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Yaku'amides A and B, Cytotoxic Linear Peptides Rich in Dehydroamino Acids from the Marine Sponge *Ceratopsion* sp.°

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Abstract: Two cytotoxic peptides, yaku'amides A (**1**) and B (**2**), were isolated from the marine sponge *Ceratopsion* sp. Their planar structures were elucidated on the basis of spectroscopic data, whereas the absolute configurations were determined by a combination of the Marfey's analysis and dansylation analysis of the total and partial acid hydrolysis products. The growth inhibitory profile of yaku'amide A against a panel of 39 human cancer cell lines was clearly unique and distinguished from other anticancer drugs.

Nonribosomal peptides compare well with polyketides in their potent biological activities, production by prokaryotes or fungi, and biosynthesis by modular enzymes.[1] Marine sponges also contain a variety of nonribosomal peptides and polyketides.[2] In the course of our search for cytotoxic compounds from marine sponges, we found that a deep-sea sponge *Ceratopsion* sp. collected at Yakushinsone in the East China Sea showed potent cytotoxicity. From the sponge we have isolated yaku'amides A and B (**1** and **2**, respectively), linear peptides with unique N- and C-termini and rich in dehydroamino acids. Their structures were elucidated by analysis of spectroscopic data and chemical degradations.

The organic layer of the extract of the sponge was subjected to a modification of the Kupchan's solvent−solvent partitioning scheme.[3] The $CHCl_3$ fraction was separated by ODS flash chromatography, silica gel column chromatography, and reversed-phase HPLC to give yaku'amides A (**1**) and B (**2**).

Yaku'amide A (**1**) had a molecular formula of $C_{83}H_{145}N_{15}O_{18}$ which was established by the HR-ESIMS. Analysis of the ^{1}H NMR spectrum measured in CD_3OD in conjunction with the HSQC spectrum revealed the presence of a large number of aliphatic methyls, five vinylic methyls, one broad methyl signal, and α-methines. In DMSO-d_6, exchangeable NH signals characteristic of a peptide were observed and the broad methyl signal was sharpened. The ^{13}C NMR spectrum showed numerous nonprotonated carbons, viz., one ketone, many amide carbonyl carbons, sp^2 carbons, and oxygenated quaternary carbons, indicating the presence of a variety of nonproteinogenic amino acid residues. Analysis of 2D NMR data revealed the presence of one residue each of Gly, Ala, and Ile and three residues of Val. In addition to these residues,

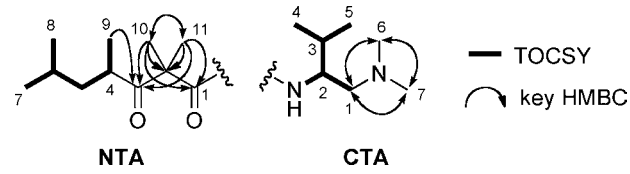

Figure 1. 2D-NMR correlations in NTA and CTA.

one residue each of α,β-dehydro-Val and β-hydroxy-Ile, two residues of β-hydroxy-Val, and three residues of α,β-dehydro-Ile were assigned. Both termini of yaku'amide A (**1**) were blocked by unique groups. The partial structure of C-4 to C-9 in the N-terminal acyl group (NTA) was deduced from the COSY and TOCSY data (Figure 1). In NTA, the HMBC correlations from H-10/H-11 to C-1, C-2, and C-3 indicated the presence of a 2,2-dimethyl malonyl moiety. These two partial structures were connected on the basis of an HMBC correlation from H-9 to C-3. COSY and TOCSY data revealed the partial structure of C-1 to C-5 of the C-terminal amine (CTA). The HMBC correlations from the *N*-methyl (H_3-6 and H_3-7) to C-1 showed the attachment of a dimethylamino group to C-1 in CTA (Figure 1).

Partial amino acid sequences were deduced by interpretation of the HMBC data (Figure 2). The HMBC correlation from H-2 of OHIle to C-1 of NTA indicated the connection between NTA and OHIle (unit **a**). The HMBC cross peaks from H-2 of Gly to C-1 of ΔIle1, from NH of ΔIle2 to C-1 of Gly, and from NH and H-2 of Val1 to C-1 of ΔIle2 indicated the sequence of ΔIle1-Gly-ΔIle2-Val1 (unit **b**). The HMBC correlation from H-2 of Ala to C-1 of ΔIle3 revealed that ΔIle3 was attached to Ala (unit **c**). The arrangement of Val2-Val3-ΔVal-CTA (unit **d**) was assigned by the HMBC cross peaks from NH of Val3 to C-1 of Val2, from NH of ΔVal to C-2 of Val3, and from NH of CTA to C-1 of ΔVal. No sequential data for Ile and two OHVal residues were obtained by the HMBC data. Then the above partial sequences were integrated on the basis of the NOESY data (Figure 2). The correlation between NH of ΔIle1 and H-2 of OHIle permitted the connection between unit **a** and unit **b**. The cross peaks between NH of Ile and NH, H-2, H-3, and H_3-4 of Val1 and between NH of Ile and NH of OHVal1 indicated the sequence of Val1-Ile-OHVal1. The connection of OHVal2 and ΔIle3 was assigned by the correlation between NH of ΔIle3 and NH, H-2, H_3-4, and H_3-5 of OHVal2, and the connection of Ala and Val2 was assigned by the correlation between NH of Val2 and NH, H-2, and H_3-3 of Ala. Finally, OHVal1 was attached to OHVal2 on the basis of the NOESY cross peaks between methyl singlets of each residue.

° Yaku'amides were named after yakushinsone, the collection site.
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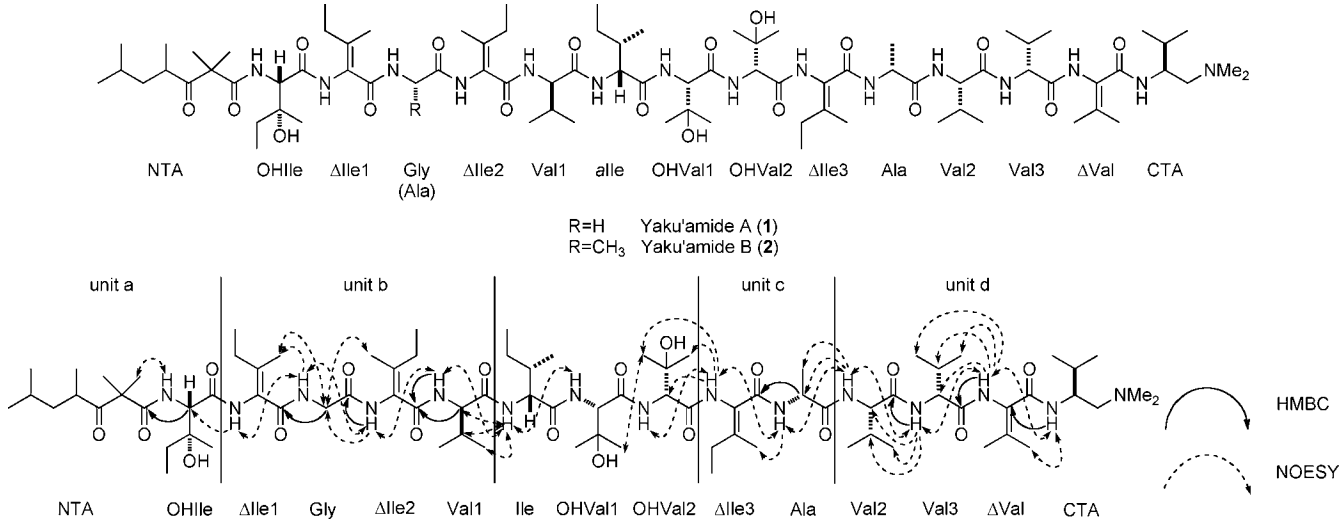

Figure 2. Structures of yaku'amides and HMBC and NOESY correlations observed in **1**.

The geometries of the double bonds in the three ΔIle residues were determined on the basis of NOESY data. The correlations between NH and H-2 of Gly and H_3-6 of ΔIle1 indicated the *Z*-configuration in ΔIle1. The *E*-configuration in ΔIle2 was assigned by the cross peak between H-2 of Gly and H_3-6 of ΔIle2. The cross peak between NH of Ala and H_3-6 of ΔIle3 indicated the *Z*-configuration in ΔIle3.

The absolute configurations of the component amino acids were determined by the Marfey's analysis.[4] The standard amino acids were assigned as D-Ala, one L-Val, two D-Val, and D-*allo*-Ile by comparing retention times with those of standards. 2*S* isomers of OHVal and CTA as well as (2*S*,3*S*)- and (2*R*,3*S*)-isomers of OHIle were synthesized as reported in the literature.[5] They were derivatized by L-FDAA and D-FDAA and used as standards for the relevant isomers and the corresponding enantiomers, respectively.[6] The presence of one L-OHVal, one D-OHVal, (2*S*,3*R*)-OHIle, and 2*S*-CTA was disclosed by comparing the retention times. In order to establish the positional assignments of OHVal and Val residues, **1** was subjected to partial acidic hydrolysis. Yaku'amide A (**1**) was hydrolyzed with 70% AcOH at 110 °C for 4 days, and the products were separated by ODS-HPLC to give **3**−**5**, whose sequences were assigned by a combination of the ESIMS and Marfey's analysis (Figure 3). The Marfey's analysis of **3** and **4** showed that OHVal2 and Val1 were both in the D-form. The configuration of Val2 was determined to be L by dansylation of **5** followed by acidic hydrolysis and chiral HPLC analysis.[7] By a process of elimination, the configurations of Val1, OHVal1, and Val3 were determined as D, L, and D, respectively. The absolute configuration at C-4 of NTA was left undefined.

OHVal2-ΔIle3-Ala-Val2-Val3-ΔVal-CTA (**3**)

Ala-Val2-Val3-ΔVal-CTA (**4**)

Val2-Val3-ΔVal-CTA (**5**)

Figure 3. Amino acid sequences of fragments obtained by partial acidic hydrolysis.

Yaku'amide B (**2**) had the molecular formula one CH_2 unit larger than that of **1**. The ^{1}H NMR spectrum of **2** was very similar to that of **1**. Analysis of 2D NMR data showed that Gly in **1** was substituted for Ala in **2**. The absolute configurations of the components were assigned by Marfey's analysis as L-Ala (1), D-Ala (1), L-Val (1), D-Val (2), D-*allo*-Ile, L-OHVal (1), D-OHVal (1), (2*S*,3*R*)-OHIle, and 2*S*-CTA. Almost identical NMR data and common absolute configurations of component amino acids suggested that they share the same configurations throughout the chains.

Yaku'amides A (**1**) and B (**2**) exhibited potent cell-growth inhibitory activity against P388 murine leukemia cells with IC_{50} values of 14 and 4 ng/mL, respectively. In order to analyze the mode of action of yaku'amide A (**1**), we examined its growth-inhibitory profile against a panel of 39 human cancer cell lines (JFCR39) that include various human cancers.[8] Because yaku'amide A (**1**) exhibits clearly a unique profile as compared to any 38 anticancer drugs,[8a] it is suggested that yaku'amide A (**1**) has a unique mode of action in its growth-inhibitory activity.

Yaku'amides are unique in containing many dehydroamino acids and β-hydroxyamino acids, both of which are reminiscent of the thiomycin class of peptides,[9] which are synthesized ribosomally and become mature after a series of post-translational modifications.[10] In contrast, the presence of several D-amino acid residues in yaku'amides suggests that they are nonribosomal. The N-terminal and C-terminal blocking groups of yaku'amides are both unprecedented as components of natural products.

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Supporting Information Available: Experimental procedures, references, and NMR spectra for yaku'amides A and B (**1** and **2**) and noncommercial amino acids synthesized in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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