Communications to the Editor

Kapakahine B: A Cyclic Hexapeptide with an α-Carboline Ring System from the Marine Sponge *Cribrochalina olemda*

Yoichi Nakao, Bryan K. S. Yeung, Wesley Y. Yoshida, and Paul J. Scheuer*

Department of Chemistry, University of Hawai'i at Manoa 2545 The Mall, Honolulu, Hawaii 96822-2275

Michelle Kelly-Borges[1]

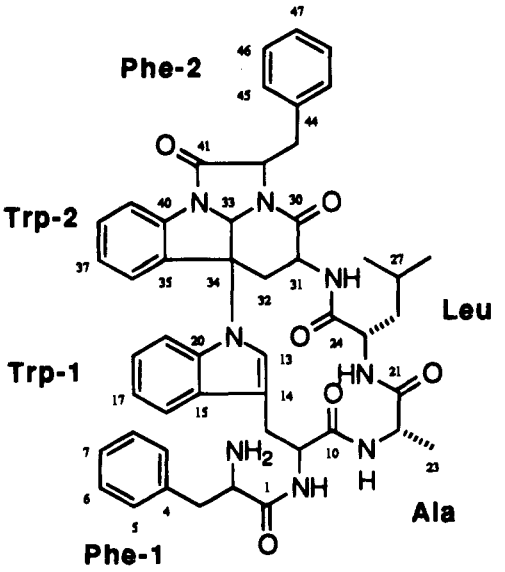
Harbor Branch Oceanographic Institution, Inc.[2] 5600 North U.S. 1, Fort Pierce, Florida 34936

Received March 6, 1995

β-Carbolines, biogenetically readily derivable from tryptophan, are a familiar structural feature of marine alkaloids.[3] Conversely, α-carbolines, also derivable from tryptophan, are rare. The grossularines, isolated from a tunicate, constitute the only previous example.[4] We now report the structure of a cyclic hexapeptide with a tryptophan-derived α-carboline which is part of a fused tetracyclic system that also includes phenylalanine-derived (Phe-2) imidazolone. Moreover, another tryptophan (Trp-1) is linked by its indole nitrogen atom to a ring juncture of the α-carboline and forms a peptide bond with a second phenylalanine (Phe-1) which bears a free amino group. Because of this non-peptide feature, a C−N bond to an indole nitrogen, we have called this compound kapakahine.[5]

Kapakahine B (**1**) was isolated from a sponge, *Cribrochalina olemda*,[6,7] collected at Pohnpei, Federated States of Micronesia, in April 1992 and re-collected in August 1993. The ethanolic extract of the frozen sponge (840 g, wet weight) was partitioned between ethyl acetate and water. The organic layer was subjected to the Kupchan separation scheme.[11] The methylene chloride fraction was chromatographed on Sephadex LH-20 (MeOH). The peptide-containing fractions (monitored by TLC) were separated by ODS HPLC (COSMOSIL 5C_{18}-AR; H_2O/MeCN, 58:42, 0.05% TFA) and yielded two peptides, kapakahine A (5.8 mg) and an impure kapakahine B, which was further purified by HPLC on an amino column (MICROSORB NH_2 80-799-C_5; $CHCl_3$/MeOH, 98:2) furnishing 0.3 mg of pure compound. This report deals with the smaller of the two peptides, kapakahine B (**1**).[12]

The molecular formula of kapakahine B (**1**) was determined as $C_{49}H_{52}N_8O_6$ by HR-FABMS [*m/z* 849.4116 $(M + H)^+$, Δ 2.8 mmu]. Initial inspection of the ^1H and ^{13}C NMR spectra indicated the peptide nature of the compound. Present were signals resonating at 4−5 ppm indicative of α-hydrogens associated with α-amino acid residues. Amino acid analysis following acid hydrolysis (6 N HCl, 110 °C) of **1** indicated that **1** contains 1 mol each of Ala and Leu and 2 mol of Phe. The upfield region included three doublet methyl signals (δ 1.39, 0.96, and 0.93) that were eventually assigned to the Ala and Leu residues on the basis of HMBC and COSY data.



Kapakahine B (1)

The downfield region of the ^1H NMR spectrum, 6−8 ppm, displayed signals that were indicative of aromatic amino acids. There were signals for two monosubstituted benzene rings [δ 7.05 (1H), 7.13 (2H), 7.21 (2H), 7.23 (2H), 7.28 (1H), and 7.31 (2H)], which are compatible with two Phe residues.

The remaining signals could be assigned to two Trp moieties on account of the characteristic signals for an ortho-substituted benzene ring. These included two triplets, δ 6.79 (H-18, J = 7.9) and δ 6.99 (H-17, J = 7.5), two doublets, δ 6.24 (H-19, J

(1) Present adress: Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, U.K.

(2) HBOI Contribution No. 1103.

(3) Baker, B. J. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Springer-Verlag: New York, NY; Vol. 10, in press.

(4) Moquin-Pattey, C.; Guyot, M. *Tetrahedron* **1989**, *45*, 3445−3450.

(5) Kapakahi (Hawaiian) = lopsided, crooked.

(6) The sample was collected from a depth of 30−40 m. It is *Cribrochalina olemda* de Laubenfels and is well described by de Laubenfels[8] and Bergquist.[9] A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, FL (Catalog No. 003:891).

(7) Interestingly, from a Papua-New Guinea *Cribrochalina* sp. Crews et al.[10] have isolated manzamines, complex alkaloids incorporating tryptophan.

(8) de Laubenfels, H. M. The Sponges of the West Central Pacific; Oregon State Monograph Studies in Zoology: Corvallis, OR, 1954; pp 77−79.

(9) Bergquist, P. R. *Pac. Sci.* **1965**, *14*, 123−204.

(10) Crews, P.; Cheng, X.-C.; Adamczeski, M.; Rodriguez, J.; Jaspars, M.; Schmitz, F. J.; Traeger, S. C.; Pordesimo, S. O. *Tetrahedron* **1994**, *50*, 13567−13574.

(11) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178−179.

(12) A total of 2.0 mg of kapakahine B was obtained from the re-collection (4 kg) by the same procedure. Kapakahine B (1): white amorphous solid; $[\alpha]^{20}_D$ −70° (*c* 0.3, MeOH); UV (MeOH) 214 nm (ε 21 000), 250 (8400), 280 (5600), 294 (4000); IR ($CHCl_3$) 3450, 2920, 2810, 1665, 1565, 1325 cm^{-1}; ^1H NMR [CD_3CN/CD_3OH (2:1)+AcOH] Phe-1 δ 4.00 (bt, 7.0; H-2), 3.13 (dd, 5.5, 14.0; H-3), 2.98 (dd, 8.4, 13.9; H-3′), 7.21 (d, 7.3; H-5, 9), 7.31 (t, 7.0; H-6, 8), 7.28 (t, 7.0; H-7); Trp-1 δ 7.83 (bd, 7.0; NH), 4.58 (bt, 7.6; H-11), 3.37 (dd, 7.9, 15.3; H-12), 2.97 (d, 15.3; H-12′), 7.22 (s; H-13), 7.42 (d, 7.9; H-16), 6.99 (t, 7.5; H-17), 6.79 (t, 7.9; H-18), 6.24 (d, 8.5; H-19); Ala δ 7.72 (d, 7.0; NH), 4.32 (quintet, 7.3; H-22), 1.39 (d, 7.3; H-23); Leu δ 7.34 (d, 8.2; NH), 4.33 (q, 7.5; H-25), 1.60 (dd, 7.3, 6.1; H-26), 1.63 (nonuplet, 6.1; H-27), 0.93 (d, 6.1; H-28), 0.96 (d, 6.1; H-29); Trp-2 δ 8.10 (d, 8.2; NH), 4.69 (dd, 8.2, 11.6; H-31), 2.92 (dd, 0.9, 14.3; H-32), 2.58 (dd, 12.8, 14.4; H-32′), 5.55 (s; H-33), 6.80 (d, 7.9; H-36), 6.95 (dt, 0.6, 8.2; H-37), 7.32 (t, 7.6; H-38), 7.58 (d, 7.9; H-39); Phe-2 δ 4.96 (t, 5.3; H-42), 3.22 (dd, 4.6, 14.0; H-43), 3.12 (dd, 5.8, 14.0; H-43′), 7.23 (d, 7.9; H-45, 49), 7.13 (t, 7.5; H-46, 48), 7.05 (t, 7.3; H-47); ^{13}C NMR [CD_3CN/CD_3OH (2:1) + AcOH] Phe-1 δ 169.2 (C-1), 55.8 (C- 2), 38.4 (C-3), 135.6 (C-4), 130.7 (C-5, 9), 130.3 (C-6, 8), 129.1 (C-7); Trp-1 δ 172.4 (C- 10), 55.4 (C-11), 27.7 (C-12), 126.4 (C-13), 111.9 (C-14), 132.2 (C-15), 120.0 (C-16), 121.1 (C-17), 123.0 (C-18), 112.2 (C-19), 135.4 (C-20); Ala δ 175.1 (C-21), 52.5 (C-22), 17.7 (C-23); Leu δ 175.4 (C-24), 53.8 (C-25), 40.8 (C-26), 26.1 (C-27), 22.7 (C-28), 23.3 (C-29); Trp-2 δ 169.5 (C-30), 49.4 (C-31), 38.9 (C-32), 83.4 (C-33), 69.3 (C-34), 135.5 (C-35), 125.2 (C-36), 127.1 (C-37), 131.6 (C-38), 115.9 (C-39), 140.6 (C-40); Phe-2 δ 173.1 (C-41), 65.8 (C-42), 37.6 (C-43), 137.3 (C-44), 130.9 (C-45, 49), 129.7 (C-46, 48), 128.6 (C-47).

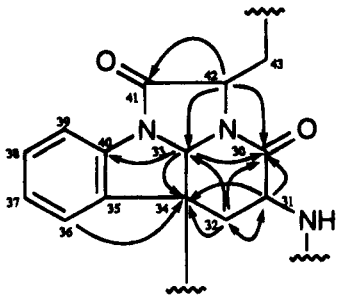


Figure 1. Important HMBC correlations of the α-carboline moiety.

= 8.5) and δ 7.42 (H-16, J = 7.9), and a singlet at δ 7.22 (H-13, s), which were shown to be connected to each other and were assigned to Trp-1 by COSY and HMBC experiments.

The remaining aromatic signals were initially assigned to a second Trp moiety; it was clear that an indole was present, but it lacked signals for aromatic carbons at C-33 and C-34. These carbons were replaced by a methine (CH-33; δ_C 83.4, δ_H 5.55) and a quaternary carbon (C-34, δ 69.3). The HMBC data pointed to a complex ring system, which somehow incorporated one Phe moiety. Critical long-range correlations were observed between H-31/C-30, H-31/C-32, H-31/C-34, H_2-32/C-30, H_2-32/C-31, H-32/C-33, H-32/C-34, H-32′/C-35, H-33/C-30, H-33/C-34, H-33/C-40, H-36/C-34, H-42/C-30, and H-42/C-33, all of which indicated the presence of an α-carboline ring system, which also incorporated the amino nitrogen of Phe-2. The nitrogen atom linked to C-31, which becomes part of a β-carboline in the manzamines,[13] is now *exo* to the piperidone and forms an amide with the carboxyl of Leu (Figure 1).

The sequence of amino acid residues was determined by spectral measurements (HMBC and ROESY) and Edman degradation. HMBC correlations from amide protons to neighboring carbonyl carbons were observed between Ala/Trp-1, Leu/Ala, and Trp-2/Leu. ROESY correlations from H-13 to H-31 and H-32′ suggested that a C−N bond was formed between the indole nitrogen atom of Trp-1 and C-34 to form a 16-membered ring.[14] This was supported by the chemical shift of C-34 (δ 69.3), which is compatible with related carbon shifts in flustramine E (δ 57.3)[15] and urochordamine A (δ 60.9).[16] An important HMBC correlation was seen between H-11/C-1, connecting Phe-1 to Trp-1. Edman degradation (Applied Biosystems 477A protein sequencer) showed a Phe residue only in the first cycle, which confirmed an N-terminal position for Phe-1 with free amine.[17] This experiment also proved that Phe-1 is the only residue exocyclic to the 16-membered ring. Adding the molecular weights of all residues resulted in a formula of $C_{49}H_{54}N_8O_7$, which is larger by 1 mol of water than the molecular weight measured by HR-FABMS. Hence, there should be one more amide bond, which is only possible between the indole nitrogen of Trp-2 and carbonyl of Phe-2 forming an imidazolone ring. This completes the planar structure of **1**.

Although the bioactivity of kapakahine B (**1**) was moderate (IC_{50} of 5.0 μg/mL; against P388 murine leukemia cells), the structure is unique and of biogenetic interest. The absolute stereochemistry was determined for Ala and Leu by Marfey's method[18] as L. Further work is in progress.[19]

Acknowledgment. We are indebted to Drs. Mark Hamann, Toshio Ichiba, and Steve Bobzin for collection and re-collection of sponges; to Candy Young of Aaron's Dive Shop and to Valentine Martin, Pelson Moses, and Jackson Roby of Marine Resources, Pohnpei, Micronesia, for their assistance with collections. Thanks are also due to Mr. Seketsu Fukuzawa and Professor Nobuhiro Fusetani of University of Tokyo for bioassay and mass spectral measurements. Financial assistance by the National Science Foundation, the Sea Grant College Program, and Pharma Mar. S.A. is gratefully acknowledged. Y.N. is financially supported by Postdoctoral Fellowships for Research Abroad awarded by the Japan Society for the Promotion of Science.

Supporting Information Available: ^1H and ^{13}C NMR, COSY, ROESY, HMQC, and HMBC spectra in [CD_3CN/CD_3OH (2:1) + AcOH], FAB-MS/MS, and protein sequencing analysis for **1** (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA950731C

(13) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc.* **1986**, *108*, 6404−6405.

(14) Kapakahine A shares the same substructure, where we observed an unambiguous C−H three-bond correlation between the singlet proton signal of Trp-1 (corresponding to H-13 of kapakahine B) and the quartenary carbon of Trp-2 (corresponding to C-34) in the selective INEPT experiment. This unambiguously connects Trp-1 and -2 through a C−N bond.

(15) Holst, P. B.; Anthoni, U.; Christophersen, C.; Nielsen, P. H. *J. Nat. Prod.* **1994**, *57*, 997−1000.

(16) Tsukamoto, S.; Hirota, H.; Kato, H.; Fusetani, N. *Tetrahedron Lett.* **1993**, *34*, 4819−4822.

(17) Kapakahine B was also subjected to a FAB-MS/MS measurement to confirm the structure. In the positive ion FAB-MS/MS experiment of **1** (from the precursor ion at *m/z* 849), we observed an intense daughter ion peak at *m/z* 730. Usually a peptide linkage is broken between amide linkages, but interestingly, this ion peak corresponds to cleavage between the α-carbon and carbonyl carbon of Phe-1 (C-1 and C-2).

(18) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(19) Complete absolute stereochemistry will be reported in a full paper which includes kapakahine A.