Deoxyamphimedine, a New Pyridoacridine Alkaloid from Two Tropical *Xestospongia* Sponges

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Marine organisms are rich sources of polycyclic pigments. Pyridoacridine alkaloids are an important class of marine-derived heteroaromatic pigments, many of which possess cytotoxic activity. Amphimedine (**1**), isolated from an *Amphimedon* sp. sponge,[1] constitutes the first example of this type of alkaloid from a marine organism. Pyridoacridines are not exclusive to sponges (phylum Porifera) and have been reported from other marine phyla, such as Urochordata, Cnidaria, and Mollusca.[2] In the search for new anticancer metabolites from marine animals, our research group recently published the isolation of neoamphimedine from a Philippine *Xestospongia* sp. and *Xestospongia* cf. *carbonaria* from Micronesia.[3] Neoamphimedine (**2**) is a new topoisomerase II inhibitor that induces catenation of plasmid DNA by mammalian topoisomerase II. We now report the isolation and structural elucidation of a new pyridoacridine alkaloid, deoxyamphimedine (**3**), along with amphimedine (**1**) and neoamphimedine (**2**), from two specimens of a *Xestospongia* sp., collected from the Philippines and Palau. Comparative bioactivity studies reveal that, unlike amphimedine (**1**) and neoamphimedine (**2**), deoxyamphimedine (**3**) appears to damage DNA through the production of reactive oxygen species.

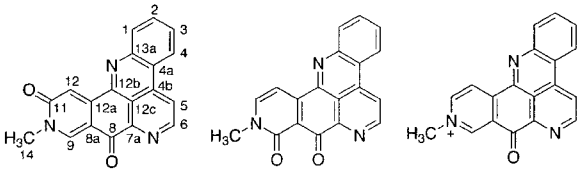
The sponge *Xestospongia* sp., collected from Baler, the Philippines, was extracted with MeOH and MeOH/$CHCl_3$ (9:1) mixtures. The pooled extracts were subjected to a solvent partition scheme to yield hexane-, $CHCl_3$-, and aqueous MeOH-solubles, as explained in the Experimental Section. The $CHCl_3$-soluble material yielded neoamphimedine (**2**). Purification of the aqueous MeOH extract by C-18 HPLC afforded deoxyamphimedine (**3**). No amphimedine (**1**) was detected in this sample.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for 2 and 3 (δ in ppm, J in Hz)

	2		**3**		
position	δ_C[a]	δ_C[b]	δ_H[c]	δ_C[c]	δ_C[b]
1	131.7 d	134.2 d	8.55 (d, 6.8)	133.3 d	136.4 d
2	131.5 d	137.5 d	8.13 (dt, 6.8, 7.7)	133.9 d	139.6 d
3	130.0 d	134.4 d	8.06 (t, 7.7)	132.9 d	136.8 d
4	122.9 d	125.7 d	8.97 (d, 7.7)	125.2 d	127.2 d
4a	121.9 s	120.8 s		124.3 s	122.7 s
4b	137.2 s	145.7 s		139.9 s	147.8 s
5	119.2 d	125.5 d	9.13 (d, 6.0)	122.9 d	127.6 d
6	149.0 d	140.4 d	9.32 (d, 6.0)	151.4 d	142.8 d
7a	146.6 s	138.7 s		149.5 s	139.7 s
8	179.0 s	175.6 s		179.2 s	174.9 s
8a	119.1 s	116.7 s		131.6 s	130.5 s
9	159.6 s	161.8 s	9.87 br s	147.9 d	148.8 d
11	144.6 d	144.2 d	9.28 (d, 6.0)	149.3 d	151.3 d
12	101.3 d	108.2 d	9.45 (d, 6.0)	124.6 d	128.2 d
12a	149.6 s	151.5 s		147.3 s	150.5 s
12b	146.4 s	145.1 s		145.5 s	144.7 s
12c	117.3 s	117.8 s		121.0 s	120.9 s
13a	145.0 s	148.0 s		146.7 s	149.4 s
14	38.1 q	40.4 q	4.64 s	49.0 q	51.8 q

[a] Measured in $CDCl_3/CD_3OD$ (2:1). [b] Measured in TFA-*d*/$CDCl_3$ (2:1). [c] Measured in CD_3OD.

The specimen of *Xestospongia* cf. *carbonaria* collected from Palau was extracted with MeOH, MeOH/$CHCl_3$ (1:1) mixtures, and $CHCl_3$, respectively. The combined organic extracts were partitioned as above. Silica gel flash column chromatography (CC) of a precipitate formed at the earliest stage of the partition protocol (see Experimental Section) afforded amphimedine (**1**). The $CHCl_3$ layer was chromatographed over silica to yield neoamphimedine (**2**). The remaining aqueous MeOH phase was subjected to a multistep gradient C-18 flash CC and resulted in the isolation of deoxyamphimedine (**3**).



1 **2** **3**

Amphimedine (**1**) was isolated as a yellow, poorly soluble powder. The chemical formula ($C_{19}H_{11}N_3O_2$) was deduced by HREIMS; ^{1}H chemical shifts and coupling patterns of **1** were identical with those reported by Schmitz et al.[1] Neoamphimedine (**2**) was obtained as a yellow-brown solid. The HREIMS and ^{1}H and ^{13}C NMR data (Table 1) of **2** were found to be superimposable with the published data.[3]

Deoxyamphimedine (**3**), also a yellow-brown amorphous solid, was obtained as a TFA salt. Its structure was elucidated predominantly by interpretation of NMR and MS data and comparison to spectral data for neoamphimedine (**2**, Table 1). HREIMS (*m*/*z* 298.0966) corresponded to the molecular formula $C_{19}H_{12}N_3O$. The positive mode FABMS displayed ions at *m*/*z* 299 and 300 suggesting in situ reduction during the ionization process, which is commonly reported for pyridoacridine alkaloids.[2] The ^{13}C NMR spectrum of **3** revealed 17 aromatic carbon atoms (Table 1), a carbonyl (δ 179.2), and an *N*-methyl

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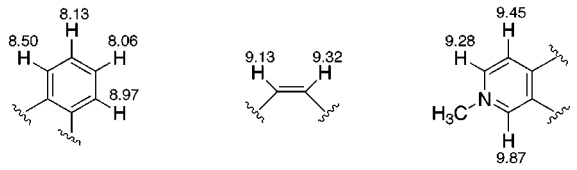

Figure 1. Spin systems within deoxyamphimedine (**3**).

(δ 49.0). The ^{1}H NMR spectrum (500 MHz) in CD_3OD or in TFA-*d*/$CDCl_3$ (2:1) contained nine aromatic proton signals and an *N*-methyl group (δ 4.64, s). A DEPT-135 experiment coupled with an HMQC measurement disclosed the existence of an extra aromatic proton in **3** in comparison to amphimedine (**1**) or neoamphimedine (**2**). A phase sensitive gradient double quantum COSY experiment indicated the presence of three spin systems (Figure 1). Four proton signals were easily assigned to the contiguous carbon atoms of a disubstituted benzene ring (C-1 through C-4) by their chemical shift, multiplicity, and the coupling constant values (δ 8.06, t, $J = 7.7$ Hz; δ 8.13, dt, $J = 6.8$, 7.7 Hz; δ 8.55, d, $J = 6.8$ Hz, and δ 8.97, d, $J = 7.7$ Hz). An isolated AB system resonating at δ 9.32 and δ 9.13 ($J = 6.0$ Hz) was attributed to the α (H-6) and β (H-5) protons of the trisubstituted pyridine system. The remaining *ortho*-coupled proton resonances at δ 9.28 (H-11) and δ 9.45 (H-12), each with a coupling constant of 6.0 Hz, formed the last spin system. H-11 also showed a long-range homonuclear coupling to an isolated singlet at δ 9.87 in the COSY spectrum. The latter signal was ascribed to H-9 as a result of the highly deshielding effect of a positively charged nitrogen atom (position 10) and the carbonyl functionality at C-8. These data also suggested that the only difference between **3** and amphimedine (**1**) or neoamphimedine (**2**) was the deoxygenation of the *N*-methyl pyridine ring. A gradient HMBC experiment optimized for $J = 10$ Hz provided additional structural information. The *N*-methyl protons showed the expected long-range correlations to C-9 (δ 147.9, d) and C-11 (δ 149.3, d). The same correlations were also observed in the 2D NOESY spectrum. The remaining three bond HMBC cross-peaks were seen from ring A protons, namely, from δ 8.55 (H-1) to δ 132.9 (C-3) and δ 124.3 (4a); from δ 8.13 (H-2) to δ 146.7 (C-13a) and δ 125.2 (C-4); from δ 8.06 (H-3) to δ 133.3 (C-1) and δ 124.3 (4a); and from δ 8.97 (H-4) to δ 133.9 (C-2). A weak four bond correlation was also observed between H-9 (δ 9.87) and C-7a (δ 149.5). Interestingly, the proton resonances at δ 9.13 and δ 9.32 did not show any long-range correlations in the HMBC spectrum, but the prominent NOE correlation between δ 8.97 (H-4) and δ 9.13 unequivocally placed them at positions C-5 and C-6, respectively.[2] Comparison of the NMR data of **3** with those of amphimedine[1] or neoamphimedine (Table 1) clearly indicated the structure of **3** to be as shown.

Deoxyamphimedine (**3**) showed cytotoxic activity against human colon tumor cells (HCT-116) with an IC_{50} of 335 nM. In a related cytotoxicity assay, deoxyamphimedine (**3**) was tested in Chinese hamster ovary cells, AA8 (wild type) and EM9 (sensitive to single strand (ss) DNA break damage).[4] EM9 cells were 4-fold more sensitive to damage from **3** than the AA8 cells, with IC_{50} values of 6 and 25 μM, respectively. The XRCC1 gene is mutated in EM9 cells, thus rendering them deficient in DNA ligase III activity. EM9 cells are therefore sensitive to ssDNA breaks caused by either topoisomerase I poisons or the generation of reactive oxygen species (ROS).[5−7] Neoamphimedine (**2**) and amphimedine (**1**) did not show enhanced toxicity in the EM9 cells and had IC_{50}'s of 248 and 319 μM in AA8 cells, respectively.

Deoxyamphimedine (**3**) was studied in a purified enzyme system, along with amphimedine (**1**) and neoamphimedine (**2**). DNA cleavage was measured as described by Matsumoto et al.[8] Deoxyamphimedine (**3**) showed the highest degree of DNA cleavage of the three compounds and did not require topoisomerase I or II for this activity. DNA cleavage required only a reducing agent and aerobic conditions and was time- and concentration-dependent. DNA damage caused by deoxamphimedine (**3**) could be prevented or significantly reduced by the addition of the antioxidants catalase, benzoic acid, glutathione, or *N*-acetyl-cysteine. Chelators, EDTA, ferrozine, and deferoxamine mesylate also afforded slight protection. These data are indicative of ROS-mediated DNA damage. It is hypothesized that ROS generation results from quinone redox cycling or Fenton reaction and is the subject of ongoing research.

Experimental Section

General Procedures. The IR spectrum was recorded as film using polyethylene IR card. All NMR spectra were obtained at 500 MHz (^{1}H) and 125 MHz (^{13}C). Chemical shifts are reported in ppm relative to residual nondeuterated solvents, CD_3OD, $CDCl_3$ (for $CDCl_3$/CD_3OD 2:1 mixture) and TFA-*d* (TFA-*d*/$CDCl_3$ 2:1 mixture). For flash chromatography, SiO_2 (Merck Kieselgel 60, particle size 0.040−0.063 mm) and C-18 material (J. T. Baker, 40 μm, 275 Å) were used. HPLC separations were performed using a Rainin Dynamax 60 Å ODS column (10 × 250 mm, 8 μm) with photodiode array detection.

Extraction and Isolation. The animal material examined first (sample number PI96-3-37) was collected at a depth of 10 m near Dibud (Baler, Philippines) in March, 1996. Identification of the sponge was done by one of us (M.K.H.). A voucher sample is kept at University of the Philippines, Diliman, Quezon City, Philippines and at the University of Utah, Salt Lake City, Utah. The frozen sponge was ground and extracted exhaustively with MeOH and MeOH/$CHCl_3$ (9:1) mixtures. The crude extract (165 mg) was partitioned between 10% water in MeOH (100 mL) and hexane (3 × 100 mL). The concentration of the aqueous MeOH was adjusted to 30% by the addition of water (40 mL) and then extracted with $CHCl_3$ (3 × 100 mL). The $CHCl_3$ extract (32.6 mg) contained primarily neoamphimedine (**2**), which was purified by C-18 HPLC employing 60% MeOH/40% aqueous TFA (0.05%) solution as eluent (9 mg yield). The aqueous MeOH phase was dried and repeatedly triturated with MeOH to remove insoluble salts. The MeOH-soluble material (102 mg) was also applied to a C-18 HPLC column. Elution with 15% MeOH/85% aqueous TFA (0.05%) solution afforded deoxyamphimedine (**3**, 8 mg).

The Palauan *Xestospongia* specimen was kindly provided by Dr. D. John Faulkner (sample number 95-040). The freeze-dried sponge (40 g) was extracted with MeOH, MeOH/$CHCl_3$ (1:1), and $CHCl_3$, respectively. The combined organic extracts were evaporated in vacuo and subjected to the solvent partition

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scheme explained above. A precipitate (250 mg) formed upon addition of 90% MeOH to the crude extract (7.4 g) and was largely amphimedine (**1**). Silica flash CC of this precipitate using increasing amounts of MeOH in CH_2Cl_2 yielded pure amphimedine (**1**, 102 mg). An aliquot (203 mg) of the $CHCl_3$-soluble fraction (420 mg) was also subjected to SiO_2 flash CC using CH_2Cl_2/MeOH gradients to provide neoamphimedine (**2**, 52 mg). The desalted aqueous MeOH layer (3.0 g) was chromatographed over C-18 (flash CC, in two portions) using a step gradient from 0% to 100% MeOH in water (0.1% TFA) solution. Deoxyamphimedine (**3**, 21 mg) was eluted with 30% aqueous MeOH.

Deoxyamphimedine (3). Yellow-brown amorphous solid; UV (MeOH) λ_{max} (log ϵ) 206 (3.92), 244 (3.93), 294 (sh, 3.52), 388 (3.51), 478 (2.59) nm; FTIR (film) 2925, 1684, 1620, 1595, 1510, 1197, 1130 cm^{-1}; ^1H NMR (500 MHz) see Table 1; ^{13}C NMR (125 MHz) see Table 1; HREIMS *m*/*z* 298.0966 $[M]^+$ (calcd for $C_{19}H_{12}N_3O$ 298.0980).

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Supporting Information Available: ^1H and ^{13}C NMR spectral data for deoxyamphimedine (**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

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