HETEROCYCLES, Vol. 88, No. 1, 2014, pp. 591 - 594. © 2014 The Japan Institute of Heterocyclic Chemistry
Received, 14th March, 2013, Accepted, 9th April, 2013, Published online, 18th April, 2013
DOI: 10.3987/COM-13-S(S)3

AAPTOLINE A, A NEW QUINOLINE ALKALOID FROM THE MARINE SPONGE *AAPTOS SUBERITOIDES*

Yodai Kudo,[1] Hikaru Kato,[1] Henki Rotinsulu,[2] Fitje Losung,[3] Remy E. P. Mangindaan,[3] Nicole J. de Voogd,[4] and Sachiko Tsukamoto[1,*]

[1]Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan; [2]Faculty of Agriculture, Universitas Pembangunan Indonesia, Manado 95361, Indonesia; [3]Faculty of Fisheries and Marine Science, Sam Ratulangi University, Kampus Bahu, Manado 95115, Indonesia; [4]Netherlands Centre for Biodiversity Naturalis, PO Box 9517, 2300 RA Leiden, The Netherlands. E-mail: sachiko@kumamoto-u.ac.jp

Dedicated to Professor Victor Snieckus on the occasion of his 77$_{th}$ birthday

Abstract – A new quinoline alkaloid, aaptoline A (**1**), was isolated from the marine sponge *Aaptos suberitoides*, along with known alkaloids, aaptamine (**2**), isoaaptamine (**3**), and demethylaaptamine (**4**). Interestingly, **1**-**4** are presumably biosynthesized from common precursors, L-DOPA and β-alanine aldehyde.

Marine organisms are recognized as a rich source of drug leads for cancer, infectious diseases, central nervous system control, cardiovascular diseases, and immune modulation.[1] During the search for biologically active compounds from marine organisms, we encountered a cytotoxic extract of the sponge *Aaptos suberitoides* collected in Indonesia. The marine sponge *A. suberitoides* is a well-known source of aaptamine (**2**)[2] and its congeners[3,4] (Figure 1). Herein, we describe the isolation and structural elucidation of a new alkaloid, aaptoline A (**1**).

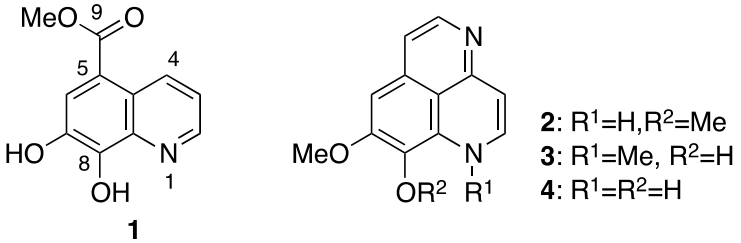


Figure 1. Structures of **1**-**4**

Table 1. ^{1}H and ^{13}C NMR data for **1** in CD_3OD

No.	δ_H (*J*, Hz)	δ_C (mult.)	HMBC [a]
2	8.92 (1H, d, 4.6)	145.4 d	
3	7.87 (1H, dd, 8.7, 4.6)	119.6 d	
4	10.02 (1H, d, 8.7)	144.1 d	2, 8a
4a		124.6 s	
5		119.1 s	
6	8.28 (1H, s)	126.8 d	4a, 5, 7, 8, 9
7		147.4 s	
8		139.2 s	
8a		132.7 s	
9		166.7 s	
10	4.00 (3H, s)	53.1 q	9

[a] HMBC correlations are from proton(s) stated for the indicated carbon(s).

The marine sponge (120 g, wet weight) was soaked in EtOH immediately after its collection. The extract was condensed and the residual aqueous solution was partitioned with EtOAc and then *n*-BuOH. The latter fraction was partitioned between *n*-hexane and 80% MeOH/H_2O. The aqueous MeOH fraction (5.7 g) was subjected to Sephadex LH-20 column chromatography, silica gel column chromatography, and ODS HPLC to afford **1** (1.8 mg) along with **2**, isoaaptamine (**3**),[3] and demethylaaptamine (**4**).[4] Compound **1** was isolated as a red amorphous solid, with the molecular formula $C_{11}H_9NO_4$ as determined by HRESITOF-MS. The UV spectrum displayed absorption maxima at 213 (log ε 4.5), 247 (4.7), and 357 (3.9) nm, suggesting the presence of a heteroaromatic chromophore. The ^{1}H NMR spectrum exhibited a methoxy signal at δ 4.00, and four aromatic signals at δ 7.87 (dd, *J* = 8.7 and 4.6 Hz, H-3), 8.28 (s, H-6), 8.92 (d, *J* = 4.6 Hz, H-2), and 10.02 (d, *J* = 8.7 Hz, H-4) (Table 1). The aromatic signals were indicated to be attached to a heteroaromatic nucleus due to their low-field chemical shifts and coupling constants.[5] The COSY spectrum indicated the presence of a 2,3-disubstituted pyridine nucleus, since the coupling constants, 8.7 and 4.6 Hz, and the chemical shifts were characteristics of a pyridine moiety and HMBC correlations between H-4 and carbons at δ 132.7 (C-8a) and 145.4 (C-2) were observed (Figure 2). HMBC correlations from the methoxy signal (δ_H 4.00, δ_C 53.1) to a carbonyl carbon (δ 166.7, C-9) and from □□6 to a quaternary carbon (δ 119.1, C-5) and the carbonyl carbon (C-9) showed the presence of a methyl ester attached to C-5. The NOESY spectrum showed a correlation between the methoxy signal and the aromatic signal at δ 10.02 (H-4). H-6 showed HMBC correlations with two oxygenated

quaternary carbons at δ 139.2 (C-8) and 147.4 (C-7) and a quaternary carbon at δ 124.6 (C-4a). Together, the above results showed that **1** was 7,8-dihydroxyquinoline-5-carboxylic acid methyl ester. The high-field resonance of H-4 is likely due to a hydrogen bond with oxygens of the methyl ester at C-5.

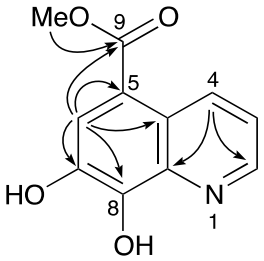
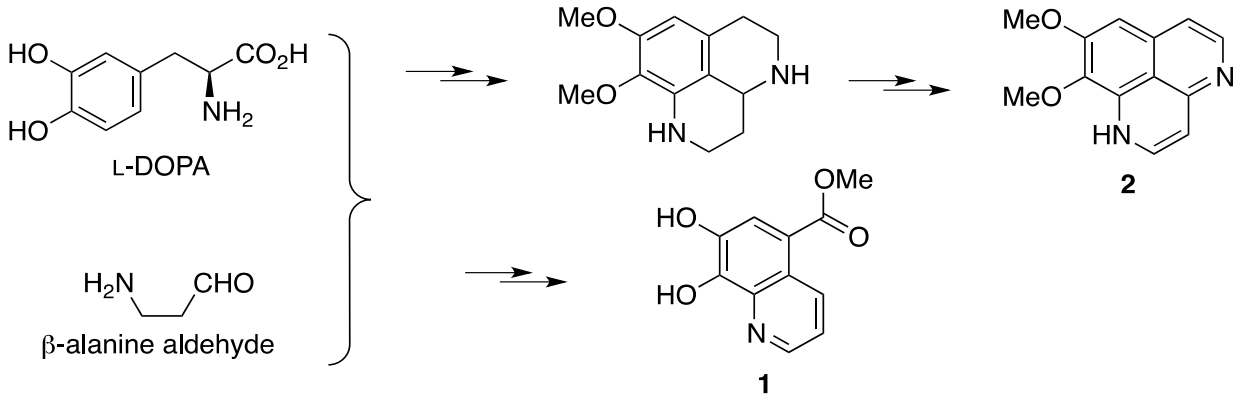


Figure 2. HMBC correlations observed for **1**

The large amounts of **2-4** explained the cytotoxicity of the sponge extract. Unfortunately, **1** decomposed before the cytotoxicity measurement. Recently, a proposed biosynthetic pathway for **2** and its congeners from L-DOPA and β-alanine aldehyde was reported (Scheme 1).[6] From the structural relationship, **1** would be also biosynthesized from these precursors.



Scheme 1. Proposed biosynthetic relationship of **1** and **2**

EXPERIMENTAL

General Experimental Procedures.

UV spectrum was measured on a JASCO V-550 spectrophotometer in MeOH. IR spectrum was measured on a JEOL JIR-6500W spectrophotometer. NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer in CD_3OD. Chemical shifts were referenced to the residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). Mass spectra were measured on a BRUKER esquire3000plus-K1 or BRUKER BIO-TOF mass spectrometer.

Material.

The marine sponge was collected at a depth of 10 m in Siladen, Indonesia, in September 2006 and soaked in EtOH immediately. The sponge was identified as *Aaptos suberitoides*. A voucher specimen (RMNH POR. 6182) has been deposited in the Netherlands Centre for Biodiversity Naturalis, The Netherlands.

Extraction and Isolation.

The marine sponge (120 g, wet weight) was extracted with EtOH. The concentrated aqueous solution was successively extracted with EtOAc and *n*-BuOH, and the latter fraction was partitioned between *n*-hexane and 80% MeOH/H_2O. The aqueous MeOH fraction (5.7 g) was subjected to Sephadex LH-20 column chromatography with 90% MeOH/H_2O (0.1% AcOH) (fraction A) and $CHCl_3$/MeOH/H_2O (6:4:1) (fraction B). Fraction A (92.1 mg) was purified by silica gel column chromatography with $CHCl_3$/MeOH/H_2O (6:4:1) followed by HPLC (COSMOSIL 5C18-AR-II (ϕ 20 x 250 mm), 0-35% MeCN-H_2O (0.1% TFA) in 100 min with a linear gradient, 6 mL/min) to afford aaptoline A (**1**, 1.8 mg, 0.0015%). Fraction B was fractionated to afford aaptamine (**2**, 20.8 mg), isoaaptamine (**3**, 27.2 mg), demethylaaptamine (**4**, 1.3 mg), and their mixture (284 mg).

Aaptoline A (1): Red amorphous solid; NMR data (CD_3OD), see Table 1. IR ν_{max} (KBr) 2924, 2360, 1684, 1437, 1186 cm^{-1}; UV λ_{max} (MeOH) (log ε) 213 nm (4.5), 247 (4.7), 357 (3.9); ESIMS *m*/*z* 220 $(M+H)^+$; HRESI-MS $(M+H)^+$ *m*/*z* 220.0568 (calcd for $C_{11}H_{10}NO_4$, 220.0610).

ACKNOWLEDGEMENTS

We thank Prof. M. Namikoshi and Dr. K. Ukai of Tohoku Pharmaceutical University and Dr. H. Kobayashi of the University of Tokyo for collection of the sponge.

REFERENCES

1. J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. G. Munro, and M. R. Prinsep, *Nat. Prod. Rep.*, 2013, **30**, 237.
2. H. Nakamura, J. Kobayashi, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett.*, 1982, **23**, 5555.
3. Y. Kashman, A. Rudi, S. Hirsh, S. Isaacs, D. Green, D. Blasberger, and S. Carmely, *New J. Chem.*, 1990, **14**, 729.
4. H. Nakamura, J. Kobayashi, Y. Ohizumi, and Y. Hirata, *J. Chem. Soc., Perkin Trans. 1*, 1987, 173.
5. E. Pretsch, T. Clerc, J. Seibl, and W. Simon, 'Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden', Springer-Verlag, Berlin, Heidelberg, New York, 1981, p. C160 and H335.
6. E. L. Larghi, M. L. Bohn, and T. S. Kaufman, *Tetrahedron*, 2009, **65**, 4257.