-	36.9	-	-	-
23	35.9	35.7	(α) 3.68 (β) 2.38	d d	20.4 20.4	36.3	(α) 3.69 (β) 2.37	d d	20.3 20.3
24	172.5	172.2	-	-	-	172.8	-	-	-
25	20.7	20.9	1.01	s	-	21.5	1.01	s	-
26	13.8	-	-	-	-	-	-	-	-
27	22.9	24.2	2.02	s	-	24.6	2.02	s	-
28	18.4	18.4	1.01	s	-	18.8	1.00	s	-
29	18.3	18.3	1.18	s	-	18.8	1.17	s	-
30	21.8	16.6	0.98	d	6.9	67.9	3.50 3.44	dd dd	6 ; 10.4 6.6; 10.4

The spectroscopic data of compound 11 were closely related to those of 11-hydroxynorzoanthamine 10 with the exception of its molecular formula $C_{30}H_{41}NO_6$ and the substitution of the methylene C-19 by a methine coupled to a new methyl group Me-26, suggesting that this compound belongs to the zoanthamine series and that its structure was 11-hydroxyzoanthamine.



Scheme 1

In a previous work we described an unusual deuterium exchange at the methylene C-11 after treatment of a sample of norzoanthamine with D_2O. Identical behaviour was observed by treatment of zoanthenol 7, 3-hydroxynorzoanthamine 8 and 30-hydroxynorzoanthamine 9 with D_2O, where the proton H-11$_\beta$ was totally exchanged at 5 min and H-11$_\alpha$ was exchanged after two days (Scheme 1). However, the 11-hydroxy derivatives of norzoanthamine 10 and zoanthamine 11 did not show deuterium exchange at C-11 after treatment with D_2O. This result reinforced the hypothesis we put forward to explain the deuterium exchange in norzoanthamine, where we proposed, necessarily, an equilibrium in solution between the lactone and the enamine forms.[6] This equilibrium starts with the highly favoured anti-elimination of the H-11$_\beta$ to yield the enamine form A with a free carboxylic acid which incorporates a deuterium atom when the lactone form is reestablished. Due to the isotope effect on cleavage of the C-D versus C-H bond, the proton H-11$_\alpha$ is preferentially removed leaving the deuterium atom in the enamine form B, this step being the rate-determining step in the overall reaction. The deuterium interchange is blocked for compounds 10 and 11 due to the presence of a β-hydroxyl group at C-11.

Table 3. NMR data for compounds 10 and 11.

	11-Hydroxynorzoantamine 10				11-Hydroxyzoanthamine 11			
n° C	δ ^{13}C	δ ^{1}H		J (Hz)	δ ^{13}C	δ ^{1}H		J (Hz)
1	45.4	(α) 3.23 (β) 3.83	br d dd	7 7; 7	45.4	(α) 3.24 (β) 3.84	br d dd	7 7; 7
2	74.1	4.61	m	-	74.0	4.61	m	-
3	38.6	(α) 1.60 (β) 1.49	ddd ddd	3.4 ; 4.5 ;12 3 ; 11.3 ; 12	38.5	(α) 1.62 (β) 1.50	ddd ddd	4.5 ; 3 ;12 3 ; 12 ;12
4	23.0	2.30	m		22.9	2.30	m	
5	44.3	(α) 2.11 (β) 1.10	dd dd	4.9 ; 12.5 12.5; 12.5	44.3	(α) 2.21 (β) 1.11	dd dd	5.2 ; 13.4 12; 13.4
6	91.3	-	-	-	91.3	-	-	-
7	30.0	(α) 1.78 (β) 1.95	ddd ddd	3; 4;13 4.5 ;13;13	29.9	(α) 1.75 (β) 1.97	ddd ddd	3 ; 4 ; 13 4 ; 13 ; 13
8	26.6	(α) 1.75 (β) 1.55	ddd ddd	4 ;13 ;13 4 ; 4.5 ; 13	26.6	(α) 1.72 (β) 1.55	ddd ddd	4 ; 13 ; 13 3 ; 4 ; 13
9	40.4	-	-	-	40.6	-	-	-
10	107.8	-	-	-	104.0	-	-	-
11	67.4	4.27	s	-	67.4	4.27	s	-
12	45.1	-	-	-	44.7	-	-	-
13	49.1	2.92	ddd	3.5 ; 11.2 ; 13	43.6	3.14	ddd	5.2; 11.5; 13.5
14	32.2	(α) 2.25 (β) 2.53	dd dd	13 ; 17 3.5 ; 17	30.8	(α) 2.19 (β) 2.49	dd dd	1.5; 17.3 3.8; 17.3
15	161.4	-	-	-	160.9	-	-	-
16	125.1	5.91	s	-	125.1	5.90	s	-
17	198.0	-	-	-	196.8	-	-	-
18	46.3	2.61	ddd	5.6;12.5;13	48.2	2.59	dd	5.2; 13.5
19	43.0	(α) 2.79 (β) 2.46	dd dd	5.6; 13.5 12.5; 13.5	45.9	3.06	m	-
20	209.5	-	-	-	212.0	-	-	-
21	55.0	3.00	s	-	49.14	3.39	s	-
22	37.0	-	-	-	36.4	-	-	-
23	36.7	(α) 3.82 (β) 2.41	d d	20.4 20.4	36.4	(α) 2.19 (β) 2.41	d d	20.7 20.7
24	172.1	-	-	-	171.7	-	-	-
25	20.4	1.00	s		20.5	0.97	s	-
26	-	-	-	-	12.3	1.18	d	6.9
27	24.4	2.01	s	-	24.9	2.00	s	-
28	20.1	1.19	s	-	20.0	1.18	s	-
29	21.3	1.46	s	-	21.4	1.51	s	-
30	21.8	0.91	d	6.5	21.7	0.91	d	6.5

Acknowledgements

A.H. Daranas is grateful to the MEC for a research fellowship. We acknowledge financial support from the Ciencia y Tecnología Marina MAR95-1981-C04-C02 and MAR98-0978. The authors thank Prof. A. Brito from Universidad de La Laguna for assistance in obtaining of samples.

Experimental Part

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.*[8] were collected in March 1996 in the intertidal zone at Punta Hidalgo, Bajamar (Tenerife, Canary Islands), and a few polyps were deposited at Departamento de Biología Marina and identified by Prof. A. Brito from 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 (600 x 70 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 Dragendorff's reagent were selected and each one was rechromatographed on a medium pressure reverse-phase Lobar LiChropred RP-8 (310 x 25 mm∅), with MeOH:H_2O (85:15) as eluent and later on a μ-Bondapack C-18 (150 x 19 mm∅) column HPLC reverse phase chromatography using as eluent CH_3CN:MeOH:H_2O in different proportions. This final purification, carried out by HPLC, yielded pure zoanthamine 1 (7.3 mg), norzoanthamine 2 (25 mg), oxyzoanthamine 3 (3.8 mg), epioxyzoanthamine 4 (2.6 mg), zoanthaminone 5 (2.8 mg) and norzoanthaminone 6 (3.6 mg) along with the new products zonthenol 7 (2.4 mg), 3-hydroxynorzoanthamine 8 (0.75 mg), 30-hydroxynorzoanthamine 9 (2.7 mg), 11-hydroxynorzoanthamine 10 (2.3 mg) and 11-hydroxyzoanthamine 11 (2.5 mg).

Compound 7, zoanthenol: amorphous white solid, $[\alpha]^{25}_D$ = +7.1 (*c* 0.24, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3600-3300 (bs), 2963, 2925, 2851, 1714, 1360, 1258 cm^{-1}; UV λ_{max}(EtOH): 204 (ε 4500), 276 (ε 300), 282 (ε 300); MS *m/z*: 493[M], 478, 480, 460, 437, 394; HRMS: 493.2810 (calc. $C_{30}H_{39}NO_5$ 493.2828); ^1H and ^{13}C-NMR (Table 1).

***Compound* 8**, 3-hydroxynorzoanthamine: amorphous white solid, $[\alpha]^{25}_D$ = +12 (*c* 0.075, Et_2O); IR υ_{max} ($CHCl_3$): 3688, 3609, 2951, 1711, 1601, 1363 cm^{-1}; MS *m/z*: 497[M], 482, 457, 438, 424, 389, 346, 279; HRMS: 497.2754 (calc. $C_{29}H_{39}NO_6$ 497.2777); ^1H and ^{13}C-NMR (Table 2).

***Compound* 9**, 30-hydroxynorzoanthamine: amorphous white solid, $[\alpha]^{25}_D$ = -1.1 (*c* 0.27, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3600-3300 (bs), 2953, 2920, 2851, 1711, 1666 cm^{-1}; MS *m/z*: 497[M], 480, 466, 438, 377, 362; HRMS: 497.2773 (calc. $C_{29}H_{39}NO_6$ 497.2777); ^1H and ^{13}C-NMR (Table 2).

***Compound* 10**, 11-hydroxynorzoanthamine: amorphous white solid, $[\alpha]^{25}_D$ = -11.5 (*c* 0.13, Et_2O); IR υ_{max} ($CHCl_3$): 3600-3300 (bs), 2954, 2925, 1715, 1668 cm^{-1}; MS *m/z*: 497[M], 481, 466, 438, 422, 404; HRMS: 497.2781 (calc. $C_{29}H_{39}NO_6$ 497.2777) ^1H and ^{13}C-NMR (Table 3).

***Compound* 11**, 11-hydroxyzoanthamine: amorphous white solid, $[\alpha]^{25}_D$ = + 1.8 (*c* 0.23, $CHCl_3$); IR υ_{max} ($CHCl_3$): 3600-3300 (bs), 1715, 1668 cm^{-1}; MS *m/z*: 511[M], 495, 480, 462, 452; HRMS: 511.2882 (calc. $C_{30}H_{41}NO_6$ 511.2933); ^1H and ^{13}C-NMR (Table 3).

References

1. Rao CB, Anjaneyula ASR, Sarma NS, Venkatateswarlu Y, Rosser RM, Faulkner DJ, Chen MH , Clardy J. J. Am. Chem. Soc. 1984, 106:7983-7984.
2. Rao CB, Anjaneyulu ASR, Sarma NS, Venkatateswarlu Y, Rosser RM, Faulkner DJ. J. Org. Chem. 1985, 50:3757-3760.
3. Rao CB, Rao DV, Raju VSN, Raju BW, Sullivan BW, Faulkner, DJ. Heterocycles 1989, 28:103-106.
4. Fukuzawa S, Hayashi Y, Uemura D, Nagastu A, Yamada K, Ijyum Y. Heterocycl. Commun. 1995, 1:207-214.
5. Kuramoto M, Hayashi K, Fujitani Y, Yamaguchi K, Tsuji T, Yamada K, Ijuin Y, Uemura D. Tetrahedron Lett. 1997, 38:5683-5686.
6. Daranas AH, Fernández JJ, Gavín JA, Norte M. Tetrahedron,1998, 54:7891-7896.
7. Atta-ur-Rahman, Alvi, K.A., Ali Abbas, S., Iqbal Choudhary, M. and Clardy, J. Tetrahedron Lett. 1989, 30:6825-6828.
8. Brito, A. Estudio taxonómico, ecológico y biogeográfico de los antozoos de la región litoral de las Islas Canarias, PhD, Universidad de La Laguna, unpublished result, 1985.