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Aplidiopsamine A, an Antiplasmodial Alkaloid from the Temperate Australian Ascidian, *Aplidiopsis confluata*

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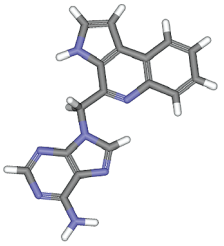
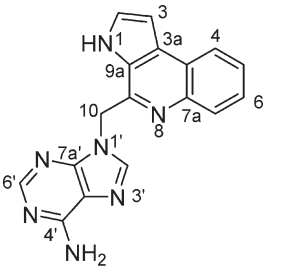


Aplidiopsamine A

A polyaromatic alkaloid, aplidiopsamine A was isolated from the temperate Australian ascidian, *Aplidiopsis confluata*, and its structure was determined from interpretation of mass, 1D and 2D NMR spectra. Aplidiopsamine A is the first alkaloid to possess the tricyclic aromatic substructure 3*H*-pyrrolo[2,3-*c*]quinoline conjugated to an adenine. Aplidiopsamine A exhibited significant inhibition of growth of chloroquine resistant and sensitive strains of the malaria parasite, *Plasmodium falciparum*, and minimal toxicity toward human cells.

Plants have historically provided an arsenal of natural products that have been used to treat infections by the blood borne parasite *Plasmodium* spp, which causes malaria.[1] Complex alkaloids containing indole, β-carboline, or quinoline moieties have been demonstrated to possess potent antimalarial activity, with quinine being the drug used most widely in the past.[1] The malaria parasite, however, is highly adaptive and this has led to increasing incidences of resistance to current drug therapies.[2] Comprehensive investigations of plants to find novel antimalarial compounds have been particularly fruitful with the sesquiterpene, artemisinin, currently being administered as a successful treatment for drug resistant strains of the malaria parasite *Plasmodium falciparum*.[3] Despite this success, there is still a need to find alternative drugs to treat resistant strains of *Plasmodium*. We recently reported on the structure and biological activity of a group of bis-indole alkaloids isolated from Australian and Papua New Guinean plants from the genus *Flindersia*.[4] These compounds show potent and selective activity against chloroquine resistant strains of *P. falciparum*., while also showing minimal human cell toxicity. Organisms from the marine environment have also been the focus of biodiscovery efforts to find new antimalarial drugs.[5] The manzamines isolated from the Indonesian sponge *Acanthostrongylophora* sp. are the most advanced preclinical candidates.[6] The observation that ascidians, marine invertebrates from the phylum Chordata, are major producers of aromatic alkaloids[7] prompted us to investigate this group to find alkaloid producing species and to test their alkaloids for antiplasmodial activity.

We have developed a simple method to identify species producing unique alkaloids and application of this methodology highlighted extracts from the colonial ascidian *Aplidiopsis confluata* collected from Tasmania in southeastern Australia as a potential source of unique alkaloids.[8,9] This note reports on the alkaloid chemistry of *A. confluata* and the antiplasmodial activity of a novel compound, aplidiopsamine A, isolated from this ascidian.



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Electrospray MS analysis of the MeOH extracts of over 400 Australian ascidian species collected from the Great Barrier Reef and Tasmania highlighted *A. confluata* from Tasmania as a specimen worth investigating further since it contained a peak that eluted early off C_{18} under acidic conditions (a characteristic of basic compounds), which produced an ion at m/z 316.1316 Da, and this indicated (through elemental composition matching and database searching) that the ascidian contained a unique alkaloid containing seven nitrogen

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TABLE 1. NMR Data for Aplidiopsamine A (1) in d_6-DMSO[a]

no.	δ_C (J_{CH}, Hz)[b]	δ_H (J, Hz)	HMBC
1		12.33 (br s)	C-3, 3a, 9a
2	127.9 (186)	7.71 (dd, 2.9, 3.1)	C-3, 3a, 9a
3	101.7 (174)	7.16 (dd, 2.9, 1.4)	C-2, 3a, 3b, 9a
3a	128.8		
3b	123.3		
4	122.9 (162)	8.22 (dd, 2.0, 8.2)	C-3a, 3b, 6, 7a
5	126.1 (156)	7.48 (ddd, 1.3, 7.1, 8.2)	C-3b, 7
6	126.0 (156)	7.43 (ddd, 2.0, 7.1, 7.1)	C-4, 7a
7	128.9 (162)	7.71 (dd, 1.3, 7.1)	C-3b, 5, 7a
7a	141.8		
9	143.3		
9a	126.5		
10	44.4 (138)	5.90 (s)	C-9, 9a, 2′, 7a′
2′	142.4 (216)	8.28 (s)	C-3a′, 7a′, 10
3a′	118.3		
4′	155.8[c]		
4′-NH_3^+		7.16 (br s)	C-3a′
5′			
6′	153.7 (204)	8.04 (s)	C-3a′, 4′, 7a′
7a′	149.4		

[a] ^{1}H NMR at 600 MHz referenced to residual DMSO solvent (δ_H 2.49) and ^{13}C NMR at 150 MHz referenced to DMSO (δ_C 39.5). [b] $^1J_{CH}$ obtained from correlations observed in a gHMBC spectrum. [c] Not observed in a ^{13}C NMR spectrum but observed in a gHMBC spectrum.

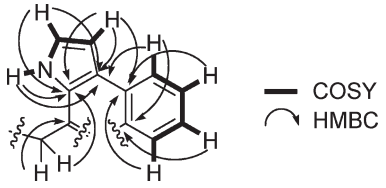

FIGURE 1. Important COSY and HMBC correlations observed for aplidiopsamine A.

atoms. Purification of the MeOH extract of *A. confluata* on Sephadex LH20 eluting with MeOH followed by C_{18} silica gel HPLC separation eluting with a linear gradient from H_2O containing 1% TFA to MeOH (containing 1% TFA) over 60 min yielded aplidiopsamine A (5.5 mg, 0.08%).

Aplidiopsamine A (**1**) was obtained as a yellow gum. A $[M + H]^+$ ion in the (+) HRESIMS at m/z 316.1316 (Δ 3.6 ppm) allowed a molecular formula of $C_{17}H_{13}N_7$ to be assigned to **1**. An absorption band at 3485 cm^{-1} in the IR spectrum suggested that the molecule contained an amine functionality. The ^{1}H NMR spectrum of **1** (Table 1) contained four aromatic doublet of doublets, two aromatic doublet of doublet of doublets, two aromatic singlets and signals for a downfield exchangeable proton singlet at δ_H 12.33, a broad exchangeable three-proton singlet at δ_H 7.16, and a sharp methylene proton singlet at δ_H 5.90. Intense COSY correlations (Figure 1) attributable to ortho coupling between aromatic protons at δ_H 8.22 (J = 2.0, 8.2 Hz) and 7.71 (J = 1.3, 7.1 Hz) and aromatic protons at δ_H 7.48 (J = 1.3, 8.2, 7.1 Hz) and 7.43 (J = 2.0, 7.1, 7.1 Hz), respectively, in addition to ortho couplings between δ_H 7.48 and 7.43 indicated that these four protons were on contiguous carbons and this suggested that **1** possessed a 1,2-disubstituted benzene ring. The exchangeable proton at δ_H 12.33 showed COSY correlations to protons at δ_H 7.71 and 7.16 and these protons were also mutually coupled. Small proton coupling constants between δ_H 7.71 and 7.16 (J = 2.9 Hz), between δ_H 7.71 and 12.33 (J = 3.1 Hz), and between δ_H 7.16 and 12.33 (J = 1.4 Hz) were consistent with these resonances being part of a 2,3-disubstituted pyrrole or indole.[10]

Sixteen of the 17 carbon resonances were observed in the ^{13}C NMR spectrum of **1** (Table 1) and correlations observed in a gHSQC spectrum allowed eight protonated aromatic carbons and one amino-methylene carbon (δ_H/δ_C 5.90/44.4) to be identified. The proton at δ_H 7.71 correlated to a carbon at δ_C 127.9 and this was in agreement with the literature for a carbon α to the nitrogen of a pyrrole or indole.[10] Further evidence for this assignment was provided by the large $^1J_{CH}$ coupling constant ($^1J_{CH}$ = 186 Hz) observed between these resonances, measured from correlations observed in a gHMBC spectrum of **1**, since nitrogen atom substituted protonated aromatic carbons show $^1J_{CH}$ couplings > 180 Hz.[10] The proton at δ_H 7.16 showed a correlation to a carbon resonance at δ_C 101.7 in the gHSQC spectrum and this was consistent with this carbon being β to a pyrrole or indole nitrogen.[10] A series of $^2J_{CH}$ and $^3J_{CH}$ correlations from H-1, H-2, and H-3 to two quaternary aromatic carbons at δ_C 128.8 and 126.5 in the gHMBC spectrum (Figure 1) further supported the presence of a 2,3-disubstituted pyrrolo group since the combination of these correlations is only possible in a five-atom aromatic heterocycle. Since the chemical shifts of the quaternary carbons (δ_C 126.5 and 128.8) were similar it was impossible to unambiguously assign the position of each carbon and either could be assigned to C-3a or C-9a.

Intense $^3J_{CH}$ correlations were observed between δ_H 8.22 and 7.43 and an aromatic quaternary carbon at δ_C 141.8 (C-7a) and between δ_H 7.48 and 7.71 and an aromatic quaternary carbon at δ_C 123.3 (C-3b) and this indicated that these two carbons (C-3b and C-7a) could be assigned to the quaternary aromatic carbons in the 1,2-disubstituted benzene ring. An additional intense $^3J_{CH}$ correlation was observed between δ_H 8.22 (H-4) and one of the quaternary pyrrole carbons (δ_C 128.8). Since H-4 is ortho to the aromatic quaternary carbon C-3b, and can only logically show an additional correlation to a carbon occupying a benzylic position, this suggested that the 1,2-disubstituted benzene ring was linked to the pyrrole by a carbon−carbon bond. A reciprocal $^3J_{CH}$ coupling between H-3 and C-3b provided further evidence for this assignment and indicated that the resonance at δ_C 128.8 was C-3a and that a bond linked C-3a and C-3b.

The two remaining unassigned protonated aromatic carbon resonances at δ_H/δ_C 8.28/142.4 ($^1J_{CH}$ = 216 Hz) and δ_H/δ_C 8.04/153.7 ($^1J_{CH}$ = 204 Hz) were assigned to carbons each substituted by two nitrogen atoms since their $^1J_{CH}$ coupling constants were > 200 Hz.[10] HMBC correlations from H-2′ to quaternary aromatic carbons at δ_C 118.3 (C-3a′) and 149.4 (C-7a′) and from H-6′ to quaternary aromatic carbons at δ_C 155.8 (C-4′) and 149.4 (C-7a′) suggested an adenine group was present in **1**.[10] A strong $^3J_{CH}$ correlation from 4′-NH_3 and a weak $^4J_{CH}$ correlation from H-6′ to C-3a′ further substantiated the presence of an adenine. The amino-methylene protons H_2-10 showed HMBC correlations to C-2′ and C-7a′ indicating that this group was directly attached to N-1′ of the adenine. Previously reported ^{13}C chemical shifts for adenine carbons in 9-alkylated adenine

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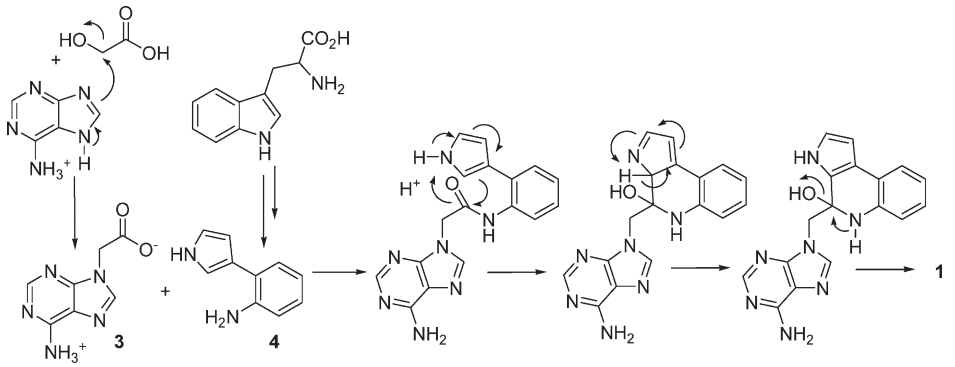
SCHEME 1. Proposed Biogenesis of Aplidiopsamine A



derivatives were compared with those observed in **1** and all were within 2 ppm, and this provided further evidence that **1** contained an alkylated adenine.[10−12]

The amino methylene protons H_2-10 also showed two HMBC correlations into carbons that were not part of the adenine moiety. Both of these carbons were sp^2 hybridized based on their chemicals shifts (δ_C 126.5, C-9a and 143.3, C-9). Since three of the four atoms attached to C-10 were accounted for by two hydrogens and the adenine nitrogen, one of the HMBC correlations must be to a carbon two bonds away and the other to a carbon three bonds away from H_2-10. C-9a was already assigned to the carbon α to the nitrogen of the pyrrole and therefore could only accommodate one additional bond and this suggested that the remaining carbon, C-9, had to be the carbon two bonds away from H_2-10 and thus form a carbon bridge between C-10 and C-9a. The structure of **1** required an additional nitrogen atom as dictated by its molecular formula, and since C-7a needed to accommodate one additional bond and C-9 needed to accommodate two additional bonds, insertion of a sp^2 hybridized nitrogen atom between C-7a and C-9 to form a quinoline ring provided the logical solution. This addition also fulfilled the requirement for two additional degrees of unsaturation (a double bond and a ring) as dictated by the molecular formula for **1**. The downfield ^{13}C chemical shifts for both C-7a (δ_C 141.8) and C-9 (δ_C 143.3) were appropriate for carbon atoms adjacent to a nitrogen atom, although both were ~8 ppm upfield of the corresponding carbons reported in quinoline.[10] This upfield shift can be attributed to the higher electron density predicted to result from delocalization of the lone pair of electrons from the amino substituent attached at C-9a to the carbons ortho and para to this substituent. The total spectroscopic evidence therefore indicated that **1** contained a 3*H*-pyrrolo[2,3-*c*]quinoline ring.

The 3*H*-pyrrolo[2,3-*c*]quinoline ring system is extremely rare having been reported previously only once before. The X-ray crystallographic structure of marinoquinoline (4-methyl-3*H*-pyrrolo[2,3-*c*]quinoline), an acetylcholine esterase inhibitor isolated from the marine gliding bacteria *Rapidithrix thailandica* from Thailand, was reported in 2006 but no spectroscopic data were published for this compound.[13] Aplidiopsamine A therefore represents only the second example of a molecule containing this unprecedented tricyclic ring system and the spectroscopic properties of this ring system are now reported for the first time. A related structure class, the indolo-[2,3-*c*]quinolines have been reported as synthetic derivatives but unfortunately no molecules from this class have had their NMR data definitively assigned using 2-D NMR techniques either.[14,15] This report therefore provides the first full NMR assignment for either the pyrrolo[2,3-*c*]quinoline or indolo-[2,3-*c*]quinoline ring systems. An additional unique feature of **1** is the conjugation of the 3*H*-pyrrolo[2,3-*c*]quinoline moiety to an adenine via a methylene bridge. Non glycoside adenine conjugates are rarely encountered in nature. Aplidiopsamine A (**1**) is likely to be biosynthesised from the condensation of 6-amino-9*H*-purine-9-acetic acid (**3**) (possibly generated from reaction of adenine and hydroxyacetic acid) with a pyrrolnitrin derivative such as 3-(*o*-aminophenyl)pyrrole (**4**) (Scheme 1). Compound **4**, recently isolated from *R. thailandica*,[16] has previously been postulated to be a dead-end product in the biosynthesis of the antibiotic pyrrolnitrin from tryptophan.[17] Pyrrolnitrin has been isolated from several strains of *Pseudomonas*, *Myxococcus* and *Burkholderia*.[18,19] The fact that microbes produce metabolites that could be considered to be precursors in the biosynthesis of **1** suggests that *A. confluata* is likely to harbor micro-organisms that produce **1**.

Aplidiopsamine A (**1**) was tested for its ability to inhibit the growth of chloroquine sensitive (3D7) and resistant (Dd2) strains of the malarial parasite, *Plasmodium falciparum*. Human cell toxicity was assessed using the normal cell line HEK-293. Aplidiopsamine A was equally active against the two malarial parasite strains (IC_{50} = 1.47 (3D7) and 1.65 μM (Dd2)), and only showed growth inhibition against HEK-293 cells at higher doses, only reaching ~100% inhibition at the highest dose tested (120 μM).[20] Aplidiopsamine A therefore represents a novel lead structure that could be further developed into a drug to treat drug-resistant malarial infections.

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Experimental Section

Animal Material. The ascidian sample *Aplidiopsis confluata* (Polyclinidae) was collected from Schooner Cove (Forester Pt) Bathurst Harbor in Western Tasmania in January 2003. A voucher specimen (TAS000320) has been lodged at Aquenal Pty Ltd. in Hobart, Tasmania.

Extraction and Fractionation. The freeze-dried and ground ascidian (6.5 g) was extracted exhaustively with MeOH (4 × 400 mL) yielding a yellow gum (0.945 g). This extract was separated on Sephadex LH20 eluting with MeOH. A total of 120 fractions were collected (10 mL each) and aliquots were analyzed by (+) ESIMS. Fractions containing an ion at *m*/*z* 316 Da were combined (15.8 mg) and separated further by HPLC on C_{18} silica gel, eluted with a gradient from 1% TFA/99% H_2O to 99% MeOH/1% TFA over 60 min, and 1 min fractions were collected. Aplidiopsamine A (**1**) (5.5 mg) eluted in fractions 34−36.

Aplidiopsamine A (1): isolated as a yellow gum (5.5 mg, 0.08%); UV (MeOH) λ_{max} 242 nm (ε 49 560), 250 (44 385), 290 (13 440), 310 (11 577), 356 (8423); IR (film) ν_{max} 3485, 2966, 2923, 2865, 1682, 1120, 1135 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR, Table 1; (+)-HRESMS *m*/*z* 316.1316 (calcd for $C_{17}H_{14}N_7$ $[MH]^+$ 316.1305).

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Supporting Information Available: Detailed description of general experimental procedures and 1D and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.