Plakinidine D, a New Pyrroloacridine Alkaloid from the Ascidian *Didemnum rubeum*

Paul W. Ford[‡] and Bradley S. Davidson*[,†]

Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322-0300, and Department of Chemistry University of Hawaii at Manoa, Honolulu, Hawaii 96822

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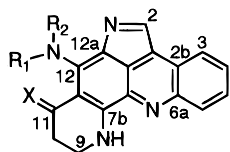
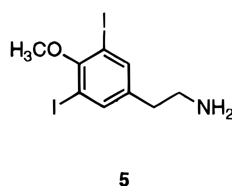
Plakinidine D (**4**), a new pyrroloacridine metabolite, was isolated together with the known compound 3,5-diiodo-4-methoxyphenethylamine (**5**) from the ascidian *Didemnum rubeum* collected near the island of Rota, Northern Mariana Islands. Spectroscopic methods were used to determine the structure of plakinidine D, which was also confirmed through the formation and characterization of the derivatives *N*-acetylplakinidine D and 11-deoxyplakinidine D. Plakinidine D represents the first metabolite in the pyrroloacridine family of compounds to be isolated from an ascidian.

In recent years, numerous polycyclic heteroaromatic compounds have been discovered from marine sponges and ascidians.[1,2] These compounds have drawn attention 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] In 1990, plakinidines A−C (**1**−**3**) were isolated from a *Plakortis* sp. sponge independently by two research groups.[6,7] They represented the first members of the pyrroloacridine class of marine alkaloids. We have recently isolated a new pyrroloacridine metabolite, plakinidine D (**4**), together with the known compound 3,5-diiodo-4-methoxyphenethylamine (**5**),[8] from the ascidian *Didemnum rubeum* Monniot and Monniot, 1996[9] (Didemnidae), collected near the island of Rota, Northern Mariana Islands. Plakinidine D represents the first metabolite in the pyrroloacridine family of compounds to be isolated from an ascidian. In this paper, we present the isolation and structure elucidation of this new marine alkaloid.



1 R_1=H, R_2=CH_3, X=O
2 R_1=CH_3, R_2=CH_3, X=O
3 R_1=H, R_2=CH_3, X=O (9,10-Didehydro)
4 R_1=H, R_2=H, X=O
6 R_1=H, R_2=Ac, X=O
7 R_1=H, R_2=H, X=H_2



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The freeze-dried ascidian tissues were steeped in ethanol, and the resulting extract was subjected to a solvent partition scheme followed by silica gel column chromatography, yielding large amounts of 3,5-diiodo-4-methoxyphenethylamine (**5**), a brown crystalline material that was identified on the basis of a comparison of the experimental data with those reported in the literature,[8] and plakinidine D (**4**), an amorphous dark red solid. HREIMS indicated that **4** had a molecular formula of $C_{17}H_{12}N_4O$, which was consistent with the presence of 17 carbon signals in the ^{13}C NMR spectrum. When taken together, the intense color and the high carbon to proton ratio suggest that compound **4** may have been a pyridoacridine alkaloid; however, whereas the ^1H NMR spectra of pyridoacridines characteristically show signals for both an *ortho*-disubstituted aromatic ring and a 2,3,4-trisubstituted pyridine ring, the spectrum recorded for plakinidine D (see Table 1) showed the signals for the substituted benzene ring (H-3, H-4, H-5, and H-6), but the pair of doublets typically observed for the pyridine ring was replaced by a single isolated aromatic proton (H-2, 8.51 ppm).

An initial review of ascidian metabolites reported in the literature uncovered many pyridoacridine alkaloids, but none contained the same substitution patterns or carbon−hydrogen atom ratio as metabolite **4**. An expanded search of metabolites obtained from other marine sources provided two compounds satisfying these requirements: the pyrroloacridine sponge metabolites plakinidines A (**1**) and B (**2**). Plakinidine A is a monomethylamine derivative, having a molecular formula of $C_{18}H_{14}N_4O$, while plakinidine B is the dimethyl amine analog having a molecular formula that includes an additional CH_2 group ($C_{19}H_{16}N_4O$). The mass spectral data for plakinidine D (**4**) was consistent with a structure corresponding to demethylplakinidine A.

Although the primary aromatic amine protons in plakinidine D were not observed in the ^1H NMR spectrum due to deuterium exchange when solvents containing TFA-*d* were used, they were observed when spectra were obtained using DMSO-d_6. In fact, three exchangable one-proton signals (11.0, 10.9, and 10.2 ppm) were observed, indicating that the primary amine was diastereotopic. This can be rationalized through the formation of a hydrogen bond between one of the amine protons and the adjacent ketone. Diastereotopic protons of a primary amine were also observed in the ^1H NMR spectrum of makaluvamine A, a related tunicate metabolite.[10] Together, the physical properties and spectroscopic data support structure **4** for the natural product.

Attempts to fully confirm that the structure of compound **4** incorporates a plakinidine-like pyrroloacridine skeleton involved both careful comparisons of the ^1H and ^{13}C NMR data for compound **4** with those reported for plakinidines A and B and interpretation of heteronuclear correlation NMR experiment data (Table 1). The

† Utah State University.
‡ University of Hawaii.
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Table 1. NMR Data for Plakinidine D (**4**)[a]

position	δ ^{13}C	δ ^{1}H (*J* in Hz)	HMBC correlations
2	131.0	8.51 (s, 1H)	
2a	121.7		H-2
2b	124.8		H-4, H-6
3	124.0	8.44 (d, 1H, *J* = 7.5 Hz)	H-5
4	129.3	7.79 (dt, 1H, *J* = 7.8, 1.3 Hz)	H-6
5	127.0	7.72 (dt, 1H, *J* = 7.8, 1.3 Hz)	H-3
6	130.8	8.27 (d, 1H, *J* = 7.5 Hz)	H-3, H-4
6a	144.3		H-3, H-4, H-5, H-6
7a	140.0		
7b	152.0		H-9
8		10.20 (br s, 1H)	
9	40.0	3.80 (t, 2H, *J* = 7.2 Hz)	H-10
10	35.9	2.80 (t, 2H, *J* = 7.2 Hz)	H-9
11	193.8		H-9, H-10
11a	99.6		H-10
12	157.6		
12a	125.1		H-2
12b	117.7		H-2
13		11.00 (br s, 1H), 10.90 (br s, 1H)	

[a] All spectra recorded in DMSO-d_6.

highly fused nature of the polycyclic heteroaromatic ring system in the plakinidines has provided challenging structure elucidation problems. Due to the preponderance of quaternary carbons and heteroatoms, it is difficult to establish with certainty the location of some carbons (i.e., C-12 and C-7a). Even a phase sensitive 2D-INADEQUATE experiment performed during the original structure work on plakinidine B (**2**) failed to establish the location of C-12b in the contiguous carbon framework,[7] and only through a crystal structure of plakinidine A (**1**) were the locations of all atoms in the molecule ultimately assigned.[7]

Unable to detect C-12 in the long-range 2D NMR experiments performed on **4**, we undertook chemical derivatizations of the natural product in hopes of producing a compound that would both allow confirmation of the proposed primary amine functionality at C-12 and be better suited for two-dimensional NMR correlation experiments. With these goals in mind, two strategies were undertaken. The first was to simply acetylate the primary amine, a step which would confirm the presence of the amine and, perhaps, provide an N-H proton signal for NMR experiments. The second was to reduce the ketone to an alcohol, placing a proton on C-11.

Acetylation of the primary amine in plakinidine D proved to be problematic. Stirring a solution of **4** in anhydrous pyridine and acetic anhydride for 2 days provided less than 10% conversion to the acetamide **6**, as indicated by TLC and ^{1}H NMR, and even the addition of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) had no effect on the reaction. Other methods for acetylation of the primary amine functionality were unsuccessful, leading to no reaction at all or loss of starting material. Ultimately, the pyridine−acetic anhydride reaction was successfully scaled up to allow isolation of a small amount of compound **6**, suitable for NMR and MS analyses.

The ^{1}H NMR spectrum of **6** showed five aromatic signals corresponding to the protons associated with the pyrroloacridine skeleton. The lone aromatic singlet (H-2), originally observed at δ 8.51 in plakinidine D, shifted downfield to δ 8.85 in the spectrum recorded for compound **6**. As expected, a signal representing the acetamide methyl group was present at δ 2.50. Of the two methylene signals, the upfield signal was observed as a triplet (H-10, 2.84 ppm) and the downfield signal was observed as a broad two proton multiplet (H-9, 4.4 ppm). Two signals attributable to N-H protons were visible at δ 10.6 and 8.2, which were assigned to the NH-8 and the newly formed acetamide proton, respectively. The existence of two additional resonances (171.4 and 24.1 ppm) in the ^{13}C NMR spectrum were also consistent with the formation of the acetamide moiety. Although formation of derivative **6** confirmed the presence of a primary amine in the natural product, the acetamide proton was too broad for detection in HMBC experiments. Furthermore, derivative **6** was surprisingly unstable in $CDCl_3$ yielding a 1:1 mixture of the starting material (**4**) and the acetamide after 3 days.

In an attempt to form the second derivative, plakinidine D (**4**) was treated with excess $NaBH_4$ in THF followed by acetic anhydride/pyridine. A single product was formed; however, tetrahydroquinoline derivative **7** was obtained instead of the expected diacetylated amino alcohol. The reduction of the ketone to a methylene under such mild conditions can be rationalized by considering that C-11 is benzylic and can also be considered a vinylogous amide.

Reduction product **7** showed a molecular ion by HREIMS which agreed with the proposed molecular formula $C_{17}H_{14}N_4$. The NMR spectral data were similar to those obtained for natural product **4** with the major exception that the carbon resonance for ketone C-11 (193.8 ppm) was replaced by an additional sp^3 aliphatic signal at δ 19.6. Further support for the structure was obtained from the ^{1}H NMR spectrum, which now showed a spin system composed of two two-proton signals triplets, one at δ 2.61 (H-11) and the other at δ 3.60 (H-9), that were both coupled to a two-proton pentet at δ 1.98 (H-10). The three exchangeable protons were visible in the ^{1}H NMR spectrum (DMSO-d_6) of **7** with the two primary amine protons coalesced into a broad singlet at δ 7.91 and the N-8 proton a very broad signal at ~10.8 ppm. All other signals in the ^{1}H and ^{13}C NMR spectra showed chemical shifts, couplings, and long-range ^{1}H−^{13}C correlations consistent with the pyrroloacridine structure **7**.

In this paper we have reported the first isolation of a pyrroloacridine alkaloid from a marine ascidian. Considering that plakinidines A−C were isolated from sponges of the genus *Plakortis*, the isolation of plakinidine D from *D. rubeum* allows for further speculation about whether an associated microorganism may be the ultimate biosynthetic origin of this interesting group of compounds.[11]

Experimental Section

General Experimental Procedures. Chromatography was carried out using silica gel, Merck 60 (60 A), 230−400 mesh, according to the procedure described by Still.[12] Reactions and chromatography fractions were analyzed by TLC, using 2 × 5 cm aluminum-backed plates covered with a 0.20 mm layer of silica gel 60 F_{254}, Art. 5554 (E. Merck, Darmstadt). Visible and UV light were used for visualization of compounds. IR spectra were recorded on a Perkin-Elmer FT-IR spectrophotom-

eter. UV spectra were obtained on either a Shimadzu UV-101 PC or a Perkin-Elmer 280 spectrophotometer in the solvent indicated at 25 °C. NMR spectra were recorded on either a General Electric QE-300 or GE GN-Omega 500 spectrometer in $CDCl_3$ (7.26 and 77.0 ppm) or in other solvents as indicated. For new compounds, purity was ascertained by both proton and ^{13}C NMR spectra. Mass spectra were recorded on a VG Analytical 70SE (EI) mass spectrometer. High-resolution mass spectral analysis was performed on samples obtained by preparative chromatographic purification.

Isolation and Purification. The frozen ascidian specimens, collected in July 1995, were freeze-dried and the lyophilized tissues (421 g) extracted with ethanol (~1.5 L). The concentrated ethanol extract was partitioned between hexane (3×100 mL) and methanol (100 mL). The methanol solution was diluted with 10% H_2O and partitioned with carbon tetrachloride (3×50 mL) and then chloroform (3 x 50 mL), and these fractions were combined and concentrated to yield 5−6 g of a dark red-brown oil that solidified upon standing. The methanol/water solution was concentrated and freeze-dried to yield a light brown solid (6.1 g). Repeated flash chromatography (silica gel 4:1 $CHCl_3$/MeOH) of the combined CCl_4/$CHCl_3$ fraction and the MeOH/H_2O residue provided pure 3,5-diiodo-4-methoxyphenethylamine (9 g) as brown crystals (after recrystallization from methanol) and plakinidine D (60 mg) as a dark red solid.

Plakinidine D (4): UV (4:1 $CHCl_3$/MeOH) λ_{max} 250 (ϵ 2040), 286 (ϵ 14 180), 340 (ϵ 22 040), 386 (ϵ 8160), 552 (ϵ 5740); IR (film) υ_{max} 3287, 1599 cm^{-1}; ^1H NMR see Table 1; ^{13}C NMR see Table 1; EIMS *m/z* (rel int) 288 (100), 259 (35), 233 (25), 191 (12), 144 (13), 69 (15); HREIMS *m/z* 288.1048 (calcd for $C_{17}H_{12}ON_4$, 288.1011).

Compound 6. A solution of plakinidine D (**4**) (25 mg) in anhydrous pyridine (10 mL) was treated with acetic anhydride (100 μL) at room temperature and the mixture stirred for 48 h. The pyridine and excess acetic anhydride were removed *in vacuo*, and the residue was purified by chromatography over silica gel with 9:1 $CHCl_3$/MeOH as solvent. The early eluting fractions afforded 2 mg (10.4% yield) of compound **6** as a bright purple solid. Later eluting column fractions provided 6−8 mg of unreacted starting material **4**: ^1H NMR ($CDCl_3$) δ 10.65 (br s, 1H), 8.85 (s, 1H), 8.38 (m, 2H), 8.13 (br s, 1H), 7.82 (m, 2H), 4.39 (br t, 2H), 2.84 (t, 2H, J = 7.2 Hz), 2.50 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 197.0, 171.4, 156.5, 150.2, 148.5, 142.0, 131.4, 130.0, 129.0, 125.0, 124.0, 42.1, 39.1, 24.1 (5 carbons not observed).

Compound 7. To a solution of compound **4** (30 mg) in THF (15 mL) was added $NaBH_4$ (40 mg), and the reaction mixture was stirred for 1.5 h at room temperature. Most of the THF was then removed by rotary evaporation and the residue dissolved in dilute aqueous NaOH (~50 mL). The basic solution was then extracted with CH_2Cl_2 (3×50 mL), and the combined organic phase was concentrated to give a crude product that was purified by chromatography over silica gel with $CHCl_3$/MeOH (4:1) as eluent, yielding 6 mg of compound **7** as a dark red solid: ^1H NMR ($CDCl_3$) δ 8.45 (s, 1H), 8.43 (dd, 1H, J = 7.1, 1.3 Hz), 8.28 (dd, 1H, J = 7.1, 1.3 Hz), 7.91 (br s, 2H), 7.80 (dd, 1H, J = 6.9, 1.3 Hz), 7.75 (dd, 1H, J = 6.9, 1.3 Hz), 3.60 (t, 2H, J = 5.3 Hz), 2.61 (t, 2H, J = 6.2 Hz), 1.98 (pent, 2H, J = 5.3 Hz); ^{13}C NMR ($CDCl_3$) δ 156.5, 150.5, 149.5, 144.4, 142.9, 131.1, 129.2, 127.4, 124.2, 124.0, 122.4, 115.1, 40.5, 20.8, 19.6 (2 carbons not observed); HREIMS *m/z* 274.1223 (calcd for $C_{17}H_{14}N_4$, 274.1183).

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