Arnoamines A and B, New Cytotoxic Pentacyclic Pyridoacridine Alkaloids from the Ascidian *Cystodytes* sp.

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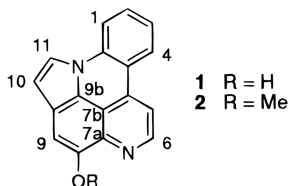
Two new cytotoxic pyridoacridine alkaloids, arnoamines A (**1**) and B (**2**), have been isolated from the ascidian *Cystodytes* sp. collected in the vicinity of Arno Atoll, Republic of Marshall Islands. Compounds **1** and **2** are proposed to be the first members of a new class of pentacyclic pyridoacridine alkaloids possessing a pyrrole ring fused to the pyridoacridine ring system. The structures of **1** and **2** were proposed on the basis of the interpretation of spectroscopic data, particularly those obtained from HMBC and NOE NMR experiments. The arnoamines were unexpectedly found to incorporate deuterium at C-10 and C-11 of the pyrrole ring when dissolved in $CDCl_3$/TFA-*d*.

Marine invertebrates, especially sponges and ascidians,[1] have been an excellent source of polycyclic heteroaromatic alkaloids.[2] Many of these compounds have generated interest both as challenging problems for structure elucidation and synthesis, as well as for their biological activities, which often involve DNA intercalation[3] or topoisomerase II inhibition.[4,5] We report the isolation and structure determination of two new cytotoxic pentacyclic pyridoacridines, arnoamines A (**1**) and B (**2**), from the brownish-purple ascidian *Cystodytes* sp.[6] collected near Arno Atoll, Republic of the Marshall Islands. In addition to being cytotoxic to several human



tumor cell lines, arnoamines A and B display an unexpected chemical reactivity in that the carbon-bound protons H-10 and H-11 exchange for deuterium when the compounds are dissolved in TFA-*d*. Compounds **1** and **2** are proposed to be the first members of a new family of pentacyclic pyridoacridine alkaloids that possess a pyrrole ring fused to the pyridoacridine skeleton.

A methanolic extract, obtained by soaking homogenized, freeze-dried ascidian tissue, was subjected to a solvent partition scheme followed by normal-phase silica gel column chromatography and reversed-phase column chromatography, yielding pure arnoamines A (**1**) and B (**2**). Neither compound would completely dissolve in typical organic solvents unless a small amount of acid (usually TFA) was added. As is usually observed for pyridoacridine alkaloids, addition of the acid induced a bathochromic shift in the visible absorption.

The HREIMS spectrum of **1** showed a molecular ion consistent with the formula $C_{17}H_{10}N_2O$, requiring 14 sites of unsaturation. A broad IR absorption band at 3416 cm^{-1} indicated the presence of a hydroxyl group. The ^1H NMR spectrum of **1** (see Table 1) showed nine signals, all resonating within the low-field region from 7.5 to 8.9 ppm, that could be divided into four spin systems. Six of the signals (H-1 to H-6) were characteristic of the protons attached to rings A and D of pyridoacridines. The third spin system consisted of a pair of signals, H-10 (δ 7.51, d, J = 3 Hz) and H-11 (8.49, d, J = 3 Hz), which were typical of the C-3 and C-2 positions of a pyrrole ring, respectively. The fourth system consists of a single proton, H-9, which is observed as a singlet at δ 8.20.

Because of the large number of quaternary carbons, connection of the proton spin systems and assignment of the carbon chemical shifts relied heavily on long-range heteronuclear correlation NMR experiments. Fortunately, the HMBC experiment showed correlations to all of the quaternary carbons (see Table 1), allowing the pentacyclic framework to be assembled. Particularly important for the positioning of the pyrrole ring on the acridine skeleton were the correlations between C-9b and H-9, H-10, and H-11 and between C-9a and both H-10 and H-11. The hydroxyl group was placed on C-8 on the basis of carbon chemical shift (δ 139.8), and the structure of **1** was established as shown.

Confirmation of the proposed structure was obtained using a series of difference NOE experiments. In fact, sequential experiments starting with H-9 showed dipolar coupling around the circumference of the molecule from H-9 ↔ H-10 ↔ H-11 ↔ H-1 ↔ H-2 ↔ H-3 ↔ H-4 ↔ H-5 ↔ H-6, strongly supporting structure **1** for arnoamine A.

The molecular formula of arnoamine B (**2**) was assigned as $C_{18}H_{12}N_2O$ on the basis of HREIMS data, indicating the presence of one additional carbon and two hydrogens, as compared with **1**. Except some slight chemical shift differences, the signals in the low-field region of the ^1H NMR spectrum of **2** (Table 1) were nearly

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Table 1. NMR Data for Arnoamines A (1) and B (2)

	1			**2**		
atom	^{13}C (mult)[a]	^{1}H (mult, *J*)	HMBC (^{13}C → ^{1}H)	^{13}C (mult)[a]	^{1}H (mult, *J*)	HMBC (^{13}C → ^{1}H)
1	116.3 (d)	8.28 (d, *J* = 8.4 Hz)	H-2, H-3, H-4	116.4 (d)	8.35 (d, *J* = 8.1 Hz)	H-3
2	135.7 (d)	8.12 (t, *J* = 7.6 Hz)	H-3, H-4	135.9 (d)	8.15 (t, *J* = 7.8 Hz)	H-4
3	126.1 (d)	7.80 (t, *J* = 7.8 Hz)	H-1	126.2 (d)	7.82 (t, *J* = 7.5 Hz)	H-1
4	127.1 (d)	8.68 (d, *J* = 7.8 Hz)	H-1, H-2, H-3	127.4 (d)	8.74 (d, *J* = 7.8 Hz)	H-2
4a	117.9 (s)		H-2, H-3, H-4, H-5	118.1 (s)		H-1, H-3
4b	141.7 (s)		H-4, H-6	142.0 (s)		H-4
5	109.6 (d)	8.35 (d, *J* = 6.1 Hz)	H-6	110.6 (d)	8.47 (br s)	
6	137.5 (d)	8.84 (d, *J* = 6.1 Hz)	H-5	137.8 (d)	8.92 (br s)	
7a	126.3 (s)		H-6, H-9	126.6 (s)		H-9
7b	113.4 (s)		H-5	113.5 (s)		H-5, H-9, H-10, H-11
8	139.8 (s)		H-9	143.8 (s)		H-9, OMe-8
9	115.6 (d)	8.20 (br s)		110.4 (d)	8.16 (s)	
9a	119.7 (s)		H-10, H-11	119.5 (s)		H-11
9b	119.5 (s)		H-9, H-10, H-11	119.3 (s)		H-9, H-10
10	110.7 (d)	7.51 (d, *J* = 3.0 Hz)	H-11	110.7 (d)	7.60 (br s)	H-11
11	121.3 (d)	8.49 (d, *J* = 3.0 Hz)	H-10	121.3 (d)	8.57 (br s)	H-10
11b	135.8 (s)		H-1, H-2, H-4	136.0 (s)		H-2
OMe-8				57.0 (q)	4.27 (s)	

[a] Carbon multiplicities were assigned using a HMQC experiment.

identical with those for **1**; however, one additional methoxyl signal was observed at δ 4.27. On the basis of NMR data, the structure of **2** was proposed to be a methoxy analogue of **1**, where the hydroxyl group on C-8 is replaced with a methoxyl. The structure and the ^{13}C NMR chemical shift assignments were strongly supported by the HMBC data.

In the course of determining the chemical structures of arnoamines A (**1**) and B (**2**), an unexpected chemical reactivity was observed. After being dissolved in a mixture of TFA-*d* and $CDCl_3$ for approximately 2 weeks, the ^{1}H NMR spectra for both compounds showed that the signals assigned to H-10 and H-11 had decreased in their integration to approximately 50% relative to other one-proton signals. Moreover, instead of each being observed as a doublet (J = 3 Hz), they were each observed as a doublet onto which a singlet was superimposed (see Figure 1a). One explanation for this result was that under the acidic conditions of the NMR solvent H-10 and H-11 had both exchanged for deuterons to approximately the same extent.

Support for the above hypothesis was obtained from the ^{13}C NMR spectra recorded for samples of **1** and **2**. Although signals for the deuterated carbons (C-10 and C-11) were not observed due to the additional spin−spin splitting, quadrupole broadening, and loss of Overhauser enhancement associated with direct deuterium substitution, secondary isotopic effects were observed. Upfield-shifted satellite signals were associated with the signals assigned to C-9a (Δ 0.08 ppm), C-10 (Δ 0.15 ppm), and C-11 (Δ 0.07 ppm) (see Figure 1b).

A direct demonstration of D/H exchange was observed by dissolving a partially deuterated sample of compound **1** in a mixture of protio-TFA and $CDCl_3$ and raising the temperature to 50 °C. After 20 min the integration for the signals assigned to H-10 and H-11 in the ^{1}H NMR spectrum had been increased to about 85% of unity and both signals had become well-resolved doublets (J = 3 Hz). Alternatively, complete deuteration was accomplished by dissolving each alkaloid in TFA-*d*. After the temperature was raised to 50 °C for 2 h, the signals assigned to H-10 and H-11 in the NMR spectra of both compounds had nearly disappeared. The EIMS spectra of the deuterated samples were identical to the original ones, except that all major ions were observed at 2 mass units higher than those observed for the protonated compounds.

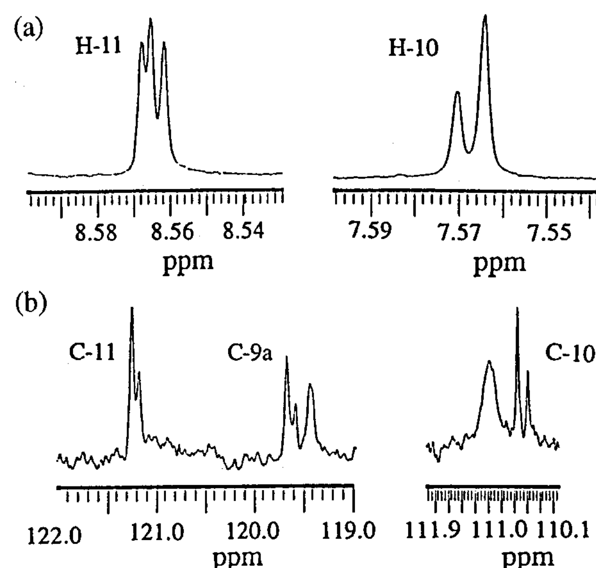
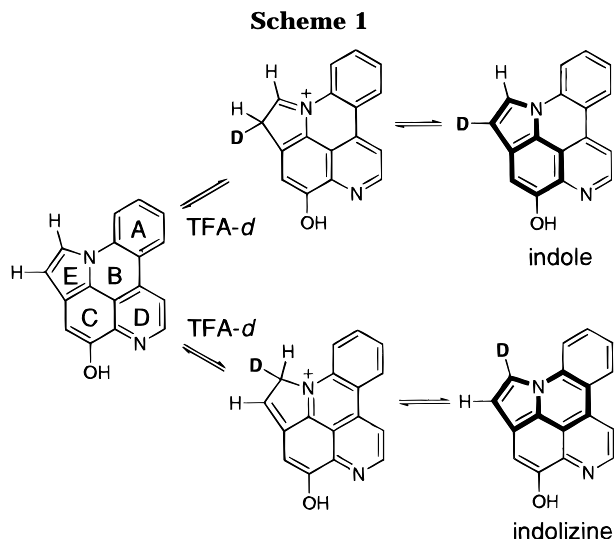


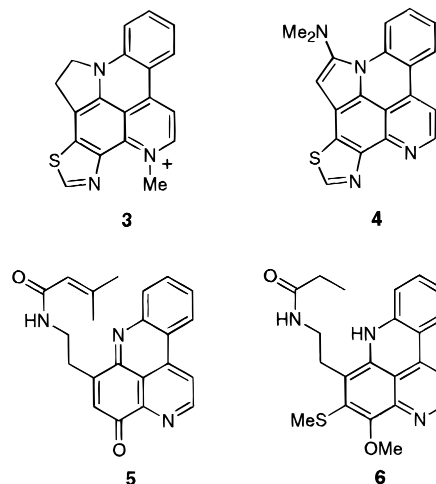
Figure 1. Selected NMR signals for partially deuterated arnoamine A (**1**): (a) ^{1}H signals for pyrrole protons H-10 and H-11, (b) ^{13}C signals for C-9a, C-10, and C-11 showing upfield satellite peaks resulting from a secondary isotope effect.

The mechanism of H/D exchange can be explained by considering the structures of both alkaloids to be a combination of various heteroaromatic ring systems (Scheme 1). For example, rings C and E resemble an indole unit and therefore can be protonated easily at position C-10, which is equivalent to position-3 of an indole ring system. In contrast, rings B and E resemble an indolizine moiety, which is preferentially protonated at position C-11, equivalent to position-3 of indolizine. In the acidic media, both positions are protonated (or deuterated) simultaneously and equally, causing the equal reduction of the H-10 and H-11 signals.

Arnoamine A exhibited selective cytotoxicity against the MCF-7 breast cancer cell line with a GI_{50} value of 0.3 μg/mL versus GI_{50}s of 2.0 and 4.0 μg/mL against the A-549 lung and HT-29 colon cell lines, respectively. Arnoamine B exhibited GI_{50}s of 5.0, 2.0, and 3.0 μg/mL against the MCF-7, A-549, and HT-29 cell lines, respec-

Scheme 1





tively. Initial assays showed differential toxicity between the DNA repair-deficient CHO cell line xrs-6[7] and the DNA repair-proficient CHO cell line BR1,[8] suggesting a mechanism of action involving topoisomerase II inhibition. Follow-up tests with purified enzyme indicated that the arnoamines inhibit the catalytic activity of topoisomerase II at concentrations higher that 90 μM but do not stabilize a cleavable complex. These results are consistent with those of other pyridoacridines which have been shown to inhibit topoisomerase II through intercalation into DNA.[4,9] The moderate activity level also conforms to the finding that pyridoacridines in the iminoquinone oxidation state are generally more potent cytotoxins and topoisomerase II inhibitors.[4]

Arnoamines A (**1**) and B (**2**) are the first pentacyclic pyridoacridine alkaloids discovered that have a pyrrole ring fused to the pyridocridine skeleton. Closely related compounds included the hexacyclic pyridoacridines cyclodercitin (**3**)[10] and stelletamine (**4**).[11] For each of these metabolites it can be proposed that the pyrrole rings are formed through cyclization of the more common amidoethyl side chain observed in the cystodytin A (**5**)[12] and the varamine A (**6**).[13]

Experimental Section

General. NMR was performed in $CDCl_3$ supplemented with ~5% TFA-*d*, unless otherwise specified. Chemical shifts are referenced to solvent peaks: 7.26 ppm (residual $CHCl_3$) and 77.0 ppm for $CDCl_3$. Mass spectral data were obtained in the EI mode.

Isolation and Purification of 1 and 2. The brownish-purple colonial ascidian *Cystodytes* sp. was collected in the vicinity of Arno Atoll, Republic of Marshall Islands, in November 1995, and preserved at −20 °C. After freeze-drying, the animals (25 g) were crushed and macerated exhaustively in MeOH (3 × 150 mL) to yield crude extract (1.5 g), which was subjected to a solvent partitioning scheme to yield hexane-, CCl_4-, and $CHCl_3$-soluble materials (123, 148, and 41 mg, respectively). The CCl_4 extract, which had the highest mass and showed the most interesting NMR spectrum, was chromatographed on a Si gel column using a gradient solvent system, from 25% hexane in EtOAc through EtOAc to 25% MeOH in EtOAc, and compound **1** (9 mg) was obtained. The last fraction from this fractionation was further chromatographed on a phenyl-bonded phase Si gel column using a gradient solvent, from 10% aqueous MeOH through MeOH to 2% TFA in MeOH, and compound **2** (37 mg) was obtained.

Arnoamine A (1): yellow glass; IR (film) ν_{max} 3418 (broad), 3094, 1681, 1200 cm^{-1}; UV (0.5% TFA in MeOH) λ_{max} (log ϵ) 278 (4.11), 401 (3.09), 486 (3.17) nm, (in 50 mM KOH in MeOH) λ_{max} (log ϵ) 258 (4.15), 328 (3.89), 431 (3.05) nm; ^{1}H and ^{13}C NMR (TFA-*d*/$CDCl_3$) see Table 1; EIMS *m/z* (rel intensity) 258 (M^+, 100), 229 (67), 201 (10), 129 (36); HREIMS calcd for $C_{17}H_{10}N_2O$ 258.0791, found 258.0794.

10,11-Dideuterioarnoamine A: EIMS *m*/*z* (rel intensity) 260 (M^+, 100), 231 (28), 203 (6); HREIMS calcd for $C_{17}H_8D_2N_2O$ 260.0917, found 260.0854.

Arnoamine B (2): yellow glass; IR (film) ν_{max} 3094, 1681, 1200 cm^{-1}; UV (0.5% TFA in MeOH) λ_{max} (log ϵ) 280 (4.15), 398 (3.15), 467 (3.22) nm, (in 50 mM KOH in MeOH) λmax (log ϵ) 254 (4.14), 309 (3.88), 400 (3.17) nm; ^{1}H and ^{13}C NMR (TFA-*d*/$CDCl_3$) see Table 1; EIMS *m*/*z* (rel intensity) 272 (M^+, 83), 271 (100), 257 (19), 242 (66), 229 (52); HREIMS calcd for $C_{18}H_{12}N_2O$ 272.0947, found 272.0946.

10,11-Dideuterioarnoamine B: EIMS *m*/*z* (rel intensity) 274 (M^+, 77), 273 (100), 259 (22), 244 (66), 231 (38); HREIMS calcd for $C_{18}H_{10}D_2N_2O$ 274.1073, found 274. 0967.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **1** and **2** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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