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A CHLOROADENINE RIBOSIDE-TYPE NUCLEOSIDE FROM the MARINE SPONGE *THEONELLA CUPOLA*

DENİZ SÜNGERİ *THEONELLA CUPOLA'DAN* KLOROADENİN RİBOZİT TİPİ BİR NÜKLEOZİT

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ABSTRACT

A nucleoside analogue, kumusine (or trachycladine A), along with cupolamide A, a cytotoxic cyclic heptapeptide, were isolated from the marine sponge Theonella cupola. Structure elucidation of the isolated compounds was performed by spectroscopic methods. This is the first report of the isolation of kumusine from the marine sponge Theonella cupola.

Key words: *Kumusine, trachycladine A, cupolamide A, marine sponge, Theonella sp.*

ÖZET

Bir nükleozit analoğu olan kumusin (veya trakikladin A), sitotoksik bir siklik heptapeptit olan kupolamid A ile birlikte, deniz süngeri Theonella cupola'dan izole edilmiştir. İzole edilen bileşiklerin yapı tayinleri spektroskopik metotlarla yapılmıştır. Bu çalışma, Theonella cupola'dan kumusinin izolasyonuna dair ilk çalışmadır.

Anahtar kelimeler: *Kumusin, trakikladin A , kupolamid A, deniz süngeri, Theonella sp.*

INTRODUCTION

Nucleosides, a family of the nitrogenous compounds, have been found to have significant biological activities (1). Among the nucleosides, chloroadenine analogues in particular are rarely found in nature and have been previously isolated only from *Streptomyces* sp. (2). The first examples of nucleoside-type compounds, spongothymidine, spongouridine and spongosine, were isolated from the marine sponge *Cryptotethia crypta* in the early 1950s (3,4). Spongouridine was also identified in the butanol extract of the gorgonian *Eunicella cavolini* (5). These compounds were the first discoveries of nucleosides in the marine sources and after twenty-five years they became the progenitors of the antiviral drugs Ara-A (vidarabine) and acyclovir (Zovirax®, Aklovir®) that are presently in clinical use.

We herein report the isolation and structural elucidation of a chloroadenine riboside 9-(2'-C-methyl-5'-deoxy-_-D-ribofuranosyl)-2-chloroadenine(I), named as kumusine or trachycladine A. Kumusine, reported to possess cytotoxic activity against P388 (IC_{50} 5.0 _g/mL), A549 (IC_{50} 2.5 _g/mL), HT29 (IC_{50} 5.0 _g/mL), and CV1 (IC_{50} 2.5 _g/mL), and moderate immunosupressive activity, has been formerly isolated from an unidentified species of *Theonella* sp. collected from Indonesia and from an Australian sponge *Trachycladus laevispirulifer* (6,7). In addition to kumusine, cupolamide A (II), a well-known cytotoxic heptapeptide, has been isolated from the same sponge. Cupolamide A was previously reported to be active against P388 murine leukemia cells (IC_{50} 7.5 _g/mL) (8).

MATERIAL AND METHODS

Instrumentation

NMR spectra were recorded on a JEOL _500 instrument at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ^{1}H NMR and ^{13}C NMR spectra are referenced to solvent signals at 3.49 and 39.1 ppm for DMSO-d_6. Chemical shifts are given in ppm relative to international standard of TMS. FABMS spectrum was obtained on a JEOL JMS-SX 102A tandem mass spectrometer. Spectral grade solvents were used for spectroscopic measurements. HPLC separations were carried out on a Hitachi L-6000 apparatus equipped with Hitachi L-4000 UV detector. Column used for preparative HPLC was reversed-phase C18 (250x10 mm, 5 _m, Capcell PAK).

Sponge Collection

A sample of the marine sponge *Theonella cupola* was collected at the depth of 35 m from Yonaguni Island, Okinawa, Japan, in September, 1996. The lemon-colored sponge was identified as *Theonella cupola* by Dr. John N.A. Hooper, Queensland Museum, Brisbane, Australia.

Extraction and Isolation

After collection of the sponge (wet weigth 2.05 kg), it was extracted with acetone, concentrated under reduced pressure to give a residue. The residue was partitioned between ethylacetate and water. Aqueous layer was washed with methanol and methanol-soluble portion (31.87 g) was applied to vacuum flash chromatography (RP 18). The fraction obtained from the elution with MeOH:$H_2$0/3:l (1.07 g) was subjected to Sephadex LH-20 column chromatography using CH_2Cl_2:MeOH/l:l as eluent. The second subfraction (9.6 mg) of this column was applied to preparative HPLC (RP 18) by eluting with $H_2$0:CH_3CN/3:1 to give compound (I) (2.3 mg) as a yellowish oil. Compound (II) (57.9 mg, white amorphous powder) was purified from the first subfraction of the Sephadex LH-20 column by preparative HPLC (RP 18) using the solvent system $H_2$0:CH_3CN/2:1.

RESULTS AND DISCUSSION

Examination of ^{1}H and ^{I3}C NMR spectra of compound (1) in DMSO-J_6 revealed a nucleoside-type structure. ^{1}H NMR spectrum displayed fourteen protons between _ 0.79 and _ 8.18, consisting of two methyl groups, one singlet and a doublet. The base-portion was determined as a C-2 substituted adenine by a broad singlet which belongs to a NH_2 moiety at _ 7.81 (2H) and a diagnostic sharp singlet, indicator of an aromatic proton nearby a nitrogen atom at _ 8.18. The chlorine substituent was identified by a 2-chloroadenine fragment ion signal in the mass spectrum. M^+ peaks at *m/z* 299 and 301 having a ratio of 3:1 pointed out the chlorine atom.

The sugar moiety of compound (1) was found to be the unprecedented branched chain furanose 2-C-methyl-D-5-deoxyribose. ^{13}C NMR spectrum showed eleven carbon resonances, corresponding to five quartet, three methyn, one methylene and two methyl carbons. The carbon chemical shift at _ 91.2 (_ 6.58 s in ^{1}H NMR) indicated that C1^{1} is an anomeric carbon. Finally,

the formula of compound (1) was established as $C_{11}H_{14}ClN_5O_3$ from FABMS spectrum (MH^+ *m/z* 300/302, 3:1 ratio). As the spectral data agreed well with the reported data, compound (1) was identified to be a known nucleoside analogue, kumusine (or tracycladine A) (Figure 1) (6,7).

Compound (2) was the most abundant component in the methanol-soluble extract of the sponge. Nine low field exchangable protons in ^1H NMR spectrum at _ 8.77, 8.15, 7.83, 8.05, 8.47, 7.56, 7.42, 7.91 and 7.14 and eight carbon resonances at _ 171.1, 170.6, 172.1, 172.3, 170.4, 171.4, 171.6, and 172.2 in the amide carbonyl region in ^{13}C NMR spectrum pointed out a peptide-like structure. Two doublet signals at _ 129.0 and _ 144.7 exhibited double intensity which was suggestive of a para-substituted benzene moiety. A sodium sulfate function was recognized by a FABMS fragment ion at *m/z* 902 [$(M+1)^+$-$S0_3Na$]. A guanidino moeity apperaed as a characteristic chemical shift at _ 156.7 in ^{13}C NMR spectrum (C-32). As the spectral data was completely same as the previously reported data, no further spectroscopic and amino acid analysis has been performed on the compound (8). Consequently, the structure of compound (2) was determined to be a known compound, named as cupolamide A, a cyclic heptapeptide comprising of two L-Valine (L-Val), one D-Leucine (D-Leucine), one D-Serine (D-Serine), and three unusual amino acid residues, D-homoarginine (Har), *trans*-4-hydvoxy-L-proline (Hyp), and L-2,4-diaminobutanoic acid (Dba) (Figure 2).

Table 1. ^1H and ^{13}C NMR data of compound (1) (in *DMSO-d_6*).

C no.	1H NMR	^{13}C NMR	C no.	1H NMR	^{13}C NMR
2	-	154.8	1'	6.58 s	91.2
4	-	151.3	2'	-	78.6
5	-	119.5	3'	4.30(d,J=8.5)	80.3
6	-	158.2	4'	4.62 (dq,J=8.4,6.2)	79.0
8	8.18 s	139.7	5'	1.68 (d, 6.5)	18.4
6-NH2	7.81 s		6'	0.79 s	20.8

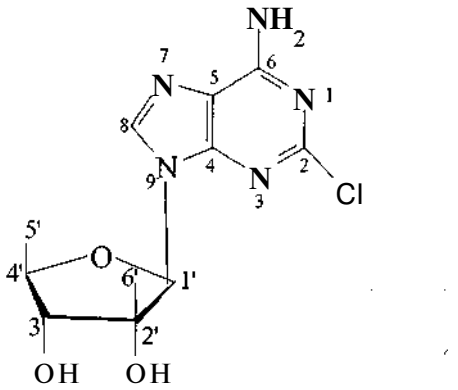

Figure 1. Kumusine (=Trachycladine A)

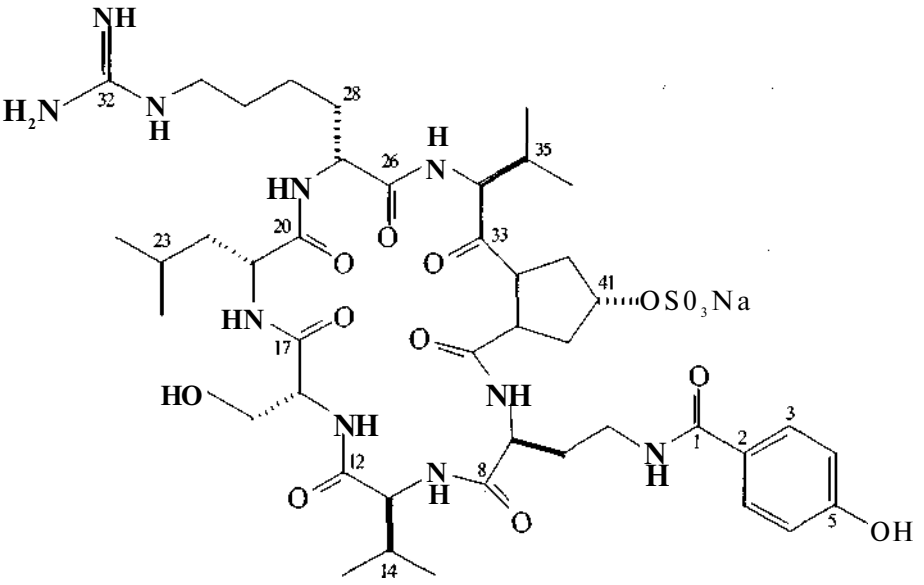

Figure 2. Cupolamide A

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