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Terrazoanthines, 2-Aminoimidazole Alkaloids from the Tropical Eastern Pacific Zoantharian *Terrazoanthus onoi*

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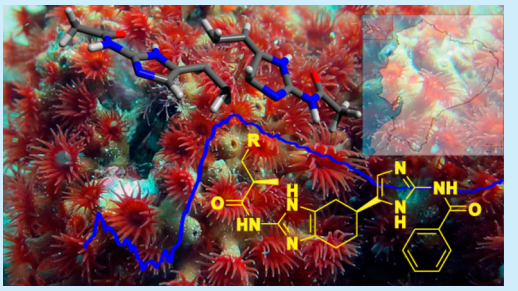
Supporting Information

ABSTRACT: The first chemical study of the common species *Terrazoanthus onoi*, present off the coast of Ecuador, led to the identification of a new family of 2-aminoimidazole alkaloids named terrazoanthines A−C (**1**−**3**). Homologues **1** and **2** feature an unprecedented 6-(imidazol-5-yl)benzo[*d*]-imidazole. Acyl substitution pattern and complete configurational assignments were deduced from comparison between experimental and theoretical ^{13}C NMR and ECD data, respectively. These compounds may represent key derivatives in the biosynthesis of zoanthoxanthins.



Zoantharians are a group of marine invertebrates (Cnidaria: Hexacorallia) widely distributed in all oceans and especially throughout the Indo-Pacific Oceans. While several studies have described their diversity around the western part of the Pacific, the Tropical Eastern part has been less investigated. Descriptions of zoantharians in this marine ecoregion were reported first from the Galapagos Islands, but overall, continental species have yet to be studied.[1,2] Interestingly, there is no chemical study reported on species of this group in this marine region so far. In the course of a national project setting up the basis for a first inventory of the bio- and chemodiversity of the Ecuadorian maritime area, zoantharians were found to be largely present and distributed off the coasts of the Peninsula of Santa Elena. After a taxonomic assessment of the main species of this group, we undertook the chemical study of one the most common zoantharian present in the marine protected area El Pelado, identified as *Terrazoanthus onoi*.[1]

To date, the main natural products reported from zoantharians include ecdysteroids,[3−5] a large family of bioactive alkaloids named zoanthamines,[6,7] aromatic guanidine alkaloids,[8−10] and some hydantoin peptidic analogues.[11,12] Chemical study of *T. onoi* was conducted with two main objectives: (i) to identify potential chemotaxonomic markers useful for the classification of zoantharians; and (ii) to identify molecules with applications in animal and human health. The first insight into the chemical content of *T. onoi* led to the isolation and structure elucidation of a new family of 2-aminoimidazole alkaloids named terrazoanthines A−C (**1**−**3**) (Figure 1). Compounds **1** and **2** feature an unprecedented 6-(imidazol-5-yl)benzo[*d*]imidazole skeleton. We report herein the isolation and structure elucidation of the three major and related metabolites produced by this species. A biosynthetic hypothesis is proposed to explain the formation of this unique skeleton. No significant antimicrobial or cytotoxic bioactivity was evidenced for these compounds through a first biological screening.

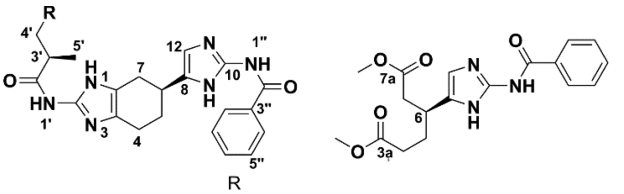


Figure 1. Structure of terrazoanthines A−C from *Terrazoanthus onoi*.

After an organic extraction of a freeze-dried sample of *T. onoi*, the extract was submitted to a first fractionation process through reversed-phase vacuum liquid chromatography. Because the methanolic fractions revealed interesting chemical profiles by UHPLC-DAD-ELSD, we undertook its purification by successive reversed-phase HPLC.

(+)-HRESIMS analysis of **1** revealed a major ion peak at *m*/*z* 407.2187, which was consistent with the molecular formula $C_{22}H_{27}N_6O_2$ $[M + H]^+$ (Δ −0.7 ppm). First inspection of the ^1H NMR spectrum evidenced a benzoyl moiety with signals at δ_H 8.07 (d, *J* = 8.0 Hz, 2H, H-4″), 7.58 (t, *J* = 8.0 Hz, 2H, H-5″), and

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Table 1. ^{1}H NMR Data for 1−3 at 600 MHz in CD_3OD (δ_H in ppm, Multiplicity, J, in Hz)

no.	1	2	3
H−N_1	8.40[a]	*a*	*a*
4	2.75, m	2.75, m	2.37, t (7.5)
5	2.36, br d (15.0)	2.36, br d (15.0)	2.03, m
	2.05, m	2.05, m	
6	3.31, m	3.31, m	3.33, m
7	3.09, dd (15.0, 3.5)	3.09, dd (15.0, 3.5)	2.78, m
	2.79, dd (15.0, 10.0)	2.79, dd (15.0, 10.0)	
H−N_9	*a*	*a*	*a*
12	7.04, s	7.04, s	7.03, s
H−$N_{1'}$	11.58[a]	*a*	*a*
3′	2.53, sext (7.0)	2.72, hept (7.0)	
4′	1.78, dquint (14.0, 7.0)	1.25, d (7.0)	
	1.56, dquint (14.0, 7.0)		
5′	1.23, d (7.0)	1.25, d (7.0)	
6′	0.97, t (7.0)		
H−$N_{1''}$	*a*	*a*	*a*
4″	8.07, d (8.0)	8.07, d (8.0)	8.03, d (8.0)
5″	7.58, t (8.0)	7.58, t (8.0)	7.58, t (8.0)
6″	7.69, t (8.0)	7.69, t (8.0)	7.69, t (8.0)
H_3CO−C-3a			3.64, s
H_3CO−C-7a			3.67, s

[a]In DMSO-d_6.

Table 2. ^{13}C NMR Data for 1−3 at 125 MHz in CD_3OD (δ_C in ppm)

no.	1	2	3
2	139.4	139.4	
3a	124.0	124.0	175.0
4	20.6	20.5	32.1
5	28.6	28.6	30.0
6	32.0	32.0	33.6
7	26.5	26.5	39.3
7a	122.9	122.9	173.7
8	134.4	134.4	133.4
10	140.9	140.9	140.6
12	111.7	111.8	113.0
2′	177.3	177.8	
3′	43.7	36.5	
4′	24.2	19.3	
5′	27.9	19.3	
6′	11.9		
2″	167.5	167.5	167.5
3″	133.0	133.0	133.0
4″	129.3	129.3	129.2
5″	130.0	130.0	130.0
6″	134.6	134.6	134.6
H_3CO−C-3a			52.2
H_3CO−C-7a			52.3

7.69 (t, J = 8.0 Hz, 1H, H-6″), with a 2-methylbutyryl unit with methyl signals at δ_H 0.97 (t, 3H, J = 7.0 Hz, H-6′) and 1.23 (d, J = 7.0 Hz, 3H, H-5′) (Table 1). Both spin-coupled systems (SCS) were confirmed by the expected COSY correlations. The two carbonyls were placed on the basis of key HMBC correlations H-4″/C-2″ and H-5′/C-2′. Even if the presence of a singlet at δ_H 7.04 (s, 1H, H-12) suggested a second aromatic ring, no clear conclusion could be ascertained based on the ^{1}H NMR spectrum.

The ^{13}C NMR spectrum of **1** revealed six additional unsaturated carbons that could only be consistent with one or two additional aromatic rings (Table 2). Because no clear HMBC correlation allowed connection of the first two SCS to these rings, we focused on the last SCS containing saturated carbons (Figure 2). The methylene at C-7 was COSY correlated to the methine at C-6 and then to two successive methylenes at C-5 and C-4. HMBC correlations with the two-terminal methylenes at C-7 and C-4 allowed closure of the cyclohexene ring through a tetrasubstituted double bond at δ_C 122.9 (C-7a) and 124.0 (C-3a). Additional H-6/C-8 and H-6/C-12 HMBC correlations placed a trisubstituted double bond at C-6. Key information was obtained from a H-12/C-10 HMBC correlation that was only consistent with the presence of a 2-aminoimidazole connected at C-6. This proposition was confirmed by the observation of similar chemical shifts described for dimers of pyrrole 2-aminoimidazoles, a large family of metabolites produced by sponges of the Axinellida or Agelasida groups.[13]

The presence of a second 2-aminoimidazole ring fused to the cyclohexene was first inferred from the molecular formula of **1**, which indicated the presence of 6 nitrogens. Even if no HMBC correlation was visible involving the quaternary carbon C-2, a clear signal at δ_C 139.4 was assigned to this last carbon.

The next issue to be addressed was the location of the two acyl groups around this bisguanidine core. We first recorded the NMR spectra of this compound in DMSO-d_6 to reveal the N−H

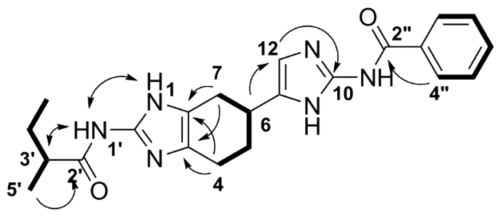

Figure 2. Key COSY (bold) and HMBC (arrow from H to C) correlations for **1**. NOE correlations are indicated with double arrows.

of the alkaloid. Four new singlets were observed in the deshielded region of the spectrum. Both H-1′/H-3′ and H-1′/H-1 ROESY correlations unambiguously placed the 2-methylbutyryl on one of the two primary amines. This substitution pattern was confirmed by the chemical shift at δ_H 8.40 (s, 1H, H-1′) observed for the signal of this first secondary amide. Similar chemical shifts were observed for pyrrole 2-aminoimidazole (nagelamides).[14] Because no HMBC was observed from all four H−N signals, we compared the experimental and theoretical ^{13}C NMR chemical shifts of all possible substitution patterns. Choosing the location of the 2-methylbutyryl on one primary amine afforded two possible substitutions for the benzoyl on the other imidazole. Among the four possible substitution patterns, the most probable locations of the acyls are shown in Figure 1 with 100% confidence (see Supporting Information, SI).[15] Confirmation was obtained when comparing the ^{13}C NMR chemical shifts of **1** with those of zoamide D.[8]

We next paid attention to the absolute configurations of **1**. The experimental ECD spectrum revealed four successive Cotton effects (Figure 3). Theoretical calculation of the ECD spectra of both enantiomers was performed using TDDFT. As depicted in Figure 3, good agreement was observed between experimental and theoretical spectra with the 6*S* absolute configuration.

Because the ECD spectra for the diastereoisomers 6*S*,3′*R* and 6*S*,3′*S* did not allow the determination of the absolute configuration at C-3′ (Figure 3), the use of the DP4 probability

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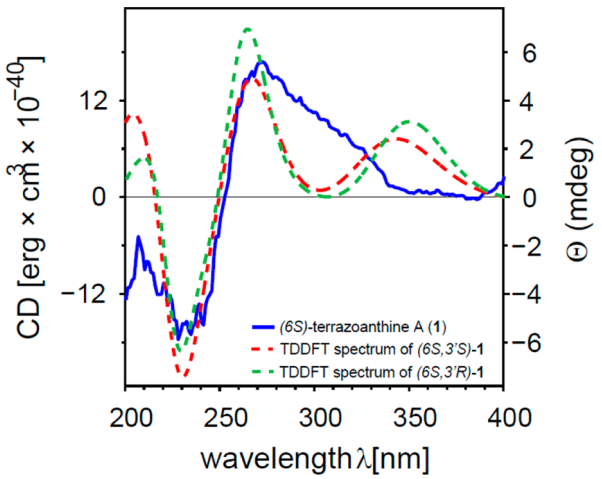


Figure 3. Comparison between the experimental (line) and theoretical (dashed) ECD spectra of two diastereoisomers of **1** at C-3′.

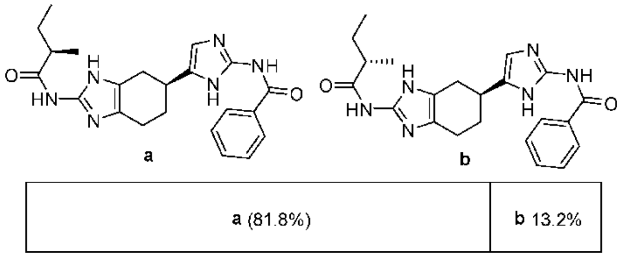


Figure 4. DP4 probabilities of ^{13}C NMR data for both diastereoisomers of **1**.

was also required to determine the relative configuration of **1**. As shown in Figure 4, the relative configuration was established as 6*S*,3′*R* with 81.8% of confidence (dia a).

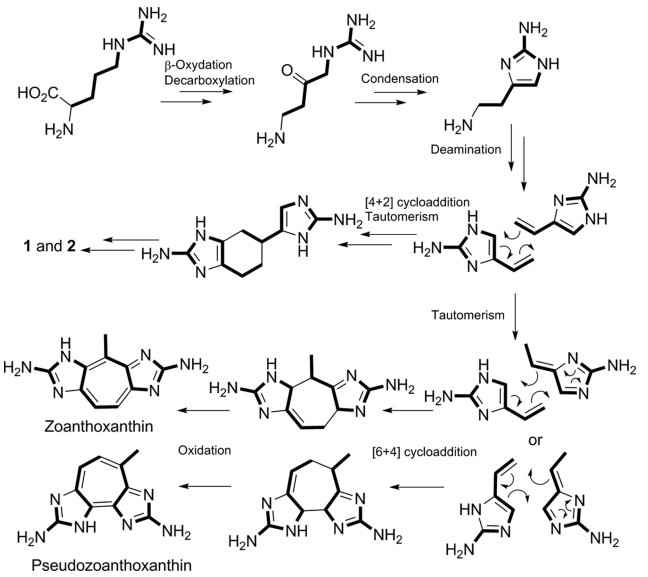
(+)-HRESIMS analysis of **2** revealed a major ion peak at *m*/*z* 393.2043, consistent with the molecular formula $C_{21}H_{24}N_6O_2$ $[M + H]^+$ (Δ 2.3 ppm). This molecular formula was consistent with the loss of a methylene unit compared to **1**. The ^{1}H NMR spectrum showed a different pattern for the methyls. Unlike **1** with two methyl signals, only one methyl signal of integration 6 was observed in the case of **2**. We then concluded that the 2-methylbutyryl substituent of the benzimidazole ring was replaced by an isobutyryl in **2** (Table 1). We assumed the same *S* absolute configuration at C-6 for **2**.

(+)-HRESIMS analysis of **3** revealed a major ion peak at *m*/*z* 360.1556, consistent with the molecular formula $C_{18}H_{22}N_3O_6$ $[M + H]^+$ (Δ 0.2 ppm). This molecular formula suggested the absence of one of the two 2-aminoimidazole rings. The ^{1}H NMR spectrum confirmed the presence of the benzoyl while the butyryl signals were absent. New signals corresponding to methoxy groups suggested the presence of two methyl ester functions. They were confirmed by H_3-7/C-7a and H_3-4/C-3a HMBC correlations. The remaining imidazole ring was still located at C-6 after COSY, HSQC, and HMBC interpretation.

Guanidine alkaloids are common in the marine environment and especially in marine invertebrates, such as sponges.[16] Their biosynthesis is still controversial due to the difficulty to perform feeding experiments or to isolate the biosynthetic genes of these compounds. The presence of two 2-aminoimidazole units in **1** and **2** is an important feature these compounds share with other marine natural products. They are clearly related to a large family of fluorescent alkaloids named zoanthoxanthins also present in several zoantharians. It is important here to underline the pioneering work of Cariello et al., who were the first to propose a C5N3 unit like arginine as a precursor of zoanthoxanthins.[10] This hypothesis was later supported by some successful biomimetic syntheses from the group of Büchi.[17,18] Horne et al. were inspired by this hypothesis to conduct other straightforward approaches toward the syntheses of these pigments.[19,20] In their work, they also proposed an alkylation at C-4 of 2-aminoimidazole derived from arginine as a possible step toward the production of C-2−2-aminoimidazole key precursors. The structures of terrazoanthines are of high biosynthetic interest as they can be seen as a clue toward the hypothesis of a C5N3 precursor. We can propose a dimerization process of a key intermediate, 4-vinylimidazol-2-amine, through a Diels−Alder-type [4 + 2] cycloaddition. The key intermediate may originate from arginine after oxidation at the β position of the arginine. The same type of oxidation but with homoarginine was proposed in the biosynthesis of pyrrole 2-aminoimidazole alkaloids.[13] While the existence of a natural Diels−Alderase was still a matter of debate until recently, a report gave some conclusive evidence of its occurrence.[21] The feasibility of this reaction was confirmed by Hartree−Fock theoretical calculations (see SI).

As proposed Büchi's group, the key 2-amino-4-vinylimidazole is in a tautomeric equilibrium with a diazafulvene analogue.[17,18] This fulvene derivative can undergo a [6 + 4] cycloaddition with the non-isomerized tautomer form, leading to the seven-membered ring after subsequent oxidation and aromatization. These unusual high-order cycloadditions are of clear biosynthetic interests as, according to the regioselectivity of the reaction, they can lead to both zoanthoxanthins and pseudozoanthoxanthins (Scheme 1). For **3**, we suggest hydrolysis of both imines present

Scheme 1. Proposed Biosynthetic Pathway for Terrazoanthines 1 and 2, Zoanthoxanthins, and Pseudozoanthoxanthins



in the benzimidazole ring leading to the loss of the guanidine and formation of a cyclohexa-1,2-dione. This dione derivative leads to the corresponding dicarboxylic acid after oxidative cleavage. Methylation of these carboxylic acids could occur either naturally or during the extraction process.

From a biological point of view, these three compounds were tested for their antimicrobial activities and their cytotoxicity

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against the human liver cancer cell line Heg2, but they were found inactive. The search for bioactivity will be extended toward the inhibition of acetylcholine esterase as some zoanthoxanthin derivatives have shown promising results for these targets.[22]

The first chemical study undertaken on a common marine invertebrate present in the coastal area of Ecuador has been extremely positive. Zoantharian *T. onoi* provided a new family of natural products named terrazoanthines. Terrazoanthines A and B feature a unique bis-2-aminoimidazole attached around a central cyclohexene. The benzoyl substituted on one primary amine seems to be a common feature of this novel family of natural products. These results show good promise in the use of metabolomics for classification of zoantharians as guanidine alkaloids, and ecdysteroids appear as common to most species of this group.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00369.

Description of the species, general experimental procedures, HRMS and ^{1}H, ^{13}C, COSY, HSQC, and HMBC NOESY NMR data for **1**−**3**; computational methods and biological assays (PDF)

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Notes

The authors declare no competing financial interest.

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