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Lobozoanthamine, a new zoanthamine-type alkaloid from the Indonesian soft coral *Lobophytum* sp.

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Abstract

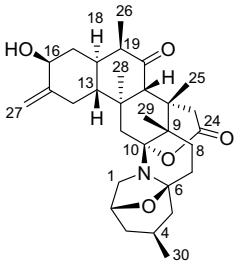
A new member of the family of zoanthamine-type alkaloids, lobozoanthamine (**1**), has been isolated from the Indonesian soft coral *Lobophytum* sp. This represents the first report of a zoanthamine-type alkaloid from a marine invertebrate different from zoanthids. The densely functionalized heptacyclic stereostructure of lobozoanthamine (**1**) has been established through the interpretation of 2D NMR data and application of the modified Mosher method.

Keywords: *Lobophytum* sp.; Indonesia; Zoanthamine; Alkaloids; Structure elucidation; NMR

Soft corals belonging to the family Alcyoniidae are the dominant reef dwelling octocorals in the Indo-West Pacific and are characterized by a great variety in colors, shapes, and sizes. These organisms are known to produce a wide array of terpenoid derivatives (mainly diterpenoids), some of which possess unique skeletal frameworks and potent bioactivities.[1–3]

In the course of our ongoing screening for bioactive secondary metabolites from Indonesian marine invertebrates,[4] we had the opportunity to analyze a specimen of *Lobophytum* sp. (Alcyonacea, Alcyoniidae) collected along the island of Siladen, in the Bunaken Marine Park of Manado (North Sulawesi, Indonesia). From the organic extract of *Lobophytum* sp. we have obtained a new alkaloid belonging to the zoanthamine class, that we have named lobozoanthamine (**1**), and herein we describe its isolation and stereostructural characterization. To our knowledge, this represents the first report of an alkaloid from soft corals belonging to the genus *Lobophytum*.

Colonies of *Lobophytum* sp. (750 g wet weight) have been repeatedly extracted with MeOH at room temperature and the obtained material has been partitioned between water and EtOAc. The organic extract (2.0 g) has been chromatographed by MPLC over silica gel using an eluent gradient system of increasing polarity from *n*-hexane to EtOAc. Fractions eluted with EtOAc/hexane



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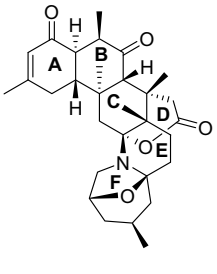

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8:2 have been further purified by analytical HPLC (EtOAc/hexane 75:25) to afford 2.8 mg of lobozoanthamine (**1**) in the pure state.

Lobozoanthamine (**1**),[5] $C_{30}H_{43}NO_5$ by HR-FABMS, showed a well-resolved ^{1}H NMR spectrum (Table 1) at 700 MHz using C_6D_6 as solvent. The ^{1}H NMR spectrum of **1** showed the presence of two broad singlets at δ_H 5.17 and 4.86, a series of multiplets between δ_H 0.90 and 4.30 and five distinct methyl signals at δ_H 0.64 (d), 0.69 (s), 0.76 (s), 0.84 (s), and 0.99 (d). The ^{13}C NMR spectrum of **1** (Table 1, C_6D_6) showed 26 signals between δ_C 13.0 and 100.0, while the remaining four signals were located in the sp^2 region of the spectrum, including an ester and a ketone carbonyl (δ_C 174.2 and 212.0, respectively). Interpretation of 1D NMR data and their translation in terms of the planar structure of compound **1** required an extensive investigation of 2D NMR spectra and was supported by comparison with data reported in the literature for other members of the same family of alkaloids.[6–10]

Table 1
^{13}C (175 MHz) and ^{1}H (700 MHz) NMR data of lobozoanthamine (**1**) (in C_6D_6)

Pos.	δ_C (mult.)	δ_H (mult., J in Hz)
1a	47.0 (CH_2)	2.85 (br d, 6.4)
1b		2.76 (t, 6.4)
2	74.2 (CH)	4.29 (m)
3a	39.0 (CH_2)	1.41 (m)
3b		1.29[a]
4	23.1 (CH)	2.38 (m)
5a	44.9 (CH_2)	2.24 (dd, 12.0, 3.5)
5b		1.11 (t, 12.0)
6	89.9 (C)	
7a	30.4 (CH_2)	1.81 (dd, 13.2, 4.4)
7b		1.53[a]
8a	23.7 (CH_2)	1.31[a]
8b		0.90 (m)
9	36.1 (C)	
10	99.8 (C)	
11a	42.9 (CH_2)	1.87 (d, 14.3)
11b		1.55 (d, 14.3)
12	39.7 (C)	
13	52.4 (CH)	1.30 (ddd, 11.0, 9.5, 2.0)
14a	33.4 (CH_2)	2.22 (t, 9.5)
14b		1.47 (dd, 9.5, 2.0)
15	160.0 (C)	
16	71.4 (CH)	3.53 (dd, 9.8, 3.0)
17a	39.2 (CH_2)	1.28[a]
17b		1.03 (dd, 11.5, 3.0)
18	38.1 (CH)	1.52[a]
19	49.5 (CH)	2.17 (m)
20	212.0 (C)	
21	54.2 (CH)	2.75 (s)
22	40.1 (C)	
23a	36.3 (CH_2)	3.95 (d, 20.0)
23b		2.27 (d, 20.0)
24	174.2 (C)	
25	20.7 (CH_3)	0.69 (s)
26	13.0 (CH_3)	0.64 (d, 7.3)
27a	105.3 (CH_2)	5.17 (br s)
27b		4.86 (br s)
28	17.7 (CH_3)	0.84 (s)
29	17.8 (CH_3)	0.76 (s)
30	21.9 (CH_3)	0.99 (d, 6.4)

[a] Overlapped with other signals.

Combined inspection of COSY and HSQC spectra of **1** revealed the presence of the three partial structures **I**–**III** depicted in Figure 1. Moiety **I** included the oxymethine at δ_H 3.53 (δ_C 71.4) and the methyl doublet at δ_H 0.64, which was linked to the relatively deshielded methine at δ_H 2.17; moiety **II** was composed of a simple dimethylene portion, while moiety **III** included the second methyl doublet (δ_H 0.99) and another oxymethine (δ_H 4.29, δ_C 74.2). In addition, COSY and HSQC spectra of **1** showed signals of an uncoupled methine (**IV**) (δ_H 2.75, δ_C 54.2) and of two uncoupled methylenes (**V** and **VI**) (δ_H 1.87 and 1.55, δ_C 42.9; δ_H 3.95 and 2.27, δ_C 36.1). With these data in hand, a 2D gradient-HMBC was the key experiment to assemble all the partial structures available, thus resulting in the building of the lobozoanthamine (**1**) planar structure and in the complete resonance assignment. The network of significant g-HMBC cross-peaks is depicted in Figure 1. Ring A was deduced by the correlations of the sp^2 methylene protons at δ_H 5.17 and 4.86 (H_2-27) with C-14, C-15, and C-16; correlations of H_3-26 with the signal at δ_C 212.0 allowed the location of the ketone carbonyl, while correlations of H_3-28 and of the methine singlet at δ_H 2.75 (H-21) delineated ring B and its linkage to CH_2-11 and C-22. Location of the two quaternary carbons resonating at δ_C 36.1 (C-9) and 99.8 (C-10) was deduced by the g-HMBC cross-peaks H_3-25/C-22, H_3-25/C-9, H_3-29/C-22, H_3-29/C-9, and H_3-29/C-10, allowing us to consequently deduce the structure of ring C. Furthermore, the g-HMBC cross-peak of H_3-25 with the methylene carbon at δ_C 36.3 (C-23), in turn attached at the carbonyl at δ_C 174.2 (cross-peak H-23/C-24) allowed the location of the methylene of CH_2COO– moiety. As for the linkage of the ester oxygen atom, it must be attached at the quaternary C-10, which resonates at δ_C 99.8, a value appropriate for a carbon linking both oxygen and nitrogen atoms. Going on, the g-HMBC correlations of (i) H_3-29 with both C-9 and C-8, (ii) H_2-1 with both C-10 and C-6, (iii) H_2-5 with both C-6 and C-7 allowed us to deduce the structure of rings E and F. Finally, the oxygen bridge within ring F was inferred by the ^3J g-HMBC correlation of H-2 with

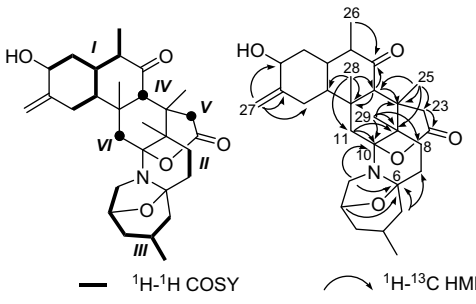

Fig. 1. ^{1}H–^{1}H COSY and $^{2,3}J_{H\rightarrow C}$ HMBC correlations of lobozoanthamine (**1**).

C-6, thus completing the body of information needed to draw the planar structure of the densely functionalized heptacyclic structure **1**, that must be considered a new member of the zoanthamine family of alkaloids.

The assignment of relative configuration to lobozoanthamine (**1**) was based on some crucial scalar coupling constants (Table 1) and on the network of spatial couplings evidenced through a 2D NMR ROESY spectrum (Fig. 2), and was supported by comparison with parallel data reported for zoanthamine (**2**) and its analogs.[7–10] The relative arrangement of the six stereogenic carbons belonging to A/B rings was deduced by the large coupling constant $J_{\text{H-13/H-18}} = 11.0$ Hz, indicative of a trans diaxial relationship, and by the ROESY cross-peaks H-13/H-21, H-21/H_3-26, H-18/H_3-28, and H-16/H-18. Analogously, the ROESY correlation of H-21 with both H_3-25 and H_3-29 indicated the cis relationship of these groups. Finally, the network of spatial correlations exhibited by protons belonging to rings E–F (see Fig. 2), closely paralleling data reported for zoanthamine (**2**) and analogs,[7–10] suggested the relative orientation of the remaining stereogenic carbons. Accordingly, the values of scalar coupling constants of rings E–F protons were almost identical to the values reported for zoanthamine (**2**) (e.g., $J_{\text{H-4/H-5b}} =$ 12.0 Hz for **1** and 13.0 Hz for **2**; $J_{\text{H-1b/H-2}} = 6.4$ Hz for **1** and 6.0 Hz for **2**).[7]

The presence of a secondary alcohol group at C-16 offered us the possibility to assess the absolute configuration of lobozoanthamine (**1**) through the application of the Mosher methodology.[11] To this aim, two aliquots of **1** were treated with (*R*)- and (*S*)-MTPA chloride in dry pyridine at room temperature overnight, providing *S* (**1a**) and *R* (**1b**) MTPA esters, respectively (Fig. 3). The ^{1}H NMR chemical shifts of **1a** and **1b** were assigned on the basis of a detailed analysis of ^{1}H–^{1}H COSY data, and the obtained distribution of $\Delta\delta$ (*S*–*R*) values (Fig. 3) for protons neighboring C-16 indicated the 16*S* configuration. Consequently, starting from the above determined relative stereochemistry, we deduced the complete absolute configuration of lobozoanthamine (**1**).

Lobozoanthamine (**1**) is a new member of the unique family of zoanthamine-type alkaloids, which appears to be unrelated to any known alkaloid framework. The biosynthetic pathway yielding these alkaloids is still a matter of debate and two conflicting hypotheses are on court:

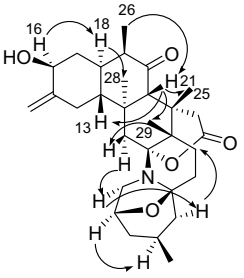

Fig. 2. Diagnostic ROESY correlations detected for lobozoanthamine (**1**).

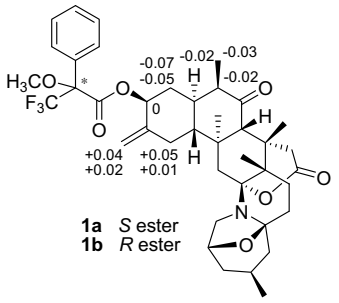

Fig. 3. Application of the modified Mosher's method for secondary alcohols on the MTPA esters of lobozoanthamine (**1a** and **1b**). $\Delta\delta$ ($\delta_S - \delta_R$) are given in ppm.

triterpenoid[6,7] versus a polyketide chain starting from a glycine unit have been indicated as precursor.[12] The intricate structure of the members of zoanthamine family and their promising pharmacological potential (anti-inflammatory activity,[7] treatment of osteoporosis,[12] and platelet-aggregation inhibition[13]) have attracted attention from a wide area of science, including a number of synthetic efforts,[14,15] which resulted in the 41-steps total synthesis of norzoanthamine.[16]

The parent compound of zoanthamine-type alkaloid family, zoanthamine (**2**), was isolated in 1984 from the marine zoanthid *Zoanthus* sp.,[6] followed by a dozen closely related analogs, invariably found from zoanthids of the genus *Zoanthus*, with the single exception of zooxanthellamine, isolated from the unicellular dinoflagellate *Symbiodinium* sp.[17] This last finding has been indicated as a point in favor of the micro-algal origin of zoanthamine alkaloids found in *Zoanthus* zoanthids, which are known to live in association with symbiotic dinoflagellates.[17] The isolation of lobozoanthamine (**1**) from the soft coral *Lobophytum* sp. is therefore particularly remarkable since it represents the first report of a zoanthamine-type alkaloid from a marine invertebrate different from zoanthids.

Since some zoanthids (in particular members of the genera *Epizoanthus* and *Parazoanthus*) are found growing on other marine invertebrates, including soft corals, a contamination of our *Lobophytum* sp. sample with a small amount of a zoanthid could be postulated. However, this hypothesis can be ruled out by considering that, according to the reported yields of zoanthamine-type alkaloids from zoanthids,[6–10] the obtained amount of **1** (2.8 mg) would have needed a so high quantity of zoanthid to be incompatible with its contaminant role. Alternatively, considering that genus *Lobophytum* is symbiotic with dinoflagellates (zooxanthellae), a symbiotic and/or a dietary algal origin of lobozoanthamine (**1**) seems possible, well fitting with the micro-algal origin proposed for the entire class of molecules.

Limited availability of lobozoanthamine (**1**) allowed us only to evaluate the activity in cytotoxicity assays. The molecule proved not to be cytotoxic ($IC_{50} > 50$ μM) against AGS (human stomach adenocarcinoma) and C6 (rat glioma) cell lines.

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