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Zoaramine, a Zoanthamine-like Alkaloid with a New Skeleton

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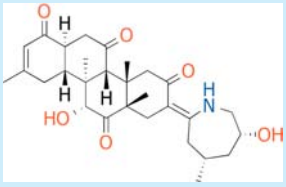
Supporting Information

ABSTRACT: Chemical investigation of an Atlantic variety of *Zoanthus* sp. led to the isolation of two new metabolites, zoaramine and zoarenone. Their structures were deduced by the use of NMR spectroscopy and computational calculation of ^{1}H and ^{13}C chemical shifts. The core of these novel compounds resembles the structure of norzoanthamine alkaloids, and their isolation represents an important step toward a better understanding of the biogenetic origin of this group of antiosteoporotic molecules.



Marine zoanthids are recognized as a prolific source of natural products that have provided a significant number of unique structures with remarkable biological activities.[1] An important group within these organisms includes the genus *Zoanthus*, where a singular family of marine alkaloids, known as zoanthamines, has been found.[2] The first example within this series, zoanthamine (**1**, Figure 1), was isolated from polyps collected at the coast of India.[3] Afterward, a number of related compounds, such as norzoanthamine (**2**), were reported.[4,5] The chemical and structural complexity of zoanthamines,[6] together with their diverse biological activities (antiosteoporotic,[7] anti-inflammatory,[8] and inhibition of platelet aggregation[9]), have attracted the attention of the scientific community. Thus, neat synthetic routes to obtain this particular carbon skeleton have been designed.[1,10,11] In fact, due to their novel mechanism of action, this group of molecules is now considered one of the most promising drug candidates for the treatment of osteoporosis, a disease with an increasing worldwide prevalence.[12] Hence, it is estimated that one in three women and one in five men over the age of 50 will sustain an osteoporotic fracture and that hip fractures are correlated with mortality rates of up to 24% in the first year after the incident.[13]

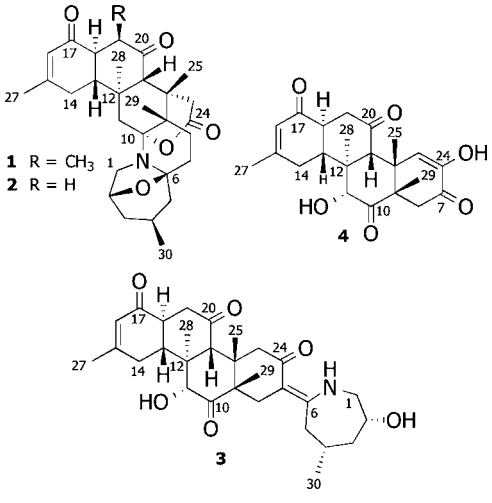


Figure 1. Structures of zoanthamine (**1**), norzoanthamine (**2**), zoaramine (**3**), and zoarenone (**4**).

In this paper, we describe the isolation of a norzoanthamine (**2**) biogenetically related alkaloid, zoaramine (**3**), which comprises a new carbon skeleton. A structurally related metabolite, zoarenone (**4**), where the C-1 → C-6 cyclic fragment that includes the nitrogen atom is absent, was also found.

Specimens of *Zoanthus* sp. collected along the coast of Punta del Hidalgo, Tenerife (28°34′35.06′′N; 16°19′43.64′′W), were extracted with MeOH at room temperature to provide an alkaloidal crude extract.[14] The alkaloid mixture (1.9 g) was chromatographed on a Sephadex LH-20 column using MeOH and subsequently with Lobar LiChroprep-RP18 and MeOH/H_2O (7:3) as eluent. The selected alkaloid fraction was further purified by HPLC using μ-Bondapack C-18 column in a ternary mixture of solvents CH_3CN/MeOH/H_2O (2:1:1) as eluent to provide 360 μg of zoaramine (**3**) and 330 μg of zoarenone (**4**).

Zoaramine (**3**) was isolated as an optically active powder $[\alpha]^{25}_{D}$ 9 (*c* 0.03, $CHCl_3$). Its molecular formula was established by ESI-HRMS (*m*/*z* 498.2848; calcd for 498.2850, $C_{29}H_{39}NO_6$, $[M + H]^+$). The NMR data of **3** resemble that of the previously isolated norzoanthamine (**2**). From the analysis of the ^{1}H and ^{13}C NMR spectra of **3** together with the examination of HSQC (multiplicity edited) and HMBC experiments, 29 carbon signals were identified as five CH_3, seven CH_2, seven CH groups (including one sp^2 carbon), and 10 quaternary carbons (assigned as four carbonyl groups, three sp^3, and three sp^2 carbons) Table 1. Therefore, according to the molecular formula, the remaining unsaturations should come from five rings.

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Table 1. ^{1}H (600.13 MHz) and ^{13}C Chemical Shifts of 3 and 4 Recorded in $CDCl_3$ at 300 K and Referenced to the Solvent (7.26 and 77. 16 ppm)

	zoaramine (3)			zoarenone (4)		
position	δ_C	δ_H	J (Hz)	δ_C	δ_H	J (Hz)
1	49.9	3.42 (2H)	m			
2	69.6	3.68	m			
3	48.1	1.41(α) 2.15(β)	10.7, 12.0, 12.6 2.0, 2.0, 12.6			
4	29.1	1.70	1.5, 2.0, 6.8, 10.1, 12.0			
5	35.1	2.29(α) 2.60(β)	10.1, 14.3 1.5, 14.3			
6	167.3					
7	95.3			191.5		
8	32.1	2.09(β) 3.03(α)	15.1 15.1	40.6	2.51(β) 2.88(α)	17.4 17.4
9	50.8			52.9		
10	212.2			211.7		
11	76.8	4.53	3.7	77.7	4.52	4.3
12	51.6			52.1		
13	53.9	2.47	5.3, 11.0, 11.8	52.0	2.47	3.5, 11.0, 1 2.0
14	33.3	2.45(α) 2.70(β)	11.0, 13.0 5.3, 13.0	33.4	2.39(α) 2.90(β)	11.0, 13.0 3.5, 13.0
15	162.2			162.5		
16	125.0	5.92		124.7	5.93	
17	198.5			197.8		
18	45.3	2.55	5.4, 11.8, 12.4	45.6	2.55	5.1, 2.5, 12.5
19	42.9	2.46(β) 2.81(α)	12.4, 14.5 5.4, 14.5	43.2	2.46(β) 2.92(α)	12.5, 13.9 5.1, 13.9
20	207.6			207.6		
21	60.6	3.00		58.8	3.09	
22	41.7			43.8		
23	43.7	2.24(α) 3.09(β)	17.9 17.9	120.7	6.74	
24	191.4			144.5		
25	21.0	1.17		21.7	1.40	
27	24.7	2.02		24.6	2.01	
28	13.0	0.87		10.7	0.81	
29	23.4	1.32		21.0	1.32	
30	24.7	1.13	6.8			
NH		12.46	5.0,5.0			
OH-11		3.92	3.7		3.91	4.3

Analysis of the COSY and HSQC spectra revealed the existence of two isolated $^{1}H-^{1}H$ spin systems. The first fragment was conveniently started from methylene CH_2-1, which was connected with H-2 and sequentially to H_2-3. H_2-3 also shows correlations with H-4 and this one (H-4) with both H_2-5 and methyl H_3-30. The second fragment was built using the coupling of methine H-13 with methylene H_2-14 and methine H-18, which was connected to H_2-19 (Figure 2). Nevertheless, due to the large number of quaternary carbons, the HMBC correlations played a central role in the structure elucidation of this molecule. The analysis was conveniently started from rings A and B (Figure 2) as their ^{13}C chemical shift and HMBC correlations matched those of norzoanthamine (**2**).

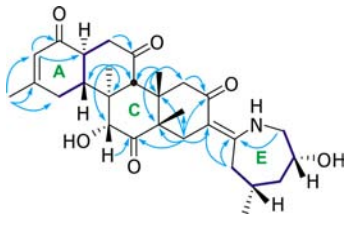

Figure 2. COSY (thick blue lines) and key HMBC (arrows) correlations observed for zoaramine (**3**). Ring nomenclature is shown using green letters.

This fragment was connected with rings C and D as follows: Correlations of H_3-25 with C-9, C-21 and C-22, together with those of H_3-28 with C-11, C-12 and C-21 and finally those of H-11 with C-10 and C-12 clearly established ring C with a carbonyl group at C-10. Correlations of H_2-8 with C-7, C-9, C-10, C-22, C-24 and C-29 as well as those of protons H_2-23 with C-9, C-22, C-24 and C-25 allowed us to build ring D locating a carbonyl group at C-24. Finally, the observed correlations between proton H_2-1 with carbon C-6, together with those of proton H_2-5 and C-6 and C-7 allowed us to build the seven-membered ring E connecting it to the previously established tetracyclic core.

The stereochemical relationships within **3** were mainly deduced from $^{1}H-^{1}H$ coupling constants values as well as dipolar correlations observed in the ROESY spectrum. Thus, the relative configurations within the C7 → C29 moiety were established as 9*S**,11*R**,12*S**,13*R**,18*S**,21*R**,22*S** based on the dipolar correlations of the methine H-21 with H-11, H-13, H_3-25, and H_3-29 and that of the methine H-18 with H_3-28 (Figure 3). The relative configuration of H-11 was secured through observation of ROE with protons H-13 and H_3-29. The regiochemistry of the C-6 → C-7 double bond was proposed as *Z* through the observation of strong ROE between H-5 at δ_H 2.60 and both H_2-8. Additionally, the configurational relationship between the hydroxyl group at C-2 and methyl C-30 was deduced from the ROE correlations observed within

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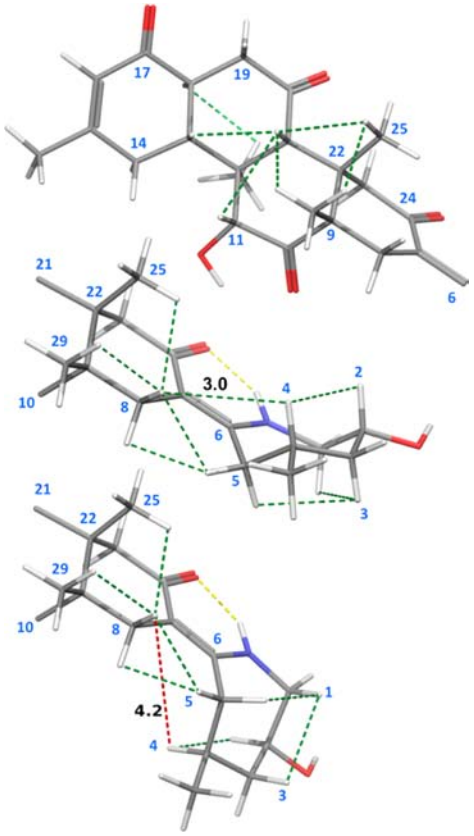

Figure 3. 3D structures of the two possible diasteroisomers of zoaramine (**3**) obtained after DFT optimization. Both C6−C29 fragments are superimposed in the upper diagram. Middle structure represents a fragment of the 2*R**,4*R** diasteroisomer. Bottom structure represents the same fragment of the 2*S**,4*S** diasteroisomer. Observed ROE are marked in green (all distances are ≤3 Å). Nonobserved ROE is marked in red. The H-bond is marked in yellow. Carbon positions are labeled in blue while distances are in black.

ring E. Thus, while H-2 showed a correlation with H-4, indicating they are in the same face, H-3 at δ_H 1.41 showed pseudo-1,3-diaxial ROE correlations with protons H-1 and H-5 at δ_H 3.42 and 2.29, placing all of them on the opposite side of the molecule. However, an important difficulty arose when the relative configurations of the C-1 → C-5 and C-7 → C-29 stereoclusters had to be connected, as both fragments are separated by a double bond. Conformational searches undertaken on the two possible diasteroisomers (2*R**,4*R** or 2*S**,4*S**) gave parallel results. In both cases, the most energetically favored structures were characterized by an almost superimposable ABC tricyclic core linked to opposite pseudo-chair conformations of the seven-membered ring (Figure 3). In addition, according to our models, one H-3 would always have two axial−equatorial couplings with its vicinal protons H-2 and H-4, while H-4 would show a trans-diaxial and an axial−equatorial coupling that would suffice the observed values (Table 1). Moreover, the equatorial proton H-5 (δ_H 2.60, $^3J_{H4H5}$ = 1.5 Hz) shows ROE with both H-8. Although H-5 is different in each model its local relationships are identical in both stereoisomers. Nevertheless, H-4 should have dipolar correlations with a different H-8 depending on the selected isomer (Figure 3, central and bottom diagrams). In fact, H-4 shows ROE only with one H-8 (H-8β, δ_H 2.09) that was secured on its relative position by observation of dipolar correlations with methyl H_3-25. Thus, while the 2*R**,4*R** isomer fits with this observation, the 2*S**,4*S** isomer does not, as the distance between H-4 and H-8β would be too large (Figure 3). As a consequence, the relative configuration of **3** should be 2*R**,4*R**,9*S**,11*R**,12*S**,13*R**,18*S**,21*R**,22*S**.

In order to reinforce the previous conclusions, we performed GIAO NMR calculations using DFT. This approach based on the comparison of calculated and experimental data has given very good results in the assignment of organic molecules.[15] Thus, we optimized the structures that came out of the conformational searches for each isomer (within 10 kJ/mol of the global minimum) using DFT at the B3LYP/6-31+G** level of theory. Next, taking into account their Boltzmann populations, NMR chemical shifts were calculated.[16] Linear regression of calculated against experimental values was undertaken, and corrected values (δ_{scaled} = (δ_{calcd} − intercept)/slope)) were obtained.[17] Our results indicated that the correlation coefficient R^2 was clearly better for the 2*R**,4*R** isomer when comparing δ_H (0.979 vs 0.941). Finally, we calculated the so-called DP4 probability that also predicted the ROE 2*R**,4*R** isomer with 99% probability, confirming our previous proposal based on ROE data.[18] It is noteworthy that the relative configuration we propose for **3** is fully consistent with that found in the lead compounds **1** and **2**.

The second compound, zoarenone (**4**), was isolated as a powder, $[\alpha]^{25}_D$ 3 (*c* 0.03, $CHCl_3$), and its molecular formula was established on the basis of the result obtained from ESI-HRMS (*m*/*z* 387.1802; calcd for 387.1802, $C_{22}H_{27}O_6$, $[M + H]^+$). The MS data were supported by analysis of its ^{13}C spectrum. Analysis of NMR data for **4** revealed an important correspondence with zoaramine (**3**), suggesting a similar backbone for both molecules. Thus, four methyls, three methylenes, and six methines, as well as nine quaternary carbons (including four carbonyl groups and two olefinic carbons), with similar chemical shifts were recognized. Moreover, the characteristic α,β-unsaturated ketone was also identified, as well as one hydroxyl group at C-11 and two carbonyl groups at C-10 and C-20. On the other hand, the main differences within this new compound were located at C-7, C-23, and C-24. Thus, taking into account the chemical shifts [C-7 (δ_C 191.5), C-23 (δ_C 120.7), C-24 (δ_C 144.5)], the existence of an enol system with a α-positioned carbonyl group was proposed. From our point of view, the presence of a carbonyl group at C-7 could be the result of an oxidative fragmentation of **3** with the corresponding loss of the nitrogenated seven-membered ring. A chemical shifts comparison and analysis of the ROESY experiment led us to the conclusion that the relative configurations of **4** are the same as those found in **3**.

Although the first member of the zoanthamine family of alkaloids was isolated in 1984, their biogenetic origin still remains uncertain. Initially, Rao et al. suggested a triterpene origin based on the observation of a 30 carbon atom skeleton.[3] On the other hand, Uemura proposed a polyketide origin for these molecules.[19] In our opinion, in the absence of further experimental data, the isolation of zoaramine reinforces the last option. Thus, starting from a common polyketide precursor (see the Supporting Information), and after a series of reactions the tricyclic ABC core system could be explained. Next, a nucleophilic attack of the amine group into the carbonoyl at C-6 results in the formation of ring E. Further tautomerization of the resulting imines into enamines would yield ring D and subsequently zoaramine (**3**). It has to be noted that the starting

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configurations at C-2 and C-4 used in our biogenetic proposal are consistent with those found in **1**, **2**, and **3** and other members of this family of compounds. Assessment of antiosteoporotic activity for compounds **3** and **4** has not been possible due to the limited amount of material available. New collections of biological samples are underway to address this problem.

■ ASSOCIATED CONTENT

Supporting Information

General experimental procedures, molecular modeling calculations, and MS and NMR data.This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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