Occurrence of Piperidine-2,6-dicarboxylic acid (*trans*) in a Red Alga, *Porphyra tenera*

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Porphyra tenera, an edible red alga, was found to contain a new acidic imino acid ($C_7H_{11}NO_4$). This compound named teneraic acid is obtained from an alcoholic extract.

The chemical structure of teneraic acid was determined to be piperidine-2,6-dicarboxylic acid by means of NMR, Mass, and IR spectrometric studies and by chemical synthesis. The configuration of the compound was proved to be a *trans* form by ORD spectrometry.

Porphyra tenera has been extensively investigated in terms of amino acid composition and the seasonal variation of the amino acids. No unusual amino acid, however, has been reported. In our survey on amino acids in various marine algae, it was recognized that an alcoholic extract of the alga contained a ninhydrin positive component, which migrated to the anode faster than aspartic acid at pH 3.1. The component was named teneraic acid.

This communication reports the isolation of teneraic acid, and the elucidation of the chemical structure and the partial configuration of the acid.

Experimental

Isolation of Teneraic Acid

The alga (1 kg) was collected in Okkirai Bay in December. The amino acid extract was initially prepared by the procedure as previously described for chordarine¹⁾. Acidic amino acids were adsorbed on a column (3 × 38 cm) of Dowex 1 × 8 in the acetate form. Elution was performed by a gradient of 0.2–2 N acetic acid. Every fraction was tested by paper electrophoresis at 400 V, pH 3.1. Ammonium tenerate was crystallized from aqueous ethanol.

Preparation of Piperidine-2,6-dicarboxylic Acid

Piperidine-2,6-dicarboxylic acid was prepared by two different methods. (1): Pyridine-2,6-dicarboxylic acid was hydrogenated with platinum black in 50% acetic acid.²⁾ The product was crystallized from aqueous ethanol. (2): Piperidine-2,6-di-

carboxylic acid was prepared by an imination reaction of α,α' -dibromopimeric acid (10 g) with ammonium carbonate (25 g) in 70% ethanol (700 ml) for two weeks at room temperature. The remaining α,α' -dibromopimeric acid was extracted with ether after acidifying the reaction mixture. The products were purified with ion exchange chromatography on Dowex 50W × 8 (3 × 120 cm) in the pyridinium form, using 0.2 M pyridinium acetate, pH 3.1 as elution buffer. The isomers of piperidine-2,6-dicarboxylic acid were separated by fractional crystallization using aqueous ethanol.

Spectrophotometric Studies

Mass spectra were obtained with a Jeol model, LMG-06H. Samples were introduced with a direct inlet probe. NMR spectra were taken with a Jeol model JNM-PMX in D_2O with DSS as standard. Infrared spectra were measured with a Jasco model, IR-AI in KBr disc. ORD spectra were taken with a Jasco model, ORD-UV-5 in H_2O .

Results and Discussion

Isolation

Paper electrophoresis revealed that glutamic acid was eluted in fractions 18 to 28 (15 ml/tube), aspartic acid in fractions 24 to 38, and teneraic acid in fractions 48 to 94 by a gradient elution of acetic acid from the anion exchanger. After concentration *in vacuo*, ammonium tenerate was crystallized from aqueous ethanol. The yield was 120 mg of colorless needles, melting at 217–

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219°C (decomp.).

Chemical Structure

The molecular weight of teneraic acid was determined to be 173 from the parent peak as shown in Fig. 1. The elemental analysis of ammonium tenerate was C: 43.17, H: 7.19, N: 14.15, O: 33.19%. The molecular formula was determined to be $C_7H_{11}N_4O_3 \cdot NH_3$ (calcd. C: 44.20, H: 7.42, N: 14.32, O: 33.65%). The mass fragmentation pattern showed intense peaks at m/e 128 (M-COOH), 82 (M-C₂H₃O₄) and 55 (C₃H₅N or C₄H₇). The NMR spectrum in D₂O gave multiplet around 2 ppm and triplet at 4.0 ppm as shown in Fig. 2A. The multiplet could be identified as the six protons on three methylene groups in cyclic members. The triplet could be assigned to two protons on identical methine groups attached to both carboxyl and imino groups. The peaks m/e 128 and 82 in Fig. 1 might be interpreted to be the

following: $CH_2-CH_2-CH_2-CH(COOH)-NH=CH$
and $CH_2-CH_2-CH=CH-NH=CH$, respectively.

All above spectral data could be accommodated in the structure, piperidine-2,6-dicarboxylic acid for teneraic acid. The chemical structure of teneraic acid was confirmed by synthesis. Piperidine-2,6-dicarboxylic acid (2.4 g) obtained from catalytic hydrogenation of pyridine-2,6-dicarboxylic acid (3 g) gave the following elemental analysis, C: 44.15, H: 6.88, N: 7.13%; calcd. for $C_7H_{11}NO_4 \cdot H_2O$, C: 44.98, H: 6.81, N: 7.33%. The imination reaction of α,α' -dibromopimeric acid (10 g) gave two compounds by fractional crystallization. The first fraction (1.1 g) was

Fig. 2. NMR spectra of (A): teneraic acid, (B): piperidine-2,6-dicarboxylic acid (*trans*) (C): piperidine-2,6-dicarboxylic acid (*cis*) in D₂O with DSS as standard.

analyzed to be the monohydrate which was identical to the compound obtained from the catalytic hydrogenation. The second fraction (300 mg) was crystallized as the ammonium salt and gave an

Fig. 1. Mass spectrum of teneraic acid.

Fig. 3. IR spectra of ammonium salt of (A): teneraic acid, (B): piperidine-2,6-dicarboxylic acid (*trans*), (C): piperidine-2,6-dicarboxylic acid (*cis*) in KBr disc.

elemental analysis similar to that of ammonium tenerate.

All synthetic compounds and teneraic acid gave identical mass spectra. However, the synthetic piperidine-2,6-dicarboxylic acid monohydrate differed from teneraic acid in NMR (Fig. 2C) and IR spectrum (Fig. 3C) while the second fraction from the fractional crystallization of the piperidine-2,6-dicarboxylic acid gave identical spectra to those of the natural compound as shown in Fig. 2B and 3B.

Configuration

Piperidine-2,6-dicarboxylic acid can exist as three isomers derived from two asymmetric carbons as shown in Fig. 4. It has been reported that the reduction of pyridine-2,6-dicarboxylic acid by platinum catalyst gives *cis* configuration only³¹, which is the *meso* form. Teneraic acid was optically active as shown in Fig. 5. Therefore, the con-

figuration can be assigned as a *trans* form. The native compound is either the L (2*s*, 6*s*) or D (2*r*, 6*r*) form, and is not a mixture. The assignment of the enantiomer is under investigation.

Fig. 4. Stereoisomers of piperidine-2,6-dicarboxylic acid.

Fig. 5. ORD spectrum of ammonium tenerate in H₂O.

This is the first publication of natural occurrence of piperidine-2,6-dicarboxylic acid (*trans*). Despite the relatively high yield (120 mg/kg wet weight of the alga), the compound in an alcoholic extract could not be detected by amino acid analysis and paper chromatography because of the very low yield of ninhydrin color. It was detected on zone electrophoresis as very faint band with ninhydrin.

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