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

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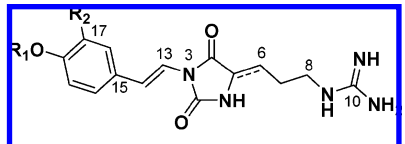
Five new hydantoin alkaloids, named parazoanthines A−E (**1**−**5**), were isolated as the major constituents of the Mediterranean sea anemone *Parazoanthus axinellae*. Their structural elucidation was achieved through NMR spectroscopic and mass spectrometric analyses. The absolute configuration of the 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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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.

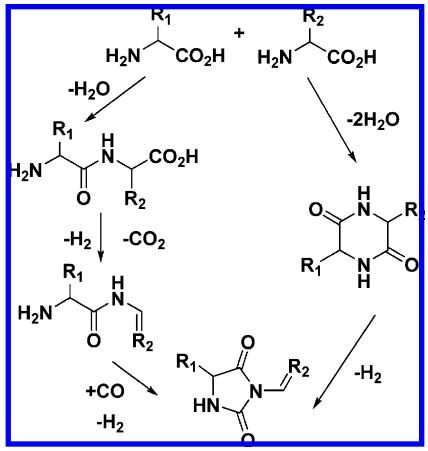
Compounds **4** and **5** were obtained as colorless oils, and their molecular formulas $C_{16}H_{20}BrN_5O_3$ and $C_{16}H_{18}BrN_5O_3$ were deduced from the clusters of two peaks (1:1) at *m*/*z* 410.08203 ($[M + H]^+$)/412.1 and *m*/*z* 408.06650 ($[M + H]^+$)/410.1, respectively, in the HRESIMS, 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

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 Sedex 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).

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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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