

Isolation of 1-Carboxymethylnicotinic Acid from the Marine Sponge *Anthosigmella* cf. *raromicrosclera* as a Cysteine Protease Inhibitor¹

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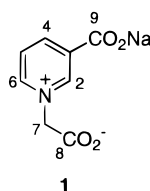
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1-Carboxymethylnicotinic acid (**1**) has been isolated from a marine sponge *Anthosigmella* cf. *raromicrosclera* as a cysteine protease inhibitor. The structure was elucidated by spectral data and chemical synthesis.

Cysteine proteases are involved in cytosolic protein metabolism; some of them are also associated with pathological conditions, for example, inflammation, muscular dystrophy, and tumors.^{2,3} Therefore, inhibitors of cysteine proteases are potential drugs for these diseases. In the course of our continuing search for drug leads from Japanese marine invertebrates, we found that the hydrophilic extract of the marine sponge *Anthosigmella* cf. *raromicrosclera* collected off the Sada Peninsula, 1000 km west of Tokyo, inhibited papain, a cysteine protease. Bioassay-directed isolation afforded an active compound that was identified as 1-carboxymethylnicotinic acid.

The EtOH extract of the sponge was partitioned between Et₂O and H₂O; the aqueous phase was further extracted with *n*-BuOH. The aqueous layer was fractionated on Sephadex G-10 (H₂O) followed by ODS (H₂O). Although the activity was not retained on ODS columns using a variety of mobile phases, a good separation was obtained with an acrylamidated Si gel column (Amide-80; CH₃CN–MeOH–100 mM ammonium formate, 70:5:25), thus yielding an active compound (**1**) as a colorless solid (1.5 mg, 1.5 × 10⁻³% wet wt).



Compound **1** had a molecular formula of C₈H₈NO₄ as established by its FABMS and NMR data.⁴ The ¹H NMR spectrum exhibited four heteroaromatic signals [δ 9.00 br s; 8.80 (d, J = 8.1 Hz); 8.72 (d, J = 5.8 Hz); 8.02 (dd, J = 5.8, 8.1 Hz)] and a deshielded methylene signal (δ 5.18 s). The framework of a β -substituted pyridine was readily constructed by the COSY spectrum. The ¹³C NMR spectrum revealed five signals assignable to pyridine (δ 147.0, 146.8, 146.0, 137.6, and 128.3), one deshielded methylene (δ 64.2), and two carboxylates (δ 171.5 and 168.5), thus satisfying the

molecular formula. The above units were connected on the basis of HMBC data; cross peaks between H7 and C-2/C-8 placed a carboxymethyl group on N1, whereas those between H2/H4 and C-9 accommodated a carboxyl group on C3. Therefore, compound **1** is 1-carboxymethylnicotinic acid. The compound was first described in 1991 as a synthetic product.⁵ Its crystal structure was reported in 1993.⁶ This is its first report from a natural source. To confirm this structure, compound **1** was prepared by condensation of nicotinic acid and iodoacetic acid. The synthetic compound was indistinguishable from the natural product in both spectral data and enzyme inhibitory activity.

1-Carboxymethylnicotinic acid inhibited papain with an IC₅₀ value of 80 mg/mL. Pipecolate derivatives,⁷ a diketopiperazine,⁷ and isoprenoid fatty acids containing phospholipids⁸ have been reported from sponges of the genus *Anthosigmella*.

Experimental Section

General Experimental Procedures. NMR spectra were recorded either on a JEOL A600 or a JEOL A500 NMR spectrometer. Chemical shifts were referenced with external dioxane (δ _C = 67.4 and δ _H = 3.70). MS were measured with a JEOL SX-102 mass spectrometer. UV spectra were recorded on a Hitachi 330 spectrophotometer.

Animal Material. The sponge was collected by hand using scuba at a depth of 20 m off the Sada Peninsula, 1000 km west of Tokyo. The sponge was identified by Dr. Rob van Soest, University of Amsterdam, as *Anthosigmella* cf. *raromicrosclera*. A voucher specimen (MA PRO 11506) was deposited at the Zoological Museum of the University of Amsterdam.

Extraction and Isolation. The frozen sponge (1.0 kg) was homogenized and extracted with EtOH (3 L × 3). The combined extracts were concentrated and partitioned between H₂O and Et₂O; the aqueous phase was further extracted with *n*-BuOH. The aqueous layer was subjected to column chromatography on Sephadex G-10 with H₂O. The fractions inhibitory against papain were combined, passed through an ODS column with H₂O, and finally separated by HPLC on Amide-80 (Tosoh) with a mixture of CH₃CN–MeOH–100 mM ammonium formate buffer (70:5:25) to yield **1** (1.5 mg).

Compound 1: colorless solid; UV (H₂O) λ _{max} 267 nm (ϵ 5,000); FABMS (positive, magic bullet matrix) m/z 204

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Table 1. ^1H and ^{13}C NMR Data for 1-Carboxymethylnicotinic Acid (**1**) in D_2O

position	^1H	^{13}C	HMBC correlation
2	8.72 (d, $J = 5.8$ Hz)	146.8	C-9
3		137.6	
4	8.80 (d, $J = 8.1$ Hz)	147.0	C-9
5	8.02 (dd, $J = 5.8, 8.1$ Hz)	128.3	C-3, C-6
6	9.00 (br s)	146.0	
7	5.18 (s)	64.2	C-2, C-8
8		171.5	
9		168.5	

(M + H)⁺ and 182 (M - Na + H)⁺; ^1H and ^{13}C NMR data, see Table 1.

Preparation of 1. To a solution of nicotinic acid (123 mg, 1 mmol) in DMF (2 mL) was added iodoacetic acid (186 mg, 1 mmol), and the mixture was stirred at 50 °C overnight. The reaction mixture was separated by Amide-80 HPLC as described above.

Enzyme Inhibitory Assay. Enzyme inhibitory assay against papain was carried out as described in the literature.⁹

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