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STRUCTURAL DETERMINATION OF CARNOSADINE, A NEW CYCLOPROPYL AMINO ACID, FROM RED ALGA *GRATELOUPIA CARNOSA*

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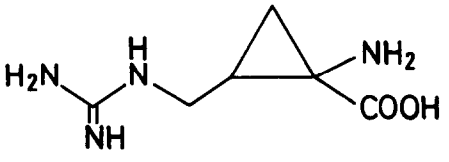
Summary: A new cyclopropyl amino acid, carnosadine, was isolated from red alga *Grateloupia carnosa* and the structure was determined to be 1-amino-2-guanidinomethylcylopropane-1-carboxylic acid from the results of NMR, mass spectrometry and coloring tests.

In our recent study on amino acids or peptides occurring in marine algae, we found two new ureido amino acids, *i.e.*, lividine (N^{ω}-carbamoyl-L-citrulline) and grateloupine (N-carbamoyl-γ-aminobutyric acid) from red algae *Grateloupia livida* and *Grateloupia filicina*, respectively.[1] Present paper describes an isolation and a structural determination of carnosadine, a new cyclopropyl amino acid from an alga in the same genus, *Grateloupia carnosa*, collected at Kada sea-coast, Wakayama Prefecture, Japan.

An aqueous extract of *Grateloupia carnosa* (dried, 2.5 Kg) was column chromatographed on Amberlite IRCG-50 to give pure carnosadine as hygroscopic powder (46 mg).[2] Carnosadine showed positive coloring tests both for ninhydrin and Sakaguchi reactions, but negative for Ehrlich reaction. This compound moved to the cathode at the same distance as arginine on paper electropherogram and was eluted in the middle of ammonia and arginine peaks in amino acid analysis. From these facts, carnosadine was assumed to be a monosubstituted guanidino amino acid.



Carnosadine

A structural determination of carnosadine was mainly achieved on the basis of NMR analyses. ^{13}C NMR spectrum revealed the presence of four alkane carbons and two carbons characteristic of guanidino (157.1 ppm) and carboxyl (171.4 ppm) groups. Of the four alkane carbons, the signal at 38.2 ppm was assigned to be of a quarternary carbon. Based on the proton selective decoupling measurement of ^{13}C NMR (Table 1), two protons centered at 1.51 and 1.58 ppm were ascertained to attach to the same carbon atom at 18.0 ppm. These protons showed the coupling with methine proton at 1.97 ppm in addition to their own geminal coupling. Furthermore, the methine proton couples with the another methylene protons splitting at 3.17 and 3.27 ppm which attach to the carbon at 39.4 ppm. Judging from the chemical shifts of these protons, the lower methylene was

Table 1. Correlations between ^{1}H and ^{13}C signals of carnosadine.

^{1}H signal	^{13}C signal	J_{C-H}(Hz)	Partial Structure
1.51(1H,t)	18.0	168	$-\overset{|}{\underset{|}{C}}-\underline{\underline{CH_2}}-\overset{|}{CH}-$
1.58(1H,q)		168	
1.97(1H,m)	24.0	169	$-CH_2-\underline{\underline{\overset{|}{CH}}}-CH_2-$
3.17(1H,q)	39.4	141	$-\overset{|}{CH}-\underline{\underline{CH_2}}-NH-C(=NH)NH_2$
3.27(1H,q)		141	

assumed to carry guanidino group rather than amino or carboxyl group.

The structure of carnosadine was unequivocally deduced to be 1-amino-2-guanidinomethylcyclopropane-1-carboxylic acid from the combination of the partial structures obtained by NMR analyses as shown in Table 1 as well as the consideration of multiplicity of three protons in higher field of ^{1}H NMR spectrum and the molecular weight measurement [*m/z* 173=$(M+H)^{+}$] by use of FAB mass spectrometry. The cyclopropyl structure was further supported from the large coupling constant between carbon and proton nuclei assigned as ring part (Table 1).

Two similar cyclopropyl amino acids have been known in nature, *i.e.*, 1-aminocyclopropane-1-carboxylic acid[4,5] from fruits as a precursor of ethylene which is an aging hormore in plants and 1-amino-2-ethylcyclopropane-1-carboxylic acid named coronamic acid[6] as a constituent amino acid of coronatine, a phytotoxin produced by *Pseudomonas coronafacience* var. *atropurpurea*. However, carnosadine is the first cyclopropyl amino acid isolated from marine algae. The studies of stereochemistry, synthesis, and biological activity of carnosadine are now in progress.

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References

1) T. Wakamiya, Y. Kobayashi, T. Shiba. K. Setogawa, and H. Matsutani, *Tetrahedron*, 40, 235 (1984).
2) Carnosadine was crystallized as di-*p*-hydroxyazobenzenesulfonate. This salt did not show a clear melting point, but sintered at 219°C decomposing at 226-232°C. Elemental analysis; Found: C, 49.12; H, 4.44; N, 15.00; S, 8.45%. Calcd for $C_{30}H_{32}N_8O_{10}S_2 \cdot 0.5H_2O$: C, 48.84; H, 4.51; N, 15.19: S, 8.69%.
3) The NMR spectra were obtained in 1N HCl by JEOL-JNM-GX 400 spectrometer.
4) L. F. Burroughs, *Nature*, 179, 360 (1957).
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