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Isolation, Structure Determination, and Biological Activity of a Novel Alkaloid, Perophoramidine, from the Philippine Ascidian *Perophora namei*

Sheryl M. Verbitski,[†] Charles L. Mayne,[‡] Rohan A. Davis,[†] Gisela P. Concepcion,[§] and Chris M. Ireland*,[†]

Departments of Medicinal Chemistry and Chemistry, University of Utah, Salt Lake City, Utah 84112, and the Marine Sciences Institute, University of the Philippines, Diliman, Quezon City 1101, Philippines

cireland@pharm.utah.edu

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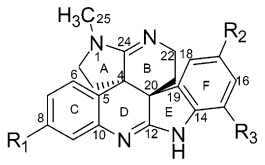
Abstract: Chemical investigation of the Philippine ascidian *Perophora namei* has resulted in the isolation of a novel polycyclic alkaloid, perophoramidine (**1**). The structure of **1** was determined by the interpretation of 1D/2D NMR and MS data. Dehalogenation of perophoramidine (**1**) by ammonium formate catalyzed transfer hydrogenation confirmed the type and number of halogen atoms present in **1**.

The study of ascidian chemistry has been driven by frequent discovery of unique and biologically active metabolites. Examples include the antitumor compound ecteinascidin-743[1] from *Ecteinascidia turbinata* and the HIV integrase inhibitor lamellarin α 20-sulfate[2] from an unidentified ascidian. As part of our continuing chemical investigation of tropical ascidians, we report here the isolation, structure determination, and biological activity of a novel polycyclic alkaloid, perophoramidine (**1**).



1 R_1=Br, R_2=Cl, R_3=Cl
2 R_1=R_2=R_3=H

The colonial ascidian *Perophora namei* Hartmeyer and Michaelson (Perophoridae) was collected in the Philippines just east of Zamboanga City near Tictauan Island. *P. namei* was exhaustively extracted with MeOH, and the dried extract was subjected to a modified Kupchan procedure,[3] resulting in a hexane, $CHCl_3$, and 30% aq MeOH fraction. The $CHCl_3$-soluble material was purified by silica gel flash chromatography using a gradient from 2% MeOH/98% $CHCl_3$ to 30% MeOH/70% $CHCl_3$. The 5% MeOH/95% $CHCl_3$ fraction was further purified by trituration using a MeOH/H_2O (7:3) mixture to yield pure perophoramidine (**1**).

Perophoramidine (**1**) was assigned a molecular formula of $C_{21}H_{17}N_4BrCl_2$ on the basis of (+)-HRFABMS analysis ($[M + H]^+$, *m/z* 475.0028, −0.0066 mmu). The (+)-LRFABMS isotopic pattern for **1** suggested the presence of one Br and two Cl's, and MeOD exchange LRFABMS indicated that perophoramidine (**1**) contained one exchangeable proton. The analysis of ^{1}H NMR (Table 1) data revealed one *N*-methyl singlet (H25), two isolated ethylene systems (H2−H3, H21−H22), and five aromatic signals that were assigned to a 1,2,4-trisubstituted (ring C) and 1,2,3,5-tetrasubstituted (ring F) benzene ring on the basis of coupling constants. The ^{13}C NMR spectrum displayed signals for 21 carbons. DEPT analysis indicated that **1** contained four methylenes, five methines, and one methyl carbon. An HMQC experiment established all one-bond ^{1}H−^{13}C connectivities.

Analysis of the gradient HMBC experiment provided connectivity between rings A and B to form a 1-methylhexahydro-1*H*-pyrrolo[2,3-*b*]pyridine system. Some key correlations establishing this ring system were observed between H3a to C24 and H3b to C20; H21a and H21b to C4, C20, and C22; and H22a and H22b to C20 and C24. Connections from the pyrrolopyridine system to rings C, D, E, and F were limited to HMBC correlations from H3a and H3b to C5 and from H21a and H21b to C12 and C19. The data interpretation was initially hampered by observation of an atypical 5-bond HMBC correlation from the *N*-methyl (H25) to C5. The hexacyclic system was confirmed by a 2D INADEQUATE experiment ($^1J_{CC}$ = 55 Hz) that provided all ^{13}C−^{13}C connections. These connectivities were determined both visually and with NMR Analyst software.[4]

Additional evidence for the type and number of halogens present in **1** was obtained by the dehalogenation of perophoramidine under hydrogenation conditions using HCO_2NH_4 and Pd/C in MeOH.[5] Purification using a C_{18} SPE cartridge afforded pure dehalogenated perophoramidine (**2**) as its TFA salt. The structure of **2** was determined following interpretation of 1D/2D NMR (Table 1) and MS data. The dehalogenated compound was assigned a molecular formula of $C_{21}H_{20}N_4$ on the basis of (+)-HRFABMS analysis ($[M + H]^+$, *m/z* 329.1758, −0.0008 mmu).

* To whom correspondence should be addressed.
[†] Department of Medicinal Chemistry, University of Utah.
[‡] Department of Chemistry, University of Utah.
[§] University of the Philippines.

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TABLE 1. NMR Data for Perophoramidine (1) and Dehalogenated Perophoramidine (2)

	perophoramidine (**1**)[a]			dehalogenated perophoramidine (**2**)[b]		
C no.	^{13}C (ppm) ($^{1}J_{cc}$, Hz)	^{1}H (ppm) (mult, *J*, Hz)	gHMBC	^{13}C (ppm)	^{1}H (ppm) (mult, *J*, Hz)	gHMBC
2a	46.6 (33.9)	3.09 (dd, 10.0, 8.8)	3, 4, 24	51.0	3.46 (dd, 10.5, 8.9)	3, 4, 24
2b		3.27 (ddd, 10.0, 9.5, 6.2)	3		3.67 (m)	3
3a	30.1 (33.9, 32.4)	1.77 (ddd, 12.1, 9.5, 8.8)	4, 5, 24	30.1	1.91 (dd, 12.5, 5.8)	4, 5, 24
3b		1.71 (dd, 12.1, 6.2)	4, 5, 20		2.10 (ddd, 12.5, 8.9, 8.7)	2, 4, 5, 20
4	45.7 (42.8, 42.6, 35.2, 32.4)			50.9		
5	125.7 (62.7, 62.4, 42.8)			123.0		
6	127.5 (62.7, 56.3)	6.74 (d, 8.3)	4, 8, 10	124.3	6.73 (dd, 7.8, 1.2)	4, 8, 10
7	127.0 (63.9, 56.3)	7.11 (dd, 8.3, 2.0)	5	124.9	7.07 (7.9, 7.8)	5
8	121.9 (67.4, 63.9)			130.3	7.34 (ddd, 7.9, 7.9, 1.2)	6, 9, 10
9	123.1 (67.4, 66.3)	7.23 (d, 2.0)	4, 5, 7, 8, 10	122.2	7.19 (d, 7.9)	4, 5, 7, 8, 10
10	138.6 (66.3, 62.4)			138.8		
12	173.2 (41.4)			169.2		
14	148.3 (77.3, 54.7)			148.2		
15	120.6 (77.3, 67.6)			113.9	7.12 (d, 7.8)	14, 16, 17, 19, 20
16	129.2 (69.1, 67.6)	7.27 (d, 1.7)	14, 15, 17, 18	130.1	7.31 (dd, 7.8, 7.8)	14, 15, 17, 19
17	128.3 (69.1, 66.3)			123.6	7.04 (dd, 7.8, 7.8)	15, 19
18	122.6 (66.3, 64.0)	6.96 (d, 1.7)	14, 15, 16, 20	123.4	7.09 (d, 7.8)	16, 20
19	135.9 (64.0, 54.7, 46.5)			128.6		
20	52.6 (46.5, 41.4, 35.2, 29.7)			48.2		
21a	25.4 (32.4, 29.7)	1.39 (dd, 13.7, 5.8)	4, 12, 19, 20, 22	24.3	1.68 (ddd, 14.1, 5.1, 1.2)	4, 19, 20
21b		2.22 (ddd, 13.7, 10.3, 7.9)	4, 12, 19, 20, 22		2.41 (ddd, 14.1, 10.6, 8.3)	4, 12, 19, 20, 22
22a	43.0 (32.4)	3.66 (ddd, 16.2, 10.3, 5.8)	20, 21, 24	38.7	3.71 (m)	20, 21, 24
22b		3.73 (br dd, 16.2, 7.9)	20, 24		3.75 (m)	21, 24
24	159.8 (42.6)			164.8		
25	31.2	3.12 (s)	2, 5, 24	33.9	3.61 (s)	2, 5, 24

[a] Spectra were recorded in $CDCl_3$ at 25 °C on a 400 MHz spectrometer. [b] Spectra were recorded in $CDCl_3$ at 26 °C on a 500 MHz spectrometer.

The distribution of the halogen atoms in **1** among the three available aromatic sites was established on the basis of ^{13}C chemical shifts using ChemNMR[6] software in conjunction with literature values.[7] The calculated chemical shifts for Br at C8 and Cl at both C15 and C17 varied only slightly from the measured values: C8, obsd 121.9 ppm, calcd 121.6 ppm; C15, obsd 122.6 ppm, calcd 121.6 ppm; C17, obsd 128.3 ppm, calcd 125.0 ppm. The ^{13}C chemical shifts for all other possible Br and Cl permutations were computed and found to have large deviations from the recorded values: with Cl at C8 (calcd 132.3 ppm); with Br at C15 (calcd 110.9 ppm); with Br at C17 (calcd 114.3 ppm). With the halogen substitution pattern determined, the planar structure for **1** was assigned.

The relative stereochemistry of perophoramidine (**1**) about the C4−C20 bond was determined to be trans on the basis of ROESY correlations between H6−H2b, H18−H3b, and H18−H22a. This assignment was supported by modeling studies using Chem3D Pro (MM2) in which the steric energy values for the trans and cis isomers for **1** were calculated to be 36 and 80 kcal/mol, respectively. Interestingly, the communesins that contain a related system except where the amidines are reduced to aminals were assigned cis stereochemistry for this region of the molecule on the basis of observed NOEs.[8] In contrast to perophoramidine, modeling of the communesin isomers showed that the cis and trans isomers have nearly identical steric energy values.

Perophoramidine (**1**) contains an unusual carbon skeleton and is the first reported metabolite from the genus *Perophora*. In addition, **1** exhibits cytotoxicity toward the HCT116 colon carcinoma cell line with an IC_{50} of 60 μM and induces apoptosis via PARP cleavage within 24 h.

Experimental Section

General Methods. NMR spectra were recorded on either a 400 spectrometer (^{1}H, 399.880 MHz; ^{13}C, 100.559 MHz) at 25 °C or a 500 spectrometer (^{1}H, 500.620 MHz; ^{13}C, 125.893 MHz) at 26 °C. The ^{1}H and ^{13}C chemical shifts are reported in parts per million relative to the reference solvent peaks at δ 7.24 and 77.00 ppm for $CDCl_3$.

Animal Material. The colonial ascidian *Perophora namei* was collected during August of 2001 by scuba (−12.2 m) east of Zamboanga City near Tictauan Island in the Philippines. Voucher specimen DZ-UFPR PEROPH 11 has been deposited at the Departamento de Zoologia, Universidade Federal do Paraná, C.P. 19020, 81.531-990, Curitiba, Brazil.

Extraction and Isolation. *P. namei* was macerated and exhaustively extracted with MeOH. The dried extract (7.58 g) was subjected to a modified Kupchan procedure,[3] resulting in a hexane (644 mg), $CHCl_3$ (729 mg), and 30% aq MeOH (6.17 g) fraction. The $CHCl_3$-soluble material was purified by silica gel flash chromatography on a column (45 × 200 mm) packed with silica (40−63 μm, 60 Å) using a gradient from 2% MeOH/98% $CHCl_3$ to 30% MeOH/70% $CHCl_3$. The 5% MeOH/95% $CHCl_3$ fraction was further purified by trituration using a MeOH/H_2O (7:3) mixture to yield pure perophoramidine (**1**, 185 mg).

Perophoramidine (1): stable, off-white amorphous solid; UV (MeOH) λ_{max} 338 (ϵ 4600), 308 (ϵ 6800), 298 (sh, ϵ 5800), 240 (sh, ϵ 10300), 220 (ϵ 13300); IR (NaCl) ν_{max} 1698, 1632, 1564, 1490, 1408, 1202 cm^{-1}; $[\alpha]_D$ +3.8 (*c* 0.733, $CHCl_3$); CD (MeOH) $[\theta]_{253}$ −19800, $[\theta]_{306}$ +24700, $[\theta]_{338}$ −3990; (+)-LRFABMS (rel int) *m*/*z* 475 (57), 477 (100), 479 (45) 481 (8); (+)-HRFABMS *m*/*z* 475.0028 ([M + H]$^+$, $C_{21}H_{17}N_4{}^{79}Br^{35}Cl_2$, −0.0066 mmu); ^{1}H and ^{13}C NMR data (Table 1).

Dehalogenation of Perophoramidine (1). To a N_2-purged solution of **1** (5.0 mg, 10.5 μmol) in MeOH (1.4 mL) were added

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HCO_2NH_4 (14.0 mg, 47.5 μmol) and 10% Pd/C (10.0 mg), and the mixture was stirred under N_2 at rt for 24 h.[5] The MeOH was removed under reduced pressure, and the resulting residue was purified on a C_{18} (40 μm, 60 Å) packed SPE cartridge (10 × 20 mm) that was initially flushed with 100% H_2O (discarded) and then flushed with 95% MeOH/5% aq TFA (0.1%). This yielded pure dehalogenated perophoramidine (**2**, 2.3 mg, 66% yield) as its TFA salt.

Dehalogenated perophoramidine (2): stable, yellow oil; UV (MeOH) λ_{max} 332 (ϵ 2300), 294 (ϵ 3100), 226 (sh, ϵ 8600), 208 (ϵ 9900); IR (NaCl) ν_{max} 1695, 1650, 1573, 1468, 1201 cm^{-1}; CD (MeOH) $[\theta]_{245}$ +4500, $[\theta]_{286}$ +8700, $[\theta]_{328}$ −3300; (+)-LRFABMS (rel int.) *m*/*z* 329 (100); (+)-HRFABMS *m*/*z* 329.1758 ($[M + H]^+$, $C_{21}H_{21}N_4$, −0.0008 mmu); ^1H and ^{13}C NMR data (Table 1).

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Supporting Information Available: ^1H and ^{13}C NMR spectra for perophoramidine (**1**) and dehalogenated perophoramidine (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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