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More Kapakahines from the Marine Sponge *Cribrochalina olemda*†

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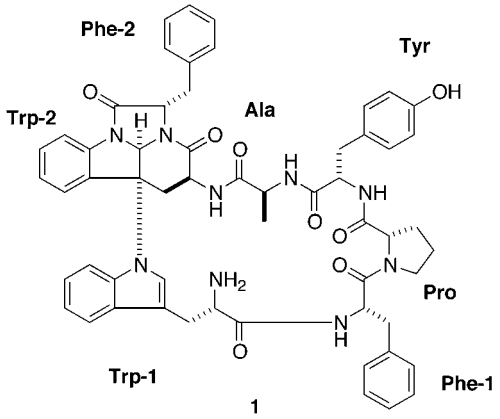
ABSTRACT



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Three new kapakahines E–G (1–3) have been isolated from the marine sponge *Cribrochalina olemda*. Limited quantities of these compounds required not only NMR analysis but also FAB-MS/MS analysis for the structure elucidation. Kapakahine E showed cytotoxicity against P388 murine leukemia cells.

Kapakahines are the cyclic peptides isolated from the marine sponge *Cribrochalina olemda*.[1] Further investigation into this sponge led to the isolation of three minor congeners of the molecules. However, the limited sample quantities required FAB-MS/MS analyses in combination with NMR techniques to complete the gross structures of these compounds. In this paper, the isolation, structure elucidation, and biological activity of these compounds are discussed.

The EtOH extract of *C. olemda* was separated by solvent partitioning followed by gel filtration and finally reversed-phase chromatography. The fractions containing the peptides were collected and purified by ODS HPLC yielding 0.9 mg of kapakahine E (**1**; 1.9×10^{-5}% yield based on wet weight), 0.8 mg of kapakahine F (**2**; 1.7×10^{-5}%), and 1.6 mg of kapakahine G (**3**; 3.3×10^{-5}%) (Chart 1).

The molecular formula of **1** was determined as $C_{57}H_{57}N_9O_8$ by HR-FABMS [$(M + H)^+$ *m/z* 996.4390 (Δ −1.8 mmu)] analysis. COSY and HMQC[2] spectra indicated that fragment **a** common to kapakahines A (**4**) and B (**5**) was also present in **1**. This was further supported by the characteristic signals of CH-4 (δ_H 7.78; δ_C 127.0), CH-39 (δ_H 4.67; δ_C 48.3), CH_2-40 (δ_H 3.59 and 2.43; δ_C 37.9), CH-41 (δ_H 5.72; δ_C 82.4), C-42 (δ_C 67.8), CH-50 (δ_H 4.95; δ_C 65.7), and two ortho-disubstituted benzene rings. Amino acid analysis

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(1) (a) Nakao, Y.; Yeung, B. K. S.; Yoshida, W. Y.; Scheuer, P. J. *J. Am. Chem. Soc.* **1995**, *117*, 8271−8272. (b) Yeung, B. K. S.; Nakao, Y.; Kinnel, R. B.; Carney, J. R.; Yoshida, W. Y.; Scheuer, P. J. *J. Org. Chem.* **1996**, *61*, 7168−7173.

(2) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.

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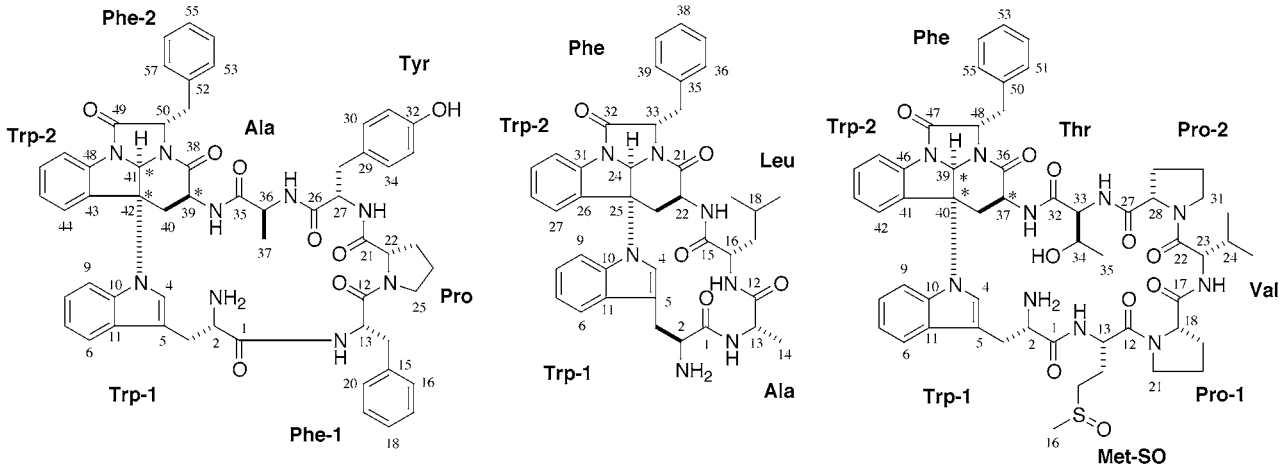
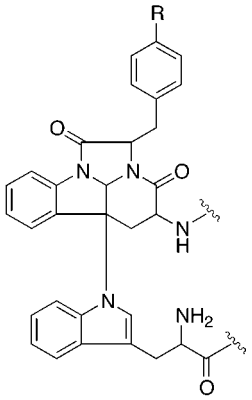
Chart 1

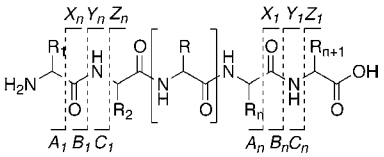


1 *: relative stereochemistry **2** **3**

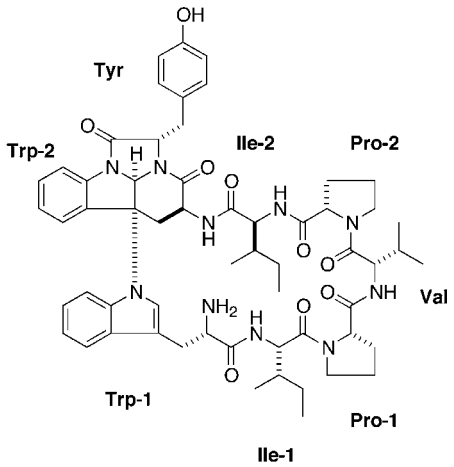
indicated that Ala, Pro, Tyr (1 equiv each), and Phe (2 equiv) were present in kapakahine E. A comparison of the chemical shifts with those of **4** and **5** enabled the assignment of some of the proton and carbon signals for the molecules. However, insufficient sample quantities precluded HMQC and HMBC[3] analysis; therefore, the sequence of each residue was determined by FAB-MS/MS analysis.



The FAB-MS/MS technique has proven to be useful in determining the amino acid sequence of a peptide.[4] In the case of linear peptides, clear fragmentation patterns from both the *N* (An, Bn, and Cn series) and *C* termini (Xn, Yn, and Zn series) can be observed in the MS/MS data.



For cyclic peptides, the FAB-MS/MS data are more complicated, and the interpretation of the spectra is more difficult. In such a case, partial structure analysis of the fragment ion peaks allows one to determine the amino acid sequence in a peptide chain. It is also helpful to know that in cyclic peptides containing Pro residues, ions corresponding to fragments with *N*-terminal Pro are higher in intensity than other fragments in FABMS.[5] In the FAB-MS/MS of kapakahine A (**4**), two sequential fragmentation patterns with N-terminal Pro (Pro-1 and -2, respectively) were observed. For kapakahine E (**1**), fragments with *N*-terminal Pro were observed at *m*/*z* 70 (Pro-CO), 261 (+Tyr), 332 (+Ala), 663 (+Trp-2 and Phe-2), and 804 (+Trp-1 −CO −NH_3), which were indicative of the sequence Pro-Tyr-Ala-fragment **a**. Another set of fragmentation patterns were also observed starting with the Phe residue next to Trp-1: *m*/*z* 148 (Phe-1), 245 (+Pro), 408 (+Tyr), 439 (+Ala), 810 (+Trp-2 and Phe-2), supporting the sequence above.



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The most useful information was obtained by comparing the MS/MS data of kapakahine E with those of kapakahines

(3) Bax, A.; Azolos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056−8063.

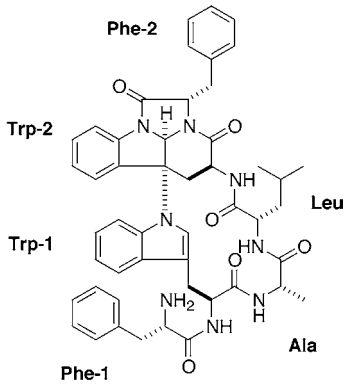
(4) Biemann, K. In *Mass Spectrometry*; McCloskey, J. A., Ed.; Methods in Enzymology 193; Academic: San Diego, 1990; pp 445−479.

(5) Konat, R. K.; Matha, B.; Winkler, J.; Kessler, H. *Liebigs Ann.* **1995**, 765−774.

A (**4**) and B (**5**), whose structures were unambiguously confirmed by NMR analysis. In the FAB-MS/MS of kapakahine A, there were two distinct fragmentation patterns for both the *N* and *C* termini derived from the cleavage of the C−N bond between Trp-1 and Trp-2. Fragmentation of the *C*-terminus started with the ion peak at *m*/*z* 348 (Y_2; Trp-2 + Tyr), followed by ions at *m*/*z* 461 (Y_3; +Ile-2), 558 (Y_4; +Pro-2), 657 (Y_5; +Val), 754 (Y_6; +Pro-1), and 895 (X_7; +Ile-1+CO). Fragmentation of the *N*-terminus started with the ion peak at *m*/*z* 130 (indole +CH_2+H of Trp-1), followed by ions at *m*/*z* 159 (A_1; +CH−NH_2), 300 (B_2; +Ile-1), and 397 (B_3; +Pro-1).

In the FAB-MS/MS of kapakahine B (**5**), the corresponding fragmentations were also observed. One started from the *C*-terminal ion peak of *m*/*z* 332 (Y_2; Trp-2 + Phe-2), followed by 445 (Y_3; +Leu) and 516 (Y_4; +Ala). The other started from the *N*-terminal ion peak at *m*/*z* 130, followed by 334 (B_2; Trp-1 + Phe-1), 405 (B_3; +Ala), and 518 (B_4; +Leu).

The important point is that one can tell which residue (Tyr or Phe) is found in fragment **a** by the Y_2 ion peak, which is further supported by other fragment ions derived from this unit. If Tyr is the constituent of the fragment **a** (in the case of kapakahine A), there are the fragment ion peaks at *m*/*z* 291, 303, 320, 331, and 348. Conversely, if Phe (in the case of kapakahine B) is the constituent, peaks at *m*/*z* 275, 287, 304, 315, and 332 will be visible. In the case of kapakahine E, both Phe and Tyr residues were present; therefore, it was expected that this observation would clearly lead to the residue in fragment **a**.



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In the FAB-MS/MS of kapakahine E, the Y_2 ion peak at *m*/*z* 332 as well as the peaks at *m*/*z* 275, 287, 304, and 315 were observed, indicating that Phe was present in fragment **a**. These peaks were followed by a series of ions at *m*/*z* 403 (Y_3; +Ala), 566 (Y_4; +Tyr), 663 (Y_5; +Pro), and 810 (Y_6; +Phe-1). Fragmentation from the *N*-terminus, i.e., ions at *m*/*z* 130, 159 (A_1; Trp-1-CO), 334 (B_2; Trp-1+Phe), 431 (B_3; +Pro), 594 (B_4; +Tyr), 665 (B_5; +Ala), supported this sequence (Figure 1), completing the planar structure depicted in **1**.

Marfey analysis[6] of kapakahine E showed L-Ala, L-Pro, L-Tyr, L-Phe (Phe-1 and 2), and a trace of L-Trp (Trp-1).

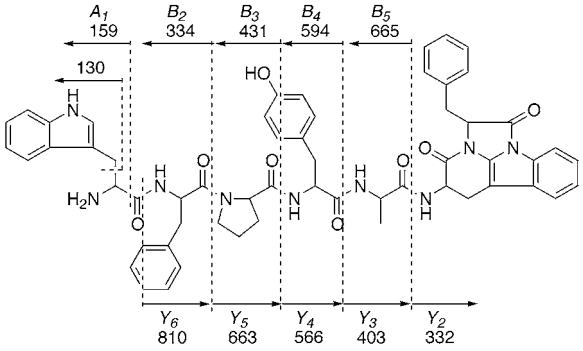

Figure 1. FAB-MS/MS fragmentation of kapakahine E (**1**).

The stereochemistry at C-39, -41, and -42 was thought to be identical to the corresponding carbons in kapakahine A (**4**) on the basis of the similar chemical shifts and NOE patterns (H-41/H-9 and H-4/H-40a).

Kapakahine F (**2**) has a molecular formula of $C_{40}H_{43}N_7O_5$, which is one Phe unit smaller than kapakahine B [HR-FABMS $(M + H)^+$ *m*/*z* 702.3384 (Δ −2.0 mmu)]. Amino acid analysis of **2** showed one residue each of Ala, Leu, and Phe. The ^{1}H NMR spectrum of **2** showed characteristic signals for fragment **a** [δ 5.69 (s, H-24) and 4.96 (dd, J = 5.8, 5.4 Hz, H-33)] together with a monosubstituted and two disubstituted benzene rings. These observations suggested the structure of **2** was identical to that of kapakahine B, except that the *N*-terminal Phe residue was missing at C-2.

FAB-MS/MS analysis of kapakahine F showed fragmentation patterns for both *C*- and *N*-termini: ion peaks at *m*/*z* 332 (Y_2; Trp-2 + Phe), 445 (Y_3; +Leu), and 516 (Y_4; +Ala), and at *m*/*z* 130, 159 (A_1; Trp-1-CO), 258 (B_2; +Ala), and 371 (B_3; +Leu), which supported structure **2** (Figure 2).

To confirm this structure and to determine the stereochemistry, kapakahine B was transformed to kapakahine F by a one-step Edman degradation.[7] The reaction mixture was separated by ODS HPLC to give a peak with a retention time identical to that of kapakahine F. By comparison of the HPLC profile, MS, and ^{1}H NMR spectrum, as well as the Marfey analysis, the structure of kapakahine F was determined as **2**.

The molecular formula of kapakahine G (**3**) was determined by HR-FABMS as $C_{55}H_{66}N_{10}O_{10}S$ [$(M + H)^+$ *m*/*z* 1059.4827 (Δ +2.6 mmu)]. Amino acid analysis of the acid hydrolysate of **3** indicated that the molecule contained one residue each of Thr, Val, Met,[8] and Phe and two Pro. ^{1}H and ^{13}C NMR spectra showed the characteristic signals for fragment **a** at C-39 (δ_H 6.44, δ_C 82.3). Additional signals for the ortho-disubstituted benzene ring, as well as the spin system for the NH protons on C-2 (δ 8.23), through the

(6) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(7) Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron Lett.* **1984**, *25*, 5165−5168.
(8) Retention times in amino acid analysis for MetSO and Met were 13.89/14.64 min (two stereoisomers at sulfoxide) and 41.81 min, respectively.

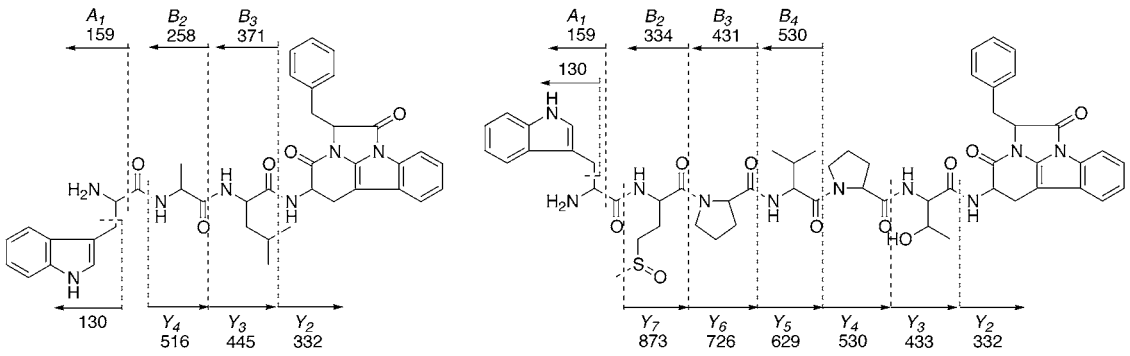

Figure 2. FAB-MS/MS fragmentation of kapakahine F (**2**) and G (**3**).

α-proton (δ 4.87, H-2), to the β-methylene protons (δ 3.50 and 3.44, H_2-3), further supported the existence of this unit.

The data above indicated a molecular formula of $C_{55}H_{66}N_{10}O_9S$, which corresponded to 16 mass units less than the molecular weight of **3**. The difference of 16 mass units was due to the presence of methioninesulfoxide (MetSO) instead of Met in **3**. A characteristic ion peak at *m*/*z* 995 corresponding to a loss of 64 ($-CH_2SO$) mass units in the MS for **3** indicated the presence of MetSO. This was supported by the ^{13}C and ^1H NMR chemical shifts at CH_2-15 (δ_C 51.2, δ_H 2.96 and 2.87) and CH_3-16 (δ_C 38.7, δ_H 2.48), which were too downfield for those of Met but were consistent with those of MetSO.[9] Furthermore, CH_2-15 exhibited a second set of ^{13}C and ^1H NMR signals (δ_C 50.9, δ_H 2.87 and 2.70) induced by the chirality of the sulfoxide moiety. This probably had an effect on the ^1H NMR resonances observed for CH-39 (δ 6.435 and 6.439) and CH-48 (δ 4.19). The doubling of the signal for H-39 and the chemical shift difference between H-39, H-48 and the corresponding protons in **1** and **2** were probably a result of the anisotropic effect of the sulfoxide. Presumably, the Met residue found in the amino acid analysis was a result of the reduction of MetSO during acid hydrolysis.

HMBC and NOE analysis disclosed the sequence of Pro-1/Val/Pro-2/Thr/Trp-2/Trp-1. Compared to the structure of kapakahine A, the remaining MetSO was most likely located between Trp-1 and Pro-1; however, no interresidue correlations were observed in the NMR spectra. Once again, FAB-MS/MS analysis was applied to show the existence of MetSO between Trp-1 and Pro-1.

In the FAB-MS/MS of **3**, the key ion peaks at *m*/*z* 332 (Y_2) and 159 (A_1) were observed. From these ion peaks, key fragmentation patterns [*m*/*z* 433 (Y_3), 530 (Y_4), 629 (Y_5), 726 (Y_6), 873 (Y_7) and *m*/*z* 334 (B_2), 431 (B_3), 530 (B_4)] were evident, thus confirming the planar structure of **3** (Figure 2). Since kapakahine G contained two Pro residues in the molecule, the two sequential fragmentation patterns containing *N*-terminal Pro [*m*/*z* 70 (Pro-1), 197 (+Val), 726 (+Pro-2, Thr, Trp-2, and Phe), 884 (+Trp-1 −CO) and 70 (Pro-2), 199 (+Thr), 530 (+Trp-2 and Phe), 688 (+Trp-1 −CO), 863 (+MetSO)] further supported the sequence above.

Marfey analysis of the acid hydrolysate for **3** indicated L-forms for the residues of Thr, Val, MetSO,[10] Phe, and Trp-1. Although the absolute stereochemistry at C-37, C-39, and C-40 is still unknown, the relative stereochemistry is identical to that of **1** on the basis of NOEs observed between H-4/H-37, H-4/H-38a, H-9/H-39, and H-37/H-39.

Kapakahines E showed moderate cytotoxicity against P388 murine leukemia cells at IC_{50} of 5.0 μg/mL; however, kapakahines F and G showed only weak cytotoxicity at this concentration.

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Supporting Information Available: Experimental procedures, structures **4** and **5**, FAB-MS/MS data for **1**−**5**, structures **1** and **3** with key NOE and HMBC correlations, NMR spectra for **1**−**3**, and ^1H NMR spectra for **2** and that prepared from **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) (a) Rashid, M. A.; Gustafson, K. R.; Boswell, J. L.; Boyd, M. R. *J. Nat. Prod.* **2000**, *63*, 956−959. (b) Mau, C. M. S.; Nakao, Y.; Yoshida, W. Y.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1996**, *61*, 6302−6304.

(10) Interestingly, MetSO was observed instead of Met in Marfey analysis of the newly prepared acid hydrolysate of **3**.