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Aaptanone, a novel zwitterionic metabolite of the aaptamine class with an oxygenated 1,6-naphthyridine core from the Vietnamese marine sponge *Aaptos aaptos*

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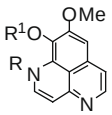
ABSTRACT

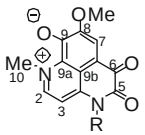
A new zwitterionic compound, aaptanone, having a rare oxygenated 1,6-naphthyridine core, has been isolated from the Vietnamese marine sponge *Aaptos aaptos*, along with the known metabolites, aaptamine, isoaaptamine, and their hydrochloride salts. The structure of aaptanone was determined as 8-methoxy-1-methyl-5,6-dioxo-5,6-dihydro-4*H*-benzo[*de*]-1,6-naphthyridine-1-ium-9-olate from spectroscopic data, X-ray analysis, and by spectroscopic analysis of an *N*-methyl derivative.

The aaptamines are a small group of biologically active marine alkaloids having a rare 1*H*-benzo[*de*]-1,6-naphthyridine skeleton. Seven aaptamines, namely, aaptamine,[1] isoaaptamine,[2] 9-demethylaaptamine,[3] 9-demethyloxyaaptamine,[3] bisdemethylaaptamine,[4] bisdemethylaaptamine-9-*O*-sulfate,[4] and 4-*N*-methylaaptamine[5] have been isolated from marine sponges mainly of the genus *Aaptos*. Moreover aaptamines were isolated from marine sponges of the genus *Suberites*,[6] *Hymeniacidon*,[7] and *Xestospongia*.[8] All these compounds have either an N-methylated or a non-N-methylated 1,6-naphthyridine core fused with a functionalized benzenoid unit. Recently, two C-3 substituted demethyloxyaaptamines, 3-phenethylamino- and 3-isopentylamino-demethyloxyaaptamines have been isolated from the Malaysian *Aaptos aaptos*.[9] Some of aaptamines have been reported to have α-adrenoceptor blocking activity,[10] anti-HIV-1 activity,[11] antimicrobial activity,[7,11] antiherpes activity,[12] sortase A inhibitory activity,[13] and anticancer activity.[14–16]

In the course of our search for antioxidants from marine organisms we have investigated an EtOH extract of the Vietnamese marine sponge *Aaptos aaptos* (order Hadromerida, family Suberitidae),[17] which showed a strong antioxidant effect in scavenging the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Bioassay-guided fractionation of the EtOH extract led to the isolation of compounds active in a DPPH scavenging, namely aaptamine (**1**) (IC_{50} 1.8×10^{-5} M), isoaaptamine (**2**) (IC_{50} 1.6×10^{-5} M), and their hydrochloride salts, which were identified by comparison of their spectral data with published values.[6,7] Moreover, the EtOH extract contained an unusual red-colored compound **3** of high polarity. In this Letter, we describe the isolation and structural elucidation of this novel compound **3**.



1 R= H, R^1= Me
2 R= Me, R^1= H



3 R= H
4 R= Me

A freeze-dried sponge sample (200 g) was extracted exhaustively with acetone followed by EtOH. The acetone extract contained the known compounds aaptamine (**1**), isoaaptamine (**2**), and their hydrochloride salts. The EtOH extract was concentrated under reduced pressure to yield a dark-red residue. This residue was chromatographed on a Polychrome-1 (powder Teflon) column with H_2O as eluent. A dark-red fraction was concentrated and triturated with acetone to precipitate a dark-red gum. This gum was dissolved in H_2O and triturated with acetone to yield a precipitate

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enriched with compound **3**, which was fractionated by vacuum flash chromatography over silica gel using 50% aqueous EtOH. Final purification was achieved by column chromatography over silica gel (Silochrome C-80) using EtOH/$CHCl_3$/H_2O (1:1:0.1) as eluent. Compound **3**, named aaptanone, gave dark purple crystals (mp >350 °C) on slow solvent evaporation (0.006% based on the dry weight of the sponge).[18] Aaptanone (**3**) produced bright dark-red solutions when dissolved in aqueous EtOH or in alkaline solution and changed from dark red to yellow in acidic solution. Evaporation of the yellow acidic solution gave a dark-red residue again.

The molecular formula $C_{13}H_{10}N_2O_4$ of aaptanone (**3**), which indicated ten double bond equivalents, was determined by interpretation of the $[M+Na]^+$ ion at *m/z* 281.0541 in combination with NMR spectroscopic data. Aaptanone (**3**) showed limited solubility in most common NMR solvents, but was soluble in D_2O containing 0.1% NaOD and in CF_3COOD. The compound was dissolved in D_2O containing 0.1% NaOD so that a reasonable signal-to-noise ratio could be obtained in heteronuclear NMR experiments.

The ^1H NMR spectrum of aaptanone (**3**) was simple in appearance. It exhibited mutually coupled doublets characteristic of a 2,3,4-trisubstituted pyridine group (δ 7.87 and δ 6.78, $^1J_{CH}$ 183 Hz and $^1J_{CH}$ 173 Hz) and an isolated proton of a benzene ring (δ 7.21, $^1J_{CH}$ 161 Hz).[6] In addition, signals due to two methyl groups connected to heteroatoms were observed. Analysis of the ^{13}C NMR and DEPT spectra indicated that **3** contained 13 carbon atoms: two methyls, three aromatic methines, and eight quaternary carbons (Table 1). Both the ^1H and ^{13}C NMR spectra indicated the presence of a methoxy group (δ_H 3.75 and δ_C 54.9) and a quaternary, rather than a tertiary, *N*-methyl group (δ_H 4.25 and δ_C 47.4). Two of the quaternary carbons (δ 170.7 and δ 169.1) clearly indicated the presence of two carbonyl groups in different chemical environments, the first signal possibly due to a ketone carbonyl group attached to an aromatic ring, the second signal possibly due to an amide carbonyl. The downfield resonance of the quaternary carbon at δ 167.9 also indicated a carbonyl group. This supposition was in agreement with the IR data of aaptanone (**3**) (1713, 1701, and 1630 cm^{-1}). Moreover, the IR spectrum suggested aaptanone (**3**) contained an NH group (3440 cm^{-1}). The HSQC spectrum allowed all the protonated carbons to be assigned. The ^1H and ^{13}C NMR spectra and the unsaturation requirements of the molecular formula indicated **3** to be tricyclic. Connectivities, deduced from HMBC correlations, readily established the presence of a modified isoaaptamine core.

The substitution pattern in **3** was substantiated by NOESY and HMBC experiments. In the NOESY spectrum of **3** an NOE between the protons of the methoxy group at δ 3.75 and the benzene proton at δ 7.21 showed that they were adjacent. In the HMBC spectrum the protons of the N_1-methyl group (δ 4.25) correlated with the carbons at δ 143.1 (C-2) and δ 128.0 (9a) indicating the position of the *N*-methyl group in a 2,3,4-trisubstituted pyridine ring. Of all the carbons of **3**, the quaternary carbon at δ 169.1 (C-5) did not show HMBC correlations with any protons. The chemical shift value of this remaining carbon signal in combination with the IR absorption at 3440 cm^{-1} suggested the presence of an amide group in **3**, therefore this carbon was located next to the NH-4 group. However, HMBC correlations did not allow unambiguous assignment of the two carbon atoms C-6 and C-9. The signal at δ 170.7 was tentatively assigned to the C-6 ketone group by a comparison of the ^{13}C NMR data of the C-3 to C-6 fragment in **3** with the literature data of known bisindole alkaloids containing an unusual α-keto enamide functionality.[19]

The ^{13}C NMR and HMBC data for aaptanone (**3**) obtained in CF_3COOD, in contrast with data obtained in D_2O containing 0.1% NaOD (Table 1), lacked evidence of the three carbonyl signals, instead revealing signals of two carbonyl groups at δ 172.2 (C-6) and δ 159.9 (C-5). The signal for the quaternary carbon C-9 appeared at δ 154.2 supporting its existence as a phenolic resonance.

To clarify the structure of aaptanone (**3**) it was methylated with MeI (acetone–K_2CO_3) to yield the methylated product **4**, mp >350 °C. Methyl derivative **4** provided additional evidence for the structure of aaptanone (**3**) since the new *N*-methyl signal gave additional three-bond HMBC correlations. High resolution ESI(+) MS analysis of **4** revealed a pseudo molecular ion $[M+Na]^+$ at *m/z* 295.0681 (calcd for $C_{14}H_{12}N_2O_4Na$: 295.0699) consistent with the molecular formula $C_{14}H_{12}N_2O_4$. Comparison of the ^1H and ^{13}C NMR data (CF_3COOD) between **3** and **4** revealed the only significant difference to be an additional resonance due to a tertiary *N*-methyl group (δ_H 3.95 and δ_C 32.7). Analysis of the HMBC data revealed key correlations between the protons of the N_4-methyl group and the flanking carbons C-3a and C-5 (Table 1) thereby supporting the true location of the amide carbonyl at C-5. The molecular formula of **4**, the presence of the quaternary N_1-methyl group and the tertiary N_4-methyl group allowed formulation of **4** as the C-9 phenolate. Consequently, the structure of aaptanone (**3**) contained a C-9 phenolate. Comparison of the IR spectrum of aaptanone (**3**) with IR spectra of synthetic zwitterionic pyridinium phenolates[20] showed that the band at 1630 cm^{-1} in **3** could belong to a phenolate group.

The zwitterionic nature of **3** explained its properties, viz. high polarity, high melting point, solubility in base and acid, and limited solubility in organic solvents. To confirm the structure of aaptanone (**3**), it was crystallized from a mixture EtOH–TFA (1:0.2) for X-ray studies. Figure 1 is a computer-generated perspective drawing of the final X-ray model of aaptanone (**3**).[21] While **3** was crystallized in the presence of TFA, X-ray analysis did not reveal any counterions in the crystal of aaptanone. This result indicated that

Table 1
^{13}C (125 MHz) and ^1H (500 MHz) NMR data for **3** and **4**

Position	δ C	δ H (*J*)	HMBC	δ C	δ H (*J*)	HMBC	δ C	δ H (*J*)	HMBC
	0.1% NaOD in D_2O **3**			CF_3COOD **3**			CF_3COOD **4**		
2	143.1 (CH)	7.87 d (6.6)	3, 3a, 9a, 10	153.4 (CH)	8.89 d (6.6)	3, 3a, 9a, 10	153.2 (CH)	8.93 d (7)	3, 3a, 9a, 10
3	112.4 (CH)	6.78 d (6.6)	2, 3a, 9b	110.7 (CH)	7.42 d (6.6)	2, 3a, 9b	109.8 (CH)	7.77 d (7)	2, 3a, 9b
3a	154.5 (C)			148.4 (C)			150.7 (C)		
5	169.1 (C)			159.9 (C)			160.0 (C)		
6	170.7 (C)			172.2 (C)			170.8 (C)		
6a	109.6 (C)			121.9 (C)			120.9 (C)		
7	109.4 (CH)	7.21 s	6, 6a, 8, 9, 9b	117.8 (CH)	8.52 s	6, 6a, 8, 9, 9b	117.2 (CH)	8.46 s	6, 8, 9, 9b
8	151.8 (C)			153.5 (C)			153.8 (C)		
9	167.9 (C)			154.2 (C)			154.2 (C)		
9a	128.0 (C)			130.9 (C)			130.8 (C)		
9b	118.6 (C)			117.3 (C)			118.1 (C)		
8-OMe	54.9 (CH_3)	3.75 s	8	59.4 (CH_3)	4.33 s	8	59.2 (CH_3)	4.30 s	8
N_1-Me (10)	47.4 (CH_3)	4.25 s	2, 9a	52.4 (CH_3)	4.93 s	2, 9a	52.4 (CH_3)	4.91 s	2, 9a
N_4-Me							32.7 (CH_3)	3.95 s	3a, 5

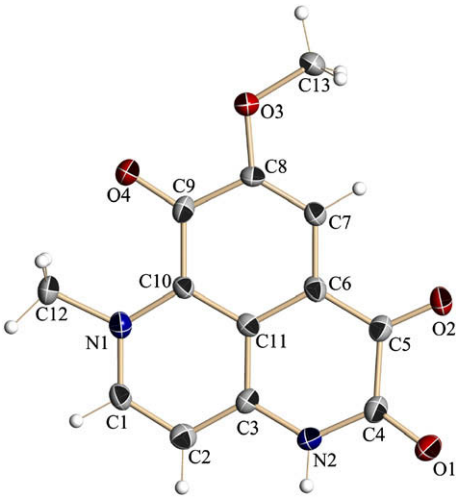

Figure 1. ORTEP diagram of aaptanone (**3**).

in the crystalline form aaptanone (**3**) clearly exists as a zwitterion. The C(9)–O(4) bond (1.259 Å) is longer than the carbonyl bonds C(4)–O(1) (1.219 Å) and C(5)–O(2) (1.241 Å), and significantly shorter than a single C(8)–O(3) bond (1.361 Å) (the atomic numbering corresponds to the X-ray model). Molecules of **3** are connected by hydrogen bonds N(2)–H(2)···O(4) (1.88 Å) forming a chain.

Thus the structure of **3** was determined to be 8-methoxy-1-methyl-5,6-dioxo-5,6-dihydro-4*H*-benzo[*de*]-1,6-naphthyridine-1-ium-9-olate. Previously, an oxidized aaptamine unit with a 5-oxo group in the 1,6-naphthyridine core was found in the spiro nonacyclic aromatic alkaloid lihouidine.[22] Aaptanone (**3**) is the first zwitterionic metabolite of the aaptamine class bearing two adjacent carbonyl groups in the 1,6-naphthyridine core. The isolation of aaptanone adds to the structural diversity of alkaloids from marine sponges.

In our measurements of bioactivity against Gram-positive and Gram-negative bacteria, compound **3** was inactive against the tested strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, and *Escherichia coli*. Compound **3** did not show cytotoxic activity against mouse Ehrlich carcinoma cells.

Acknowledgments

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17. The sponge was collected manually (SCUBA) at a depth of 3–6 m from Vietnamese waters, Cu Lao Re Island, in October 1987 during the 5th scientific cruise of R/V 'Academik Oparin'. The sponge was freeze-dried and stored at −20 °C until used. Taxonomic identification was provided by Vladimir Krasokhin (PIBOC). A voucher specimen (05-86) is currently on deposit at PIBOC.
18. Aaptanone (**3**):UV–vis (H_2O) λ_{max} (log ε) 250 (3.34), 294 (3.54), 320 sh (3.44), 350 sh (3.15), 527 (3.27) nm; UV–vis (H_2O/OH^-) λ_{max} (log ε) 251 (3.36), 293 (3.54), 320 sh (3.41), 350 sh (3.15), 527 (3.27) nm; UV–vis (H_2O/H^+) (log ε) 247 (4.20), 267 (4.40), 302 sh (3.34), 360 (3.41), 446 (3.96) nm; IR (KBr) ν_{max} 3440, 1713, 1701, 1630, 1568, 1546, 1500, 1482, 1468 cm^{-1}; HRESI(+)MS: *m/z* 281.0541 $[M+Na]^+$, calcd for $C_{13}H_{10}N_2O_4Na$: 281.0538.
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21. Crystallographic data (excluding structure factors) for the structure in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 718376. Copies of these data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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