Parazoanthines A−E, Hydantoin Alkaloids from the Mediterranean Sea Anemone *Parazoanthus axinellae*

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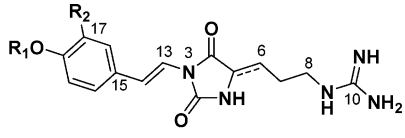
Received July 18, 2009

Five new hydantoin alkaloids, named parazoanthines A−E (**1**−**5**), were isolated as the major constituents of the Mediterranean sea anemone *Parazoanthus axinellae*. Their structural elucidation was achieved through NMR spectroscopic and mass spectrometric analyses. The absolute configuration of the chiral compounds **1** and **4** was determined by comparison between experimental and TDDFT-calculated CD spectra. The configuration of the trisubstituted double bond of **2**, **3**, and **5** was deduced from the $^3J_{H6-C4}$ coupling constant value. This family of alkaloids represents the first example of natural 3,5-disubstituted hydantoins that do not exhibit a methyl at N-3. All compounds were tested for their natural toxicity (Microtox assay), and parazoanthine C (**3**) exhibited the highest natural toxicity.

Relatively few chemical studies of zoanthids have been reported so far, despite evidence of their rich natural products chemistry.[1] Colonial sea anemones of the genus *Parazoanthus* have been identified in almost all the oceans, and they often have been described as epibionts of marine sponges belonging to the *Agelas* or *Axinella* genera. As sponges are known to exude toxic compounds, these zoanthids must have developed adaptative tools to minimize effects of such toxins. Two groups of compounds have been described from *Parazoanthus* species: fluorescent guanidine alkaloids of the zoanthoxanthin families,[2−7] and ecdysteroids.[8] As part of an ongoing research program to investigate the chemodiversity of Mediterranean invertebrates,[9] specimens of *Parazoanthus axinellae* were collected near Marseilles (Plane Island) as epibionts of the sponge *Axinella damicornis*. Because the LC-MS analyses of their crude extracts evidenced brominated compounds as major constituents, we decided to undertake the full chemical study of this species, from which only zoanthoxanthins have been previously described.[2,3] We report herein the isolation and structural characterization of a new class of alkaloids named parazoanthines A−E (**1**−**5**) with a rare 3,5-disubstituted hydantoin core.



	R_1	R_2	
Parazoanthine A (1):	H	H	
Parazoanthine B (2):	H	H	$\Delta^{5,6}$
Parazoanthine C (3):	CH_3	H	$\Delta^{5,6}$
Parazoanthine D (4):	CH_3	Br	
Parazoanthine E (5):	CH_3	Br	$\Delta^{5,6}$

Results and Discussion

Colonies of *P. axinellae* were carefully separated from the sponge *A. damicornis*. The fresh organisms were extracted with CH_2Cl_2/MeOH (1:1), and the extract was purified by C_{18} reversed-phase HPLC to yield the five new parazoanthines A−E (**1**−**5**) along with the known paragracine, zoanthoxanthin, and pseudonorzoanthoxanthin.[2,3]

Compound **1** was isolated as an optically active colorless oil, and its molecular formula $C_{15}H_{19}N_5O_3$ was deduced from HRESIMS data (*m/z* 318.15547 $[M + H]^+$). From the nine unsaturations four were assigned to a *para*-substituted phenol group due to the characteristic ^1H and ^{13}C NMR signals at δ_H 7.25 (2H, d, $J = 8.5$ Hz, H-16 and H-20), 6.76 (2H, d, $J = 8.5$ Hz, H-17 and H-19), δ_C 127.5 (C, C-15), 128.3 (CH, C-16 and C-20), 116.6 (CH, C-17 and C-19), and 158.6 (C, C-18) (Table 1). A disubstituted double bond was further evidenced by the ^1H and ^{13}C NMR signals at δ_H 7.42 (1H, d, $J = 15.0$ Hz, H-14), 6.95 (1H, d, $J = 15.0$ Hz, H-13), δ_C 121.7 (CH, C-14), and 116.5 (CH, C-13) ppm. A HMBC correlation between the equivalent protons H-16 and H-20 and C-14 allowed us to locate the double bond adjacent to the phenol ring, while the downfield shift of the H-13 doublet (δ_H 6.95) suggested the presence of a nitrogen atom at the other side of the double bond. The large $J_{H13-H14}$ (15.0 Hz) coupling constant was indicative of an *E* configuration for this double bond. Even if NMR signals at C-14 were expected to be more shielded than those at C-13 due to the mesomeric donor effect of the N-3, the opposite was observed. An explanation may be given by (i) the decrease in the donor effect of the nitrogen due to the electron-withdrawing effect of both α-carbonyl moieties and (ii) the involvement of the opposite mesomeric donor effect associated with the *para*-hydroxy group. The same observation can be made with makaluvamine E, which shows closely related functional groups.[10] The presence of an additional C_4 alkyl chain was indicated by the sequential H-5/H_2-6/H_2-7/H_2-8 COSY correlations. The sixth unsaturation was assigned to a guanidine function placed at the C-8 end of this alkyl chain due to a key H_2-8/C-10 HMBC correlation. Confirmation was made by the observation of a characteristic fragment by ESIMS-MS at *m/z* 260.1 $[M + H - CH_4N_3]^+$. Additional HMBC correlations between H-5/C-4, H-5/C-2, H-13/C-4, and H-13/C-2 allowed us to link both chains to the same carbonyl moieties. A 3,5-disubstituted hydantoin core was the only structural cycle in accordance with the latter HMBC correlations, the signals at δ_C 174.0 (C, C-4) and 157.3 (C, C-2), and the molecular formula. To assign the absolute configuration at C-5, we decided to use circular dichroism (CD) analyses because of the presence of a carbonyl function adjacent to the stereocenter.[11] Both enantiomers were consequently submitted to geometry optimization by the DFT

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10.1021/np900437y CCC: $40.75
Published on Web 08/26/2009

Table 1. ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR Data for **1**−**5**

	parazoanthine A (**1**)		parazoanthine B (**2**)		parazoanthine C (**3**)		parazoanthine D (**4**)		parazoanthine E (**5**)	
position	δ_C, mult.	δ_H, m (*J* in Hz)	δ_C	δ_H, m (*J* in Hz)	δ_C	δ_H, m (*J* in Hz)	δ_C	δ_H, m (*J* in Hz)	δ_C	δ_H, m (*J* in Hz)
2	157.3, qC		154.5		154.5		157.1		154.2	
4	174.0, qC		162.8		162.9		173.9		162.7	
5	56.9, CH	4.16, t (5.5)	131.3		131.3		57.0	4.18, t (5.5)	131.0	
6a	29.9, CH_2	1.92, m	110.2	5.80, t (7.5)	110.2	5.82, t (7.5)	29.9	1.92, m	110.4	5.81, t (7.5)
6b		1.81, m						1.79, m		
7a	25.2, CH_2	1.75, m	27.4	2.53, q (7.0)	27.5	2.53, q (7.0)	25.2	1.75, m	27.5	2.55, q (7.0)
7b		1.66, m						1.65, m		
8	41.9, CH_2	3.23, t (7.0)	41.2	3.37, t (7.0)	41.3	3.37, t (7.0)	41.9	3.23, t (7.0)	41.2	3.39, t (7.0)
10	158.6, qC		158.8		158.8		158.7		158.6	
13	116.5, CH	6.95, d (15.0)	116.2	7.00, d (15.0)	116.9	7.05, d (15.0)	118.4	7.03, d (15.0)	117.9	7.06, d (15.0)
14	121.7, CH	7.42, d (15.0)	121.9	7.44, d (15.0)	121.5	7.47, d (15.0)	119.6	7.43, d (15.0)	119.8	7.44, d (15.0)
15	127.5, qC		128.3		129.5		131.2		131.2	
16	128.3, CH	7.25, d (8.5)	128.4	7.26, d (8.5)	128.4	7.36, d (8.5)	131.5	7.60, d (2.0)	131.5	7.61, d (2.0)
17	116.6, CH	6.76, d (8.5)	116.6	6.76, d (8.5)	115.3	6.90, d (8.5)	112.9		112.9	
18	158.5, qC		158.6		161.0		157.0		157.0	
19	116.6, CH	6.76, d (8.5)	116.6	6.76, d (8.5)	115.3	6.90, d (8.5)	113.5	7.01, d (8.5)	113.5	7.01, d (8.5)
20	128.3, CH	7.25, d (8.5)	128.4	7.26, d (8.5)	128.4	7.36, d (8.5)	127.5	7.37, dd (8.5, 2.0)	127.5	7.37, dd (8.5, 2.0)
CH_3-O					55.7	3.80, s	56.8	3.88, s	56.7	3.88, s

B3LYP/6-31++G(d, p) approach, and their CD spectra were calculated using the TDDFT B3LYP/6-31++G(d, p) approach.[12,13] The experimental CD spectrum of **1** exhibited a negative Cotton effect at 281 nm and was in excellent agreement with the calculated CD spectrum of **1** with the *S* absolute configuration at C-5.[14] Interestingly, a similar negative Cotton effect has already been observed with hydantoin amino acids with the *S* configuration.[15]

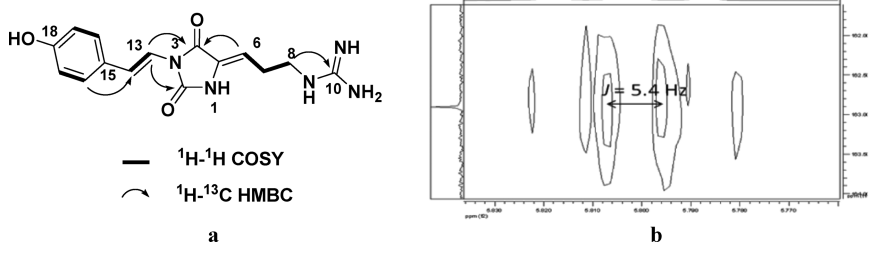
Compound **2** was isolated as a colorless oil, and its molecular formula $C_{15}H_{17}N_5O_3$ was deduced from HRESIMS data (*m*/*z* 316.13928 $[M + H]^+$). NMR data were very similar to those of parazoanthine A (**1**), and an additional unsaturation was evidenced by the replacement of the ^{1}H and ^{13}C signals of the methine at C-5 and the methylene at C-6 in the NMR spectra of **1** by signals at δ_H 5.80 (1H, t, $J = 7.5$ Hz, H-6), δ_C 131.3 (C, C-5), and 110.2 (CH, C-6) (Table 1). Compound **2** was then assumed to be the $\Delta^{5,6}$ derivative of **1**, which was confirmed by key COSY and HMBC correlations (Figure 1a). In order to assign the configuration of the trisubstituted C-5/C-6 double bond, the coupling constant $^3J_{H6-C4}$ has been measured by a non-proton-decoupled ^{1}H−^{13}C HMBC experiment. The obtained value of 5.4 Hz was consistent with a *cis* configuration between H-6 and C-4, indicating a *Z* configuration of the double bond (Figure 1b).[16]

Compound **3** was isolated as a colorless oil, and its molecular formula $C_{16}H_{19}N_5O_3$ was deduced from HRESIMS data (*m*/*z* 330.15640 $[M + H]^+$). The molecular formula suggested the addition of a methylene unit compared to **2**, and the close NMR data suggested a strong similarity between both compounds. The replacement of the hydroxy group in **2** by a methoxy group in **3** was supported by the new NMR signals at δ_H 3.80 (3H, s, $C\underline{H_3}$-O) and δ_C 55.7 (CH_3, $\underline{C}H_3$-O) and was further confirmed by the key CH_3-O/C-18 HMBC correlation.

Compounds **4** and **5** were obtained as colorless oils, and their molecular formulas $C_{16}H_{20}BrN_5O_3$ and $C_{16}H_{18}BrN_5O_3$ were deduced from the clusters of two peaks (1:1) at *m*/*z* 410.08203 ($[M + H]^+$)/412.1 and *m*/*z* 408.06650 ($[M + H]^+$)/410.1, respectively, in the HRESIMS, indicative of monobrominated compounds. On the basis of their NMR spectra, it appeared that the aromatic part of **1**−**3** was clearly modified in the case of **4** and **5**. A trisubstituted *ortho*/*para* benzene ring was indeed evidenced by the characteristic ^{1}H NMR aromatic signals pattern (Table 1). Comparison of aromatic NMR signals between **4** and **5**, on one hand, and **3**, on the other hand, allowed location of the bromine atom at the *ortho* position of the methoxy group due to characteristic ^{13}C NMR chemical shifts at δ_C 113.5 (C, C-17), 131.5 (CH, C-16), and 157.0 (C, C-18). The difference between **4** and **5** arises from the presence of a *Z* $\Delta^{5,6}$ double bond for **5** as for **2** and **3**. The absolute configuration at C-5 of the reduced compound **4** was assumed to be identical to that of **1** because the CD spectrum showed the same characteristic negative Cotton effect at 280 nm.

The originality of this family of alkaloids lies in the 3,5-disubstituted hydantoin core, where all other examples of closely related natural products were nonsubstituted at N-3 or bore a methyl group at this position. Furthermore, hydantoins have been postulated as a key connection between the origin of peptides and purines.[17] These structural features raised the issue of the biosynthetic origin of the hydantoin core. Two hypothetical pathways are described in Scheme 1. The key reaction of the first pathway is a monocarbonylation occurring after peptidic ligation of two amino acids. The second hypothetical pathway involves the ring contraction of a putative diketopiperazine formed by a double condensation between two amino acids. Diketopiperazines are common marine natural products produced by microorganisms, but the ring contraction remains unproven in this family of compounds.[18]

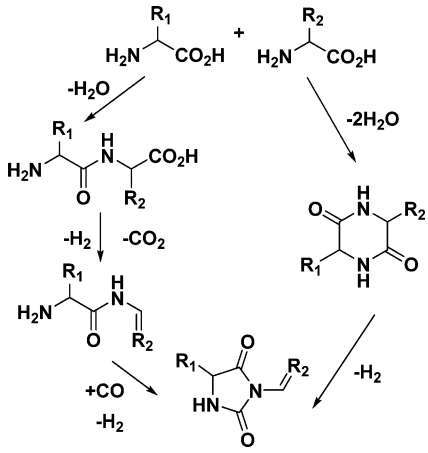
All compounds were tested for their antitumor (MDA-MB-231, HT-29, and A-549) and antimalarial (FcB1) activities, and none of them exhibited significant bioactivity. Nevertheless, during a screening of marine organisms for their natural toxicity, *P. axinellae* was found to exhibit the most bioactive extract among the



a b

Figure 1. (a) Key COSY and HMBC correlations of parazoanthine B (**2**). (b) Non-proton-decoupled H-6/C-4 HMBC correlation.

Scheme 1. Hypothetical Pathways for Hydantoin Biosynthesis



cnidarians.[19] We then decided to evaluate all the isolated compounds in this Microtox assay.[20] Parazoanthine C (**3**) showed the highest natural toxicity (EC_{50} = 1.64 μM) and may consequently be responsible for the results obtained with the extract.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 343 polarimeter equipped with a 10 cm microcell. UV measurements were performed on a Varian Cary 300 Scan UV−visible spectrophotometer. CD spectra were measured using a JASCO J-810 spectropolarimeter. IR spectra were obtained with a PerkinElmer Paragon 1000 FT-IR spectrophotometer. NMR experiments were performed on a Bruker Avance 500 MHz spectrometer. Chemical shifts (δ in ppm) are referenced to the carbon (δ_C 49.0) and residual proton (δ_H 3.31) signals of CD_3OD, the solvent, with multiplicity (s singlet, d doublet, t triplet, q quadruplet, and m multiplet). Low-resolution electrospray ionization (ESI) mass spectra were obtained with a Bruker Esquire 3000 Plus spectrometer in the positive mode. High-resolution mass spectra (HRESIMS) were conducted on a LTQ Orbitrap mass spectrometer (Thermo Finnigan). HPLC purification was carried out on a Waters 600 system equipped with a Waters 717 plus autosampler, a Waters 996 photodiode array detector, and a Sedere 55 evaporative light-scattering detector (Sedere, France).

Animal Material. Colonies of *Parazoanthus axinellae* (Schmidt, 1862) (Parazoanthidae) were collected as epibionts of the sponge *Axinella damicornis* by scuba (−20 m) off the Marseilles coast (Plane Island, France) in June 2008. A voucher specimen (no. 080623Ma4-28) has been deposited at the Centre d'Océanologie de Marseille, France.

Extraction and Isolation. The colonies of *P. axinellae* were carefully separated from the sponge *A. damicornis*. The material (17.2 g) was extracted three times with CH_2Cl_2/MeOH (1:1) at room temperature, yielding 1.1 g of extract after evaporation. The extract was fractionated by RP-C_{18} flash chromatography (elution with a decreasing polarity gradient of H_2O/MeOH from 1:0 to 0:1, then MeOH/CH_2Cl_2 from 1:0 to 0:1). The H_2O/MeOH (1:3) (195 mg) fraction was then subjected to RP-C_{18} semipreparative HPLC (Phenomenex, Luna C_{18}, 250 mm × 10 mm, 5 μm) with a gradient of H_2O/MeOH/TFA (from 28:72:0.1 to 30:70:0.1, flow 3.0 mL min^{-1}) to afford pure compounds **1** (1.4 mg), **2** (2.9 mg), **3** (1.4 mg), **4** (1.7 mg), and **5** (2.9 mg).

Computational Methods. Quantum chemical calculations were performed on both enantiomers of compound **1**. The Gaussian03W package[21] has been used for the conformational search as well as for circular dichroism calculations. Density functional theory (DFT) with the B3LYP functional[12] and Pople's 6.31++G(d, p) basis set[13] was used on the lowest energy conformer. TDDFT was employed to calculate excitation energy (in eV) and rotatory strength R in dipole velocity (R_{vel}) and dipole length (R_{len}) forms. The calculated rotatory strengths were simulated in an ECD curve by using a corrected Gaussian function.

$$\Delta\varepsilon(E) = \frac{1}{2.296 \times 10^{-39}\sqrt{2\pi\Delta}} \sum_a \Delta E_{0a} R_{0a} e^{-\left(\frac{(E - \Delta E_{0a})^2}{2\Delta}\right)}$$

where Δ is half the width of the band at $1/\varepsilon$ peak height expressed in energy units. The parameters ΔE_{0a} and R_{0a} are the excitation energies and the rotatory strengths for transition from 0 to a, respectively, Δ = 0.1 eV and R_{vel} were used.

Parazoanthine A (1): colorless oil; $[\alpha]^{20}_D$ −13 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 265 (3.94) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 281 (−0.19) nm; IR (film) ν_{max} 3365, 2953, 2917, 2854, 1714, 1657, 1624 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 318.2 $[M + H]^+$; HRESIMS *m/z* 318.15547 $[M + H]^+$ (calcd for $C_{15}H_{20}N_5O_3$, 318.15607, Δ −1.9 ppm).

Parazoanthine B (2): colorless oil; UV (MeOH) λ_{max} (log ε) 255 (3.20), 232 (3.39) nm; IR (film) ν_{max} 3345, 2957, 2924, 2854, 1719, 1669 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 316.2 $[M + H]^+$; HRESIMS *m/z* 316.13928 $[M + H]^+$ (calcd for $C_{15}H_{18}N_5O_3$, 316.14042, Δ −3.5 ppm).

Parazoanthine C (3): colorless oil; UV (MeOH) λ_{max} (log ε) 254 (3.91), 230 (3.25) nm; IR (film) ν_{max} 3365, 2953, 2917, 2854, 1714, 1657, 1624 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 330.2 $[M + H]^+$; HRESIMS *m/z* 330.15640 $[M + H]^+$ (calcd for $C_{16}H_{20}N_5O_3$, 330.15607, Δ 0.9 ppm).

Parazoanthine D (4): colorless oil; $[\alpha]^{20}_D$ −9.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 270 (3.21) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 279 (−0.62) nm; IR (film) ν_{max} 3345, 2957, 2924, 2854, 1719, 1669 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 410.1 (100), 412.1 (100) $[M + H]^+$; HRESIMS *m/z* 410.08203 $[M + H]^+$ (calcd for $C_{16}H_{21}BrN_5O_3$, 410.08223, Δ −0.5 ppm).

Parazoanthine E (5): colorless oil; UV (MeOH) λ_{max} (log ε) 264 (3.45), 235 (3.10) nm; IR (film) ν_{max} 3365, 2953, 2917, 2854, 1714, 1657, 1624 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 408.1 (100), 410.1 (100) $[M + H]^+$; HRESIMS *m/z* 408.06650 (calcd for $C_{16}H_{19}BrN_5O_4$, 408.06658, Δ −0.2 ppm).

Acknowledgment. Financial support was provided by the French ANR Biodiversité 2006 (ECIMAR project, www.ecimar.org). We are grateful to IOC/UNESCO and the Provence-Alpes-Côte d'Azur region for financial support provided by two grants (E.L.R. and N.C., respectively). The authors thank J. Ivanisevic, T. Perez, and A. Al Mourabit for fruitful discussions, M. Gaysinski for recording the NMR spectra, and J.-M. Guigonis for recording the HRESIMS spectra.

Supporting Information Available: Spectroscopic data; ^1H, ^{13}C, and 2D NMR spectra for **1**−**5**, experimental and calculated CD spectra for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP900437Y