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Haouamines A and B: A New Class of Alkaloids from the Ascidian *Aplidium haouarianum*

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The chemical study of the ascidian *Aplidium haouarianum* has led to the isolation of the new metabolites haouamines A (**1**) and B (**2**) which belong to a novel class of alkaloids. The structure of **1** was established by interpretation of its spectroscopic data and those of the *N*-methyl derivative **3**, and confirmed by X-ray crystallographic analysis. The structure of **2** was deduced by spectroscopic study of its peracetyl derivative **2a**. In solution each haouamine exists as an unseparable mixture of two interconverting isomers derived by the presence of a highly strained 3-aza-[7]-paracyclophane moiety in their structures. Compound **1** exhibits a selective cytotoxic activity against the HT-29 human colon carcinoma cell line.

Introduction

Although first accounts on natural products from ascidians can be found in the mid-1970s, it was not until 10 years later when ascidians emerged as the target of an increasing number of chemical investigations. These studies have evidenced that the secondary metabolism of these organisms is dominated by the production of nitrogen-containing metabolites which often belong to structurally unprecedented families of natural products.[1] Furthermore, some of these metabolites are also prominent from a biomedical point of view, in particular as promising antitumor compounds, with several representatives currently under preclinical or clinical evaluation.[2]

Nitrogenous metabolites isolated from ascidians of the genus *Aplidium* fall into very diverse structural types. The first of these metabolites to be isolated was aplidiasphingosine, a compound likely derived from the condensation of a linear diterpenic acid and serine.[3] Several *Aplidium* species have also been source of nucleosides, ranging from common ones[4] to the complex derivatives shimofuridines A−G.[5] Amino acid derivatives include simple iodotyrosine-derived compounds[6] and the cyclodepsipeptide aplidine from *A. albicans*, which is one of the most renown marine metabolites because of its antitumor properties.[7] An interesting family of *Aplidium* bioactive metabolites are the lobatamides, macrocyclic compounds that contain an uncommon methylated oxime moiety and that have shown a promising cytotoxic activity.[8] Furthermore, ascidians of the genus *Aplidium* have been sources of a variety of alkaloids containing thiazol,[9] imidazol[9,10] pyridoacridine,[4b] indol-pyrimidine[11] or piperidine nuclei.[12]

As a part of our project directed toward the search for pharmacologically active natural products from ascidians of the southern coast of Spain, we report here the results of the chemical investigation of specimens of the tunicate *Aplidium haouarianum* collected off Tarifa Island (Cádiz, Spain). These studies have led to the isolation of two new metabolites, haouamines A (**1**) and B (**2**), which belong to an unprecedented class of alkaloids. The structures of the haouamines are characterized by possessing a 3-aza-[7]-paracyclophane moiety which demonstrates intriguing stereochemical features in this family of compounds.

Results and Discussion

Specimens of *A. haouarianum* were collected by hand using SCUBA diving at Tarifa Island and were im-

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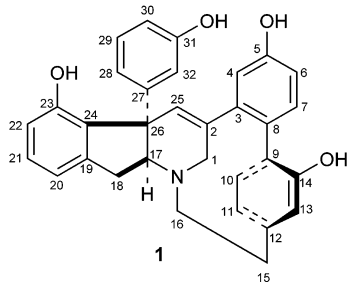

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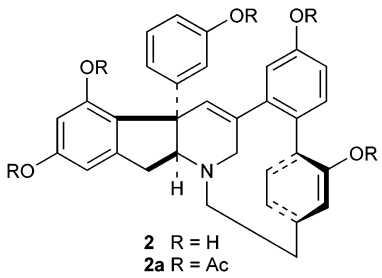

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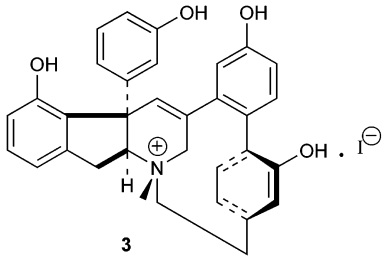

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mediately frozen. The frozen material was extracted with MeOH, and after solvent evaporation under reduced pressure, the resulting aqueous residue was extracted with Et_2O. Column chromatography of the organic extract led to several fractions that were tested in bioassays directed to detect in vitro cytotoxicity against P-388 mouse lymphoma, A-549 human lung carcinoma, and HT-29 human colon carcinoma cell lines. The more polar fractions, showing a significant cytotoxicity with IC_{50} values ranging from 0.5 to 1 μg/mL, were subjected to column chromatography and subsequent HPLC separations to yield the new alkaloids haouamine A (**1**, 150 mg) and haouamine B (**2**, 5.8 mg).

Compound **1** was obtained as a solid whose ^{1}H NMR spectrum recorded in CD_3COCD_3 was rather complex, showing two series of signals that suggested the presence of two components with similar structure in a 2:1 ratio. However, none of the chromatographic conditions assayed, including Sephadex LH-20 and HPLC (silica gel, RP-18, and CN columns) provided either separation of the apparent mixture or a change in the ratio of the components. Furthermore, by changing from CD_3COCD_3 to CD_3OD, C_5D_5N, or DMSO-d_6 as NMR solvents the two series of signals persisted, but the ratio between them changed to 1:3 when DMSO-d_6 was employed. This result indicated that in solution compound **1** was involved in a dynamic equilibrium process between two interconverting forms.

All attempts to simplify the NMR spectra by changing the temperature of the sample were unfruitful and only led to a loss of signal resolution. Therefore, we decided to acomplish structure elucidation by analysis of the NMR spectra of the mixture of the two interconverting forms. Among the different NMR solvents previously assayed, CD_3COCD_3 was chosen since in this solvent the spectra showed the more favorable dispersion of the signals generated by each form of the compound **1**, whose molecular formula $C_{32}H_{27}NO_4$, was determined by HRCIMS.

The structure of the major form of compound **1** in CD_3COCD_3, named isomer I, was defined as follows. The ^{13}C NMR spectrum of compound **1** showed, for isomer I, 26 signals between 158 and 113 ppm (Table 1) that together with a complex series of signals in the ^{1}H NMR spectrum between 7.3 and 5.5 ppm were attributable to the presence of four aromatic rings and one double bond in the molecule. These functional groups accounted for 17 of the 20 unsaturations deduced from the molecular formula.

As a first task we tried, with the aid of HMQC, COSY, and ^{1}H−^{13}C HMBC spectra, to assign the ^{1}H and ^{13}C NMR signals due to each aromatic ring and therefore establish their substitution patterns. However, the high complexity of the aromatic region of the ^{1}H NMR and ^{1}H−^{13}C HMBC spectra, together with the well-known fact that intense ^{1}H−^{13}C HMBC cross-peaks can be due, not only to three, but also to two bond correlations, did not allow the unambiguous assignment of the NMR signals of the aromatic rings. It was then considered that an HSQC-TOCSY experiment could give suitable additional information. The HSQC-TOCSY is a three-dimensional (3D) experiment that combines a homonuclear correlation experiment with inverse detected heteronuclear bond shift correlations. In this experiment one-bond correlations are shown as positive cross-peaks while long-range correlations due to the corresponding spin systems are inverted.[13] In this way, the HSQC-TOCSY spectrum of **1** allowed us to correlate each signal corresponding to protonated carbons of the aromatic rings with the signals due to protons at ortho and meta positions.

Thus, a careful analysis of the HMQC, DQFCOSY, HSQC-TOCSY, and ^{1}H−^{13}C HMBC spectra allowed us to assign the ^{1}H and ^{13}C NMR signals due to each aromatic ring. Furthermore, considering the four oxygen atoms of the molecular formula, the carbon signals at δ 157.6 (s), 156.5 (s), 155.8 (s), and 154.5 (s) were attributed to four aromatic carbons bearing hydroxyl groups. These data defined the presence in the molecule of the rings A−D showed in Figure 1.

The two remaining downfield signals of the ^{13}C NMR spectrum, a singlet at δ 140.5 and a doublet at δ 128.7 that was correlated in the HMQC spectrum with the signal at δ 6.49 (d, 1H, J = 2.6 Hz), were assigned to a trisubstituted double bond. In addition, the ^{13}C NMR spectrum showed between 75 and 34 ppm the signals of six sp^3 carbons identified as one quaternary carbon, one methyne, and four methylenes, whose proton signals appeared in the ^{1}H NMR spectrum between 3.3 and 0.8 ppm. The correlations observed in the DQFCOSY for all these signals allowed the definition of the independent spin systems E, F, and G shown in Figure 1.

The connection of all these subunits and the quaternary sp^3 carbon above-mentioned, to form the partial structure shown in Figure 2 was performed through a

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TABLE 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) Data for Haouamine A (1) in CD_3COCD_3[a,b]

		isomer I		isomer II	
ring	number	δ_H *mult.* (*J*, Hz)	δ_C *mult.*	δ_H *mult.*, (*J*, Hz)	δ_C *mult.*
A	1	2.39 *dd* (15.1, 2.6) 0.84 *d* (15.1)	53.0 *t*	2.29 *dd* (15.7, 2.5) 0.43 *d* (15.8)	44.2 *t*
	2	–	140.5 *s*	–	142.7 *s*
	3	–	145.3 *s*	–	145.8 *s*
	4	6.77 *d* (2.8)	117.2 *d*	6.53 *d* (2.8)	116.9 *d*
	5	–	156.5 *s*	–	156.6 *s*
	6	6.80 *dd* (8.1, 2.7)	113.7 *d*	6.78 *m*	113.1 *d*
	7	7.27 *d* (8.1)	127.8 *d*	7.47 *d* (8.2)	130.0 *d*
	8	–	131.1 *s*	–	130.0 *s*
	9	–	127.4 *s*	–	124.7 *s*
B	10	6.96 *d* (7.9)	131.9 *d*	7.16 *d* (7.6)	134.1 *d*
	11	6.82 *dd* (7.8, 1.5)	122.0 *d*	6.74 *dd* (7.8, 1.5)	129.1 *d*
	12	–	143.8 *s*	–	144.1 *s*
	13	5.58 *d* (1.6)	126.0 *d*	6.75 *d* (1.3)	116.2 *d*
	14	–	155.8 *s*	–	157.7 *s*
	15	2.62 *dd* (11.6, 4.3) 2.19 *ddd* (11.3, 11.3, 6.0)	40.0 *t*	2.56 *dd* (11.7, 5.0) 2.43 *ddd* (10.9, 10.9, 6.9)	38.1 *t*
	16	3.07 *dd* (14.1, 5.9) 1.79 *ddd* (14.2, 11.2, 4.4)	56.0 *t*	2.83 *dd* (13.5, 6.8) 2.06 *ddd* (13.5, 10.6, 5.1)	56.6 *t*
	17	3.22 *d* (5.4)	74.7 *d*	3.74 *dd* (7.9, 7.9)	73.1 *d*
	18	2.95 *d* (16.4) 2.87 *dd* (16.4, 5.7)	34.9 *t*	3.00 *dd* (16.6, 7.0) 2.77 *dd* (16.9, 8.9)	*c*
	19	–	144.9 *s*	–	144.2 *s*
C	20	6.89 *br d* (7.5)	116.1 *d*	6.67 *br d* (6.9)	116.6 *d*
	21	7.15 *dd* (7.6, 7.6)	128.6 *d*	7.01 *dd* (7.8, 7.8)	128.4 *d*
	22	6.76 *br d* (7.2)	114.5 *d*	6.61 *br d* (7.9)	113.7 *d*
	23	–	154.5 *s*	–	154.1 *s*
	24	–	132.3 *s*	–	133.0 *s*
	25	6.49 *d* (2.6)	128.7 *d*	6.16 *d* (2.6)	128.3 *d*
	26	–	62.5 *s*	–	58.4 *s*
	27	–	147.2 *s*	–	152.6 *s*
	28	6.52 *br d* (7.8)	118.6 *d*	6.79 *m*	118.2 *d*
D	29	7.03 *dd* (7.9, 7.9)	129.1 *d*	7.12 *dd* (7.9, 7.9)	128.9 *d*
	30	6.62 *br dd* (7.3, 2.5)	113.2 *d*	6.65 *br dd* (8.8, 2.5)	112.6 *d*
	31	–	157.6 *s*	–	157.3 *s*
	32	6.57 *dd* (2.2, 1.9)	114.5 *d*	6.83 *m*	114.1 *d*
	OH	8.4–7.5 *br s*	–	8.4–7.5 *br s*	–

[a] Assignments aided by DQFCOSY, HMQC, ^{1}H–^{13}C HMBC, and HSQC-TOCSY experiments. [b] Isomer I is the major form of haouamine A (**1**) in CD_3COCD_3. [c] Obscured by the solvent signal.

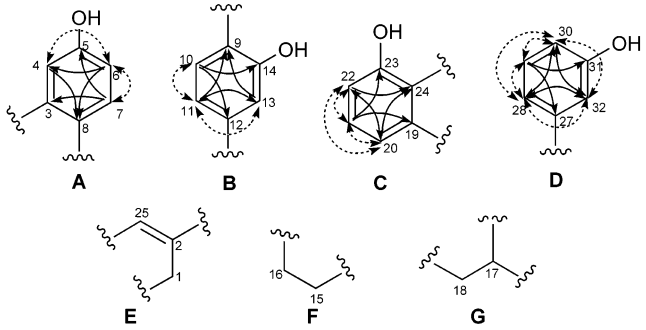

FIGURE 1. Structural subunits of haouamine A (**1**). Plain arrows indicate ^{1}H–^{13}C HMBC correlations. Dashed arrows indicate HSQC-TOCSY correlations.

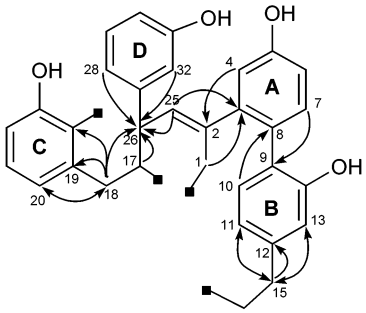

FIGURE 2. Partial structure of haouamine A (**1**). Plain arrows indicate ^{1}H–^{13}C HMBC correlations.

detailed analysis of the correlations exhibited by the ^{1}H–^{13}C HMBC spectrum. Several decisive correlations were the following ones. The A-ring proton signal at δ 7.27 (H-7) was correlated with the B-ring carbon signal at δ 127.4 (C-9), whereas the B-ring proton signal at δ 6.96 (H-10) was correlated with the A-ring carbon signal at δ 131.1 (C-8), indicating that rings A and B were connected through a single bond (Figure 2). Furthermore, the spin system F was attached to C-12 of ring B based on the correlations observed between the methylene proton signals at δ 2.62 (H-15a) and 2.19 (H-15b) and the B-ring carbon signals at δ 143.8 (C-12), 126.0 (C-13), and 122.0 (C-11). On the other hand, the olefinic proton signal of subunit E at δ 6.49 (H-25) was correlated with the A-ring carbon signal at δ 145.3 (C-3) and with a sp^3 quaternary carbon signal at δ 62.5 (C-26), which in turn, showed correlations with the ring D proton signals at δ 6.57 (H-32) and 6.52 (H-28) and with the methylene proton signal of subunit G at δ 2.95 (H-18a). This latter signal showed additional correlations with the ring C carbon signals at δ 144.9 (C-19), 132.3 (C-24), and 116.1 (C-20).

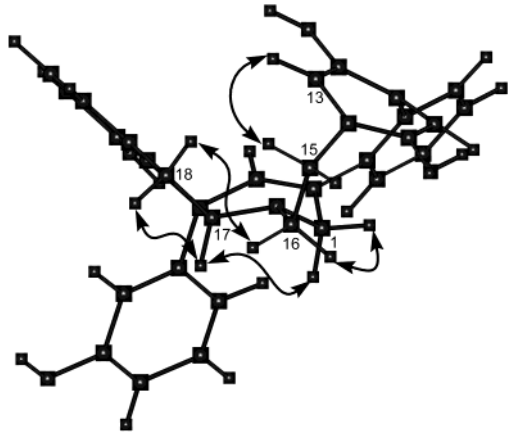

FIGURE 3. Structure of haouamine A (**1**) (isomer I) obtained by molecular mechanic calculations, showing selected ROESY correlations.

At this point all the carbon atoms of the molecular formula were assigned. Additional correlations observed in the $^1H-^{13}C$ HMBC spectrum indicated that C-1, C-16 and C-17 were connected to a common atom. Thus, among other correlations, the methylene proton signal δ 0.84 (H-1b) was correlated with the carbon signals at δ 74.7 (C-17) and 56.0 (C-16), while the methylene proton signal at δ 3.07 (H-16a) was correlated with the carbon signals at δ 74.7 (C-17) and 53.0 (C-1). It was therefore concluded that these three carbon atoms were connected to the nitrogen atom of the molecule. This assumption, which was confirmed upon observation in the $^1H-^{15}N$ HMBC spectrum of three bond couplings between the nitrogen atom signal at δ 43.0 and the signals due to protons H-15a, H-18a, and H-18b at δ 2.62, 2.95, and 2.87, respectively, implied the existence in the structure of **1** of an unique 3-aza-[7]-paracyclophane moiety involving aromatic ring B.

The plane structure of the isomer I was concluded by attachment of the quaternary carbons C-24 and C-26 to form a five-membered ring that justified the remaining unsaturation of the molecule. Finally, the ROESY correlations observed between the signal of H-17 and the signals of the D-ring protons H-28 and H-32, indicated a cis orientation for H-17 and the aromatic ring D.

Following a similar rationale to that previously developed, it was concluded that the minor form of compound **1** in CD_3COCD_3, or isomer II, possessed the same atom connectivities and stereochemical relationship between H-17 and the ring D as that described for isomer I. Therefore, the origin of the coexistence of these interconverting isomers seemed to be related either with a restricted rotation of bonds or with a slow pyramidal inversion of nitrogen in the highly strained 3-aza-[7]-paracyclophane system.

A careful reexamination of the correlations exhibited in the ROESY spectrum (CD_3COCD_3) of compound **1** suggested that isomer I could have an stereochemistry as represented in Figure 3. Specially diagnostic were the cross-peaks between the signals at δ 3.22 and 2.39 and between the signals at δ 3.07 and 2.95, assigned to the protons H-17, H-1a, H-16a, and H-18a of isomer I, respectively. Furthermore, the unusual upfield shift of H-1b and H-13 at δ 0.84 and 5.58, respectively, could also be justified in this stereostructure, where H-1b and H-13 are in the ring currents of rings B and C, respectively.

Treatment of **1** with MeI led to a compound that was identified as the quaternary ammonium iodide **3**, whose ^1H and ^{13}C NMR spectra showed no duplicity of signals. The structure of the *N*-methyl derivative **3** was determined by a detailed analysis of its ^1H NMR, ^{13}C NMR, COSY, HMQC, $^1H-^{13}C$ HMBC, and ROESY spectra that closely resembled those of isomer I of haouamine A (**1**). Furthermore, the ROESY spectrum showed, as in the case of isomer I of compound **1**, a correlation between the signals corresponding to H-17 and H-1a. This correlation together with the cross-peak between the *N*-methyl group signal and the H-13 signal, defined that compound **3** possessed the *N*-methyl group sticking up to the ring B. The absence of duplicity of NMR signals in compound **3**, where pyramidal inversion is prevented, might be consistent with the proposal that both isomers of haouamine A (**1**) result from a slow pyramidal inversion at the bridgehead amine, though it can also be claimed that the *N*-methyl group oriented as described above could have blocked atropoisomerism.

All these data led us to propose the structure **1** for haouamine A, a compound that in solution exists as an unseparable mixture of two interconverting isomers, generated either by the pyramidal inversion of the nitrogen or by atropoisomerism of the 3-aza-[7]-paracyclophane system. The unprecedented structural characteristics of this alkaloid prompted us to perform additional attempts of crystalization. As a result, we obtained a collection of crystals suitable for a crystallographic analysis that confirmed in all respects the proposed structure (Figure 4).[14]

Repeated preliminary efforts to isolate the minor component of *A. haouarianum* led to the obtention of a small amount of compound **2** as a solid, whose ^1H NMR spectrum was similar to that of **1,** also showing two series of signals. However, the structure characterization of **2** was complicated by the small amount of metabolite purified and the general low stability of these compounds. For this reason, a portion of the remaining fraction containing compound **2** was subjected to acetylation and subsequent HPLC separation to obtain the more stable peracetylated derivative **2a** whose molecular formula, $C_{42}H_{37}NO_{10}$, was established by HRCIMS.

In the ^1H NMR spectrum of **2a** (Table 2) recorded in $CDCl_3$ two series of signals, attributable to the presence in solution of two interconverting isomers, were also present. This spectrum showed 10 singlets between 1.6 and 2.4 ppm, integrating for 3H each, that were assigned to acetoxyl groups, five for each isomer, indicating that compound **2a** was a pentaacetate. It was therefore deduced that haouamine B (**2**) contained one more hydroxyl group than haouamine A (**1**). A careful analysis of COSY, HMQC, $^1H-^{13}C$ HMBC, and ROESY spectra of **2a**, following a similar rationale as that previously

(14) The structure determined by X-ray crystal analysis of haouamine A (**1**) corresponds to the one assigned by NMR studies for isomer I. Interestingly, when two aliquots of crystals were dissolved in CD_3COCD_3 and DMSO-d_6, respectively, both isomers were detected by NMR in the ratios above-described (2:1 in CD_3COCD_3 and 1:3 in DMSO-d_6).

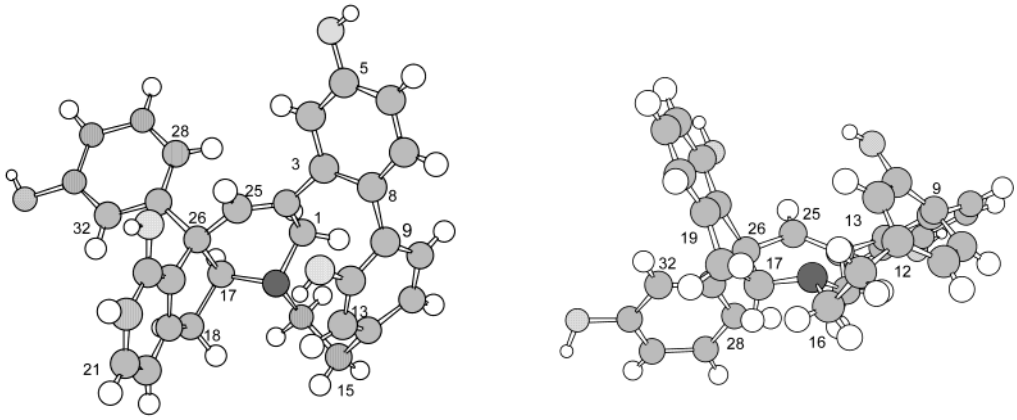

FIGURE 4. Two perspectives of the molecular structure of haouamine A (**1**) as determined by X-ray diffraction analysis.

described for compound **1**, enabled the determination that haouamine B (**2**) possesed an identical structure to haouamine A (**1**) except for the presence of an additional hydroxyl group located at C-21 of ring C.

An interesting question arises about the biosynthetic origin of this novel class of alkaloids. It is well-known that tyrosine is the precursor of a wide number of alkaloids whose structures are characterized by possessing the Ar−C_2−N subunit derived from Tyr, commonly via dopamine, which is often accompanied with additional Ar−C_1 or Ar−C_2 moieties derived from a partial degradation of the amino acids Phe or Tyr. Furthermore, the aromatic ring of all these subunits is usually oxygenated at 4-, 3,4-, or 3,4,5- positions.

From a biosynthetic point of view, in the structures of haouamines can be readily identified one Ar−C_2−N and three additional Ar−C_2 subunits that could suggest that these compounds are derived from the aromatic amino acids Phe/Tyr. However, the oxygenation pattern of all these units, hydroxylated at the meta position with respect to the C_2 chain, does not allow establishing a relationship between haouamines and the amino acid tyrosine. Thus, the biosynthesis of these alkaloids should imply either an unusual loss of the C-4 hydroxyl group of tyrosine along the biosynthetic pathway or the involvement of an undescribed natural amino acid precursor exclusively meta hydroxylated. However, the possibility that other different precursors might be involved in the biosynthesis of haouamines cannot be ruled out.

The new metabolites isolated from *A. haouarianum* were tested against the tumor cell lines of human lung carcinoma A-549, human colon carcinoma HT-29 and HCT-116, mice endothelial cells MS-1, and human prostate carcinoma PC-3. Haouamine A (**1**) showed a high and selective activity against the HT-29 cell line with IC_{50} = 0.1 μg/mL, while haouamine B (**2**) was only slightly cytotoxic against the MS-1 cell line with IC_{50} = 5 μg/mL.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR and 2D-NMR spectra were registered using $CDCl_3$, CD_3COCD_3, or CD_3OD as solvents. ^{1}H and ^{13}C NMR chemical shifts were referenced using the corresponding solvent signals (δ 7.26 and δ 77.0 for $CDCl_3$, δ 2.04 and δ 29.8 for CD_3COCD_3, and δ 3.30 and δ 49.0 for CD_3OD). DQFCOSY, ^{1}H−^{13}C HMBC (9 Hz), ^{1}H−^{15}N HMBC, and HSQC-TOCSY were performed using standard pulse sequences. In high-performance liquid chromatography (HPLC) separations, LiChrosorb Si-60 was used in normal phase mode, and LiChrospher RP-18, in reversed phase mode using a differential refractometer. All solvents were distilled from glass prior to use. Silica gel (60−200 μm) and precoated thin-layer chromatography plates were used for column chromatography and TLC, respectively.

Extraction and Isolation. Specimens of the tunicate *Aplidium haouarianum* were collected by hand using SCUBA near Tarifa Island (Spain) in May 1996 and immediately frozen. The frozen material was extracted with MeOH at room temperature. After solvent evaporation the aqueous residue was extracted with Et_2O to give an oily brown residue (3.0 g) that was chromatographed on a silica gel column using solvents of increasing polarities from hexane to Et_2O and, subsequently, $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH (7:3), and MeOH. The fraction eluted with hexane/Et_2O (1:9) was subjected to CC with a $CHCl_3$/MeOH gradient elution to give 150 mg of pure haouamine A (**1**). A portion of the fraction of the general chromatography eluted with $CHCl_3$/MeOH (7:3) was purified by CC with a $CHCl_3$/MeOH gradient elution, followed by normal phase HPLC separation eluting with $CHCl_3$/MeOH (9:1) to yield 5.8 mg of haouamine B (**2**). As haouamine B proved to be rather unstable in solution, the remaining portion of the fraction containing this compound was acetylated to prevent decomposition.

Haouamine A (1): white solid; dec 170 °C; $[\alpha]^{27}_D$ −52.0 (*c* 0.4, MeOH); UV (MeOH) λ_{max} 205 (ϵ 62000), 275 (11000) nm; IR (film) 3267 (br), 1594, 1413, 1280, 1235, 1184 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; LREIMS *m*/*z* (%) 489 (15) [M$^+$], 487 (80), 477 (59), 224 (67), 149 (84), 57 (100); HR-CIMS *m*/*z* 489.1909 [M$^+$], calcd for $C_{32}H_{27}NO_4$, 489.1940.

X-ray Crystallographic Data of 1. Suitable crystals of haouamine A (1) for X-ray analysis were obtained from MeOH. Crystal system monoclinic; space group $P2_1$; unit cell dimensions a = 9.3195(18) Å, α = 90°; b = 9.9686(19) Å, β = 104.141-(3)°; c = 15.378(3) Å, γ = 90°; volume 1385.4(5) Å^3; Z = 2; D_{calcd} = 1.327 Mg/m^3. Single-crystal data were collected on a Detector Area System with graphite-monochromated Mo Kα radiation (λ 0.71073 Å) at 100 K.

Acetylation of the Fraction Containing Compound 2. A portion (30 mg) of the fraction of the general chromatography eluted with $CHCl_3$/MeOH (7:3) which contained haouamine B (**2**) was dissolved in dry pyridine (0.5 mL) and treated with distilled acetic anhydride (0.5 mL). The mixture was stirred at room temperature for 3 h. After evaporation of the solvent under reduced pressure the residue was purified by HPLC (Hexane/EtOAc, 3:2) to yield peracetyl haouamine B (**2a,** 18.5 mg).

TABLE 2. ^{1}H NMR(400 MHz) and ^{13}C NMR (100 MHz) Data for Peracetyl Haouamine B (2a) in $CDCl_3$[a,b]

		isomer I		isomer II	
ring	number	δ_H *mult.* (*J*, Hz)	δ_C *mult.*	δ_H *mult.* (*J*, Hz)	δ_C *mult.*
A	1	2.48 *dd* (15.5, 2.7) 0.79 *d* (15.4)	52.5 *t*	2.32 *dd* (16.6, 2.4) 0.28 *d* (16.6)	43.7 *t*
	2	−	140.4 *s*	−	142.9 *s*
	3	−	144.2 *s*	−	145.1 *s*
	4	6.90 *m*	122.1 *d*	6.83 *d* (2.4)	123.2 *d*
	5	−	149.9 *s*	−	150.1 *s*
	6	7.09 *m*	120.5 *d*	7.07 *dd* (8.3, 2.5)	120.0 *d*
	7	7.54 *d* (8.2)	127.6 *d*	7.55 *d* (8.2)	127.6 *d*
	8	−	135.9 *s*	−	134.7 *s*
	9	−	131.9 *s*	−	130.6 *s*
B	10	7.19 *d* (8.1)	130.9 *d*	7.31 *d* (7.9)	133.2 *d*
	11	7.15 *dd* (8.2, 1.5)	127.1 *d*	6.93 *dd* (8.0, 1.5)	133.8 *d*
	12	−	143.8 *s*	−	143.9 *s*
	13	5.83 *d* (1.3)	129.2 *d*	7.01 *d* (1.4)	122.8 *d*
	14	−	148.3 *s*	−	150.4 *s*
	15	2.78 *m* 2.29 *m*	39.8 *t*	2.63 *dd* (12.0, 5.1) 2.53 *ddd* (12.0, 10.4, 7.2)	37.8 *t*
	16	3.05 *m* 1.78 *m*	56.3 *t*	2.78 *dd* (11.9, 5.3) 2.10 *m*	56.4 *t*
	17	3.35 *br d* (2.9)	75.1 *d*	3.92 *dd* (8.2, 8.1)	74.1 *d*
	18	2.98 *dd* (17.2, 4.9) 2.93 *d* (17.2)	34.6 *t*	3.08 *dd* (16.7, 7.8) 2.77 *dd* (16.7, 8.6)	28.4 *t*
C	19	−	141.4 *s*	−	140.9 *s*
	20	*c*	123.0 *d*[e]	7.08 *m*	123.1 *d*[f]
	21	−	*d*	−	150.4 *s*
	22	*c*	121.8 *d*[e]	7.08 *m*	123.0 *d*[f]
	23	−	*d*	−	150.4 *s*
	24	−	138.2 *s*	−	139.2 *s*
D	25	6.17 *d* (2.6)	128.9 *d*	5.86 *d* (2.5)	127.4 *d*
	26	−	62.5 *s*	−	58.0 *s*
	27	−	144.8 *s*	−	141.6 *s*
	28	6.89 *m*	124.8 *d*	7.04 *br d* (7.7)	124.7 *d*
	29	7.24 *dd* (8.1, 8.1)	129.2 *d*	7.32 *dd* (8.0, 7.9)	129.1 *d*
	30	6.90 *m*	120.1 *d*	6.98 *ddd* (8.0, 2.3, 0.9)	119.4 *d*
	31	−	150.7 *s*	−	150.3 *s*
	32	6.68 *dd* (2.1, 1.9)	120.5 *d*	7.07 *br s*	120.5 *d*
	CH$_3$CO−	2.32 *s*	21.1 *q*	2.27 *s*	21.1 *q*
	CH$_3$*CO*−	−	169.5 *s*	−	169.6 *s*
	CH$_3$*CO*−	2.25 *s*	21.1 *q*	2.26 *s*	21.1 *q*
	CH$_3$*CO*−	−	169.5 *s*	−	169.6 *s*
	CH$_3$*CO*−	2.20 *s*	21.1 *q*	2.19 *s*	20.8 *q*
	CH$_3$*CO*−	−	169.0 *s*	−	168.4 *s*
	CH$_3$*CO*−	1.92 *s*	20.2 *q*	2.17 *s*	20.8 *q*
	CH$_3$*CO*−	−	166.8 *s*	−	168.4 *s*
	CH$_3$*CO*−	1.83 *s*	20.2 *q*	1.65 *s*	19.4 *q*
	CH$_3$*CO*−	−	167.9 *s*	−	166.8 *s*

[a] Assignments aided by COSY, HMQC, and ^{1}H−^{13}C HMBC experiments. [b] Isomer II is the major form of **2a** in $CDCl_3$. [c] Obscured by the solvent signal. [d] Not detected. [e,f] Values with the same superscript may be interchanged.

Peracetyl Haouamine B (2a): white solid; $[\alpha]^{26}_D$ −27.1 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} 204 (ϵ 48100), 238 (20300) nm; IR (film) 1771, 1605, 1480, 1201, 1117, 1032 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 2; LR-CIMS *m*/*z* (%) 716 (11) [M^+ + H], 715 (100), 674 (78), 631 (20); HR-CIMS: *m*/*z* 716.2505 [M^+ + H], calcd for $C_{42}H_{38}NO_{10}$, 716.2496.

Methylation of Haouamine A (1). A solution of haouamine A (49.4 mg, 0.092 mmol) in acetone (20 mL) was treated with K_2CO_3 (13.5 mg, 0.098 mmol) and MeI (200 μL, 3.213 mmol) and the mixture refluxed for 6 h. After filtration and solvent evaporation the crude mixture was chromatographed on a small silica gel column with mixtures of $CHCl_3$/MeOH from 99:1 to 95:5, followed by reversed phase HPLC (MeOH/H_2O, 4:1) of selected fractions to yield 3.2 mg of *N*-methylhaouamine A iodide (**3**).

***N*-methylhaouamine A iodide (3):** white solid; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.23 (d, *J* = 8.7, H-7), 7.09 (dd, *J* = 7.7, 7.6, H-21), 6.99 (dd, *J* = 7.9, 7.8, H-29), 6.98 (d, *J* = 7.9, H-10), 6.88 (br d, *J* = 7.8, H-11), 6.84 (br d, *J* = 7.2, H-20), 6.74 (m, H-4 and H-6), 6.57 (br d, *J* = 7.8, H-22), 6.53 (m, H-30), 6.52 (br s, H-25), 6.48 (ddd, *J* = 7.7, 1.7, 1.0, H-28), 6.45 (dd, *J* = 2.0, 1.9, H-32), 6.14 (d, *J* = 1.1, H-13), 3.09 (d, *J* = 5.6, H-17), 3.00 (dd, *J* = 16.7, 6.0, H-18a), 2.95 (m, H-16a), 2.94 (d, *J* = 16.7, H-18b), 2.92 (s, NCH_3), 2.74 (dd, *J* = 11.5, 4.5, H-15a), 2.44 (m, H-15b), 2.13 (dd, *J* = 15.0, 2.5, H-1a), 1.76 (ddd, *J* = 14.0, 10.8, 4.8, H-16b), 0.64 (d, *J* = 15.0, H-1b); ^{13}C NMR (CD_3-OD, 100 MHz) δ 160.1 (s, C-14), 158.4 (s, C-31), 157.2 (s, C-5), 156.0 (s, C-23), 149.0 (s, C-27), 145.8 (s, C-3), 145.6 (s, C-19), 145.0 (s, C-12), 142.1 (s, C-2), 133.4 (s, C-24), 132.8 (s, C-8), 132.7 (d, C-10), 131.5 (s, C-9), 130.0 (d, C-25 and C-29), 129.6 (d, C-21), 127.9 (d, C-7), 122.5 (d, C-11), 120.5 (d, C-13), 119.4 (d, C-28), 118.3 (d, C-4 or C-6), 116.7 (d, C-20), 115.0 (d, C-32), 114.9 (d, C-22), 114.4 (d, C-6 or C-4), 113.8 (d, C-30), 76.3 (d, C-17), 63.4 (s, C-26), 57.8 (t, C-16), 54.1 (q, NCH_3), 53.9 (t, C-1), 41.3 (t, C-15), 37.0 (t, C-18).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1** and **2a**. X-ray crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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